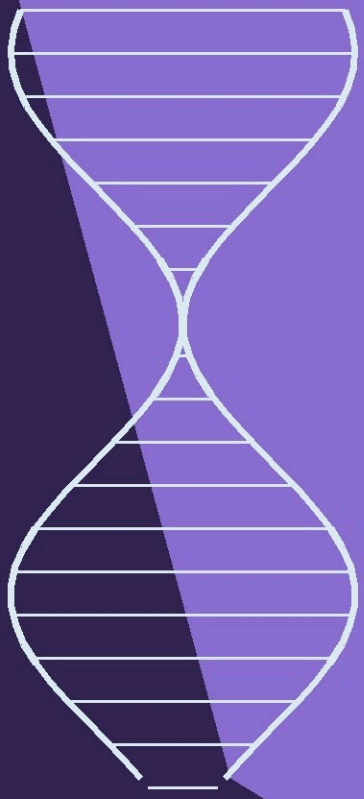
CDIL HEALTH SCIENCES PROFESSIONAL TEXTBOOK SERIES

VOLUME 10F-A

Clinical Immunology & Serology

Clinical Laboratory Science



Dr. Charles D. Bunbury II
Author

PLATINUM REVISION MASTER
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C. Douglas Institute of Learning
Making Education Easier

CDIL HEALTH SCIENCES
PROFESSIONAL TEXTBOOK SERIES

VOLUME 10F-A

Clinical Immunology & Serology

Mechanisms, interpretation and laboratory reasoning

Dr. Charles D. Bunbury II

C. Douglas Institute of Learning
Making Education Easier

CORRECTED ILLUSTRATED STUDENT EDITION

September 2026 • Controlled six-book production release

An educational text, not an authorisation to practise or a substitute for current local law, validated clinical procedures and supervised competence. Fictional teaching cases are not patient records.

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How to learn from this book

Read the mechanism before the case. Identify what each measurement actually observes, then ask which biological or analytical explanations could produce the pattern. The worked answers show how to move from observations to a defensible interpretation without hiding uncertainty.

Use each figure actively: trace the arrows, explain why a compartment or control matters, and identify what the model deliberately leaves out. In a reaction panel, choose cells that separate competing targets rather than merely counting positive wells. In a calculation, write the denominator and units before evaluating the number.

For retrieval, close the text and answer the case or mastery question in your own words. Compare the mechanism, limitations and proposed action with the reasoned answer. A correct label without a defensible explanation is not the same as professional competence.

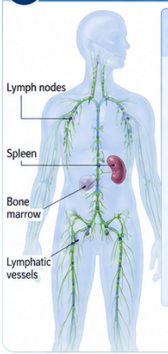
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Clinical Immunology and Serology

The Immune System in Health and Disease

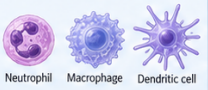
From recognition to response to diagnosis

1 Overview of the Immune System



Innate Immunity
(rapid, non-specific)

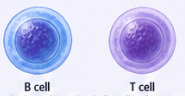
- Present at birth
- Recognizes common microbial patterns (PAMPs)
- Rapid response (minutes–hours)
- Limits infection and activates adaptive immunity



Physical barriers • Phagocytosis
Inflammation • Complement

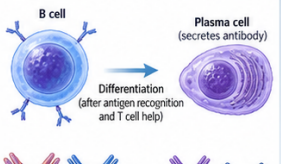
Adaptive Immunity
(specific, memory)

- Develops after antigen exposure
- Highly specific for antigens
- Slower onset (days)
- Memory for long-term protection



Innate and adaptive immunity work together for optimal protection

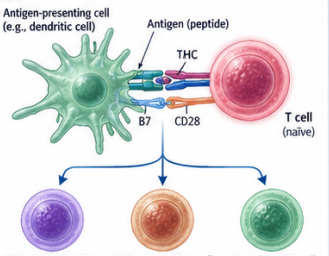
2 B Cells and Antibodies





Antibodies (immunoglobulins) bind specific antigens and mediate neutralization, opsonization, complement activation, and agglutination.

3 T Cells and Cellular Immunity

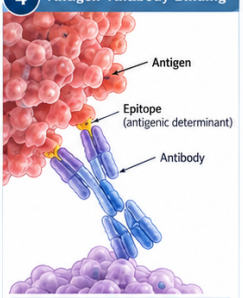


CD4⁺ T helper (Th)
- Secretes cytokines
- Helps B cells
- Activates macrophages

CD8⁺ cytotoxic T cell (Tc)
- Kills infected or abnormal cells
- Important in viral infections and cancer

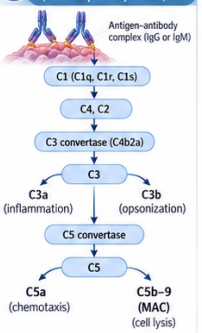
Regulatory T cell (Treg)
- Suppresses excess immune responses
- Maintains tolerance

4 Antigen–Antibody Binding



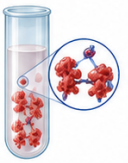
Antibodies bind specifically to epitopes on antigens through noncovalent interactions (e.g., hydrogen bonds, ionic bonds, hydrophobic forces).

5 Complement Cascade
(classical pathway shown)



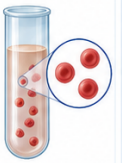
6 Agglutination

Positive agglutination
(antigen + specific antibody)



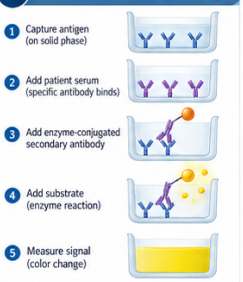
Visible clumping due to cross-linking of antigens by multivalent antibodies (e.g., in blood typing or serologic tests).

Negative reaction
(no specific antibody)



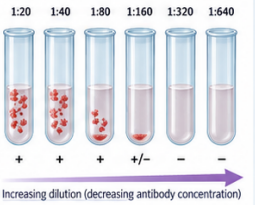
No agglutination; cells or particles remain dispersed.

7 ELISA / Immunoassay Concept



Signal intensity is proportional to the amount of specific antibody or antigen in the sample (e.g., for diagnosis or titer determination).

8 Serum Dilution and Interpretation



Titer = **highest dilution with a positive reaction.**

In this example, the antibody titer is **1:160.**

Figure 1. Clinical Immunology and Serology: key concepts and laboratory applications.
The immune system consists of innate and adaptive components that work together to defend against infection and maintain homeostasis. B cells produce antibodies, T cells mediate cellular immunity, and the complement system amplifies responses. Antigen–antibody interactions underlie many serologic tests, including agglutination and immunoassays such as ELISA. Interpretation of serum dilutions allows determination of antibody titers, which can aid in diagnosis, monitoring, and assessment of immune status.

Immunity informs diagnosis.
Serology translates immunity into answers.

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1 | Immune Architecture: Where Defence Is Organised

A blood sample can count circulating lymphocytes, but it cannot show the whole immune system. Most immune activity is organised in tissues: at an epithelial surface, inside a draining lymph node, around a splenic vessel, or within an inflamed organ. The location matters because cells can be present in normal numbers yet fail to meet antigen, migrate to the appropriate compartment, or receive the signals needed to become effective. This chapter connects the anatomical compartments with the laboratory observations that they help explain. [FA01, FA02]

1.1 Barriers are active tissues

Intact skin separates the external environment from living tissue. Its protection is not simply the presence of a covering. Closely apposed epithelial cells, an organised extracellular barrier, surface chemistry, antimicrobial substances and resident immune cells work together. A puncture bypasses this protection even when the instrument is too small to leave an obvious wound. The resulting risk depends on what was introduced, the tissue entered and the host response; the visible size of the puncture is not a measure of microbiological safety.

Mucosal surfaces face a different problem. The respiratory and gastrointestinal tracts must exchange material with the environment. They cannot simply become impermeable. Mucus, antimicrobial products, epithelial renewal and local immune responses provide protection while preserving physiological function. In the conducting respiratory tract, ciliary movement helps transport mucus and trapped material. The gastrointestinal tract uses different regional mechanisms, including mucus, peristalsis, secretions and organised mucosal immunity. It is incorrect to describe all mucosal surfaces as if they had the same ciliated clearance apparatus.

Commensal organisms are also part of this environment. Their presence does not mean that inflammation must occur. Immune regulation helps maintain a relationship in which microbial products

are tolerated in appropriate locations but invasion or tissue damage can trigger a response. A microorganism that is harmless on one surface can be dangerous in blood or a normally sterile compartment. Consequently, specimen source belongs in the interpretation of a microbiological or immunological result. The name of an organism without its anatomical context is incomplete information.

1.2 Primary lymphoid organs establish the repertoire

Bone marrow produces blood cells from haematopoietic stem and progenitor cells. B-cell development takes place predominantly in marrow, whereas T-cell precursors migrate to the thymus for important stages of maturation. The familiar branching diagram of myeloid and lymphoid development is a useful teaching map, not a claim that every cell passes through an identical, sharply separated route. Tissue-resident populations and inflammatory conditions introduce additional biological complexity.

Developing lymphocytes generate diverse antigen receptors. Diversity is valuable because the body cannot predict every antigen that it will encounter. It also creates risk: some receptors will recognise self structures. Development therefore includes selection processes that reduce dangerous self-reactivity while retaining useful cells. In the thymus, conventional T cells are selected in relation to self major histocompatibility complex molecules and the strength of interactions with self peptide–MHC complexes. Selection does not make autoimmunity impossible. Peripheral regulation remains necessary throughout life.

A small thymus on an image, a low lymphocyte count, and an abnormal T-cell functional test answer different questions. Imaging concerns anatomy; the count concerns a circulating population; functional testing concerns performance under specified laboratory conditions. None can be substituted automatically for the others. When a child has serious, recurrent or unusual infections, clinicians combine the history, examination and appropriate investigations rather than declaring immune competence from a single normal number.

1.3 Secondary lymphoid organs bring rare partners together

A lymph node receives lymph from a drainage territory. Antigen and antigen-bearing cells arriving from that territory encounter lymphocytes in organised microenvironments. B-cell follicles and T-cell-rich areas are not random collections of cells. Chemokines and adhesion systems help position cells so that the required interactions are more likely to occur. Antigen recognition is rare at the level of any one naive lymphocyte; organised trafficking greatly improves the efficiency of finding the relevant clone.

Activated B cells can participate in germinal-centre responses. Within these structures, proliferation, selection and interactions with helper cells support the development of more effective antibody responses and memory. The dark and light zones seen in an appropriate histological section reflect different aspects of this organised process. They are not simply "infected" and "uninfected" halves of a node. A histological image is a two-dimensional sample of a three-dimensional structure, so a section may not display every zone equally well.

The spleen samples blood rather than acting as a substitute for a lymph node draining an injured limb. Its red-pulp functions include filtering circulating cells, while white-pulp compartments support immune responses. The spleen is particularly important in defence against certain bloodstream infections, including infection with encapsulated bacteria. A person without effective splenic function may have a normal total white-cell count and still have an important susceptibility. The laboratory must not translate "within the reference interval" into "no immune risk."

Mucosa-associated lymphoid tissues organise responses at sites of repeated environmental exposure. Local antibody production, epithelial transport and homing of activated cells help direct protection to those surfaces. A serum immunoglobulin concentration is therefore not a complete measurement of every mucosal defence. The amount measured in blood, the isotype, the specificity and the compartment in which the antibody acts all matter.

1.4 Movement is part of immune function

Cells reach tissues by regulated movement. At an inflamed vessel, changes in endothelial adhesion molecules and local chemotactic signals help leukocytes slow, adhere, cross the endothelium and migrate. The simplified sequence of rolling, firm adhesion and transmigration is useful because it identifies distinct points of failure. A circulating neutrophil may be capable of killing a microbe in a tube yet fail to reach infected tissue efficiently in the patient.

Lymphocytes also recirculate between blood and lymphoid tissues. A blood count is a snapshot of the cells present in the sampled compartment at that moment. Stress responses, medications, infection and redistribution can change that snapshot. For a subset result, the denominator must be clear: a percentage of lymphocytes is not the same as a percentage of all leukocytes, and neither is an absolute count. A change in another population can alter a percentage even when the number of cells of interest is unchanged.

For example, suppose a synthetic sample contains 2.0 × 10^9 lymphocytes/L and 30% of those lymphocytes are CD4-positive T cells. The derived absolute CD4 count is 0.60 × 10^9/L, or 600 cells/µL. If the lymphocyte count subsequently falls to 1.0 × 10^9/L while the CD4 fraction rises to 40%, the absolute count is 400 cells/µL. The percentage rose while the absolute count fell. These invented values illustrate arithmetic, not a treatment threshold.

1.5 From architecture to a responsible laboratory report

Laboratory assessment begins by identifying the question. A complete blood count helps describe circulating cells. Flow cytometry can identify selected populations and, in some assays, evaluate function. Quantitative immunoglobulin tests measure classes of circulating proteins. Antigen-specific tests ask narrower questions about recognition or exposure. Complement investigations assess another set of proteins and pathways. A normal result in one category cannot exclude a defect in every other category.

Preanalytical information is especially important when the question concerns cells. Collection time, anticoagulant, transport conditions and delay can affect the suitability of a sample. The accepted conditions are method-specific and must come from the laboratory's validated procedure. A textbook should explain why those conditions matter; it should not invent a universal stability period for all lymphocyte assays.

The professional report preserves the distinction between observation and interpretation. "The CD19-positive B-cell count is below this laboratory's age-appropriate interval" is an observation with defined scope. "The patient cannot make antibodies" is a much broader conclusion requiring additional evidence. The second statement may be wrong even when the first is correct. The most useful interpretation connects an abnormal finding with the clinical question, identifies relevant limitations and indicates when specialist evaluation is required.

From tissue entry to a germinal-centre response

Follow a protein antigen that enters through a damaged skin barrier. A local antigen-presenting cell can acquire material, receive inflammatory signals and migrate toward a draining lymph node. The cell does not carry a miniature copy of the entire infection into every organ. Its migration links a particular tissue territory to an organised site where rare antigen-responsive lymphocytes can encounter the relevant information. The lymph node is therefore a biological meeting place, not simply a filter that mechanically traps every dangerous particle. [FA02, FA04]

Naive T cells enter lymphoid tissue through specialised vascular routes and move through regions in which antigen-presenting cells are available. Their movement is guided by chemokine receptors and adhesion interactions. Recognition of an appropriate peptide–MHC complex, together with the necessary costimulatory and cytokine signals, can initiate activation. A T cell that receives no relevant recognition signal does not become specific for that antigen merely because it passed through an inflamed node.

B cells recognise native antigen through their surface immunoglobulin receptors. A B cell that binds a protein antigen can internalise it, process it and display peptides to an appropriate helper T cell. This creates a linked interaction: the B cell's receptor and the T cell's receptor need not recognise the same physical epitope, but their recognition is connected through the antigen taken up by the B cell. The distinction helps explain why antibody recognition of a protein surface and T-cell recognition of a processed peptide are different events.

Some activated B cells develop into antibody-secreting cells relatively early. Others enter germinal-centre reactions, where proliferation, somatic hypermutation and selection contribute to the development of higher-affinity populations. Follicular dendritic cells retain antigen in a form accessible to B cells; despite their name, they are not simply the same lineage as conventional dendritic cells presenting peptide to naive T cells. T follicular helper cells provide important help during selection. Confusing these two kinds of "dendritic" cell obscures the organisation of the reaction.

A germinal centre is often described as having dark and light zones. The zones reflect different dominant activities and cell distributions, not an impenetrable wall between two fixed populations. B cells can cycle through stages of proliferation, receptor change and selection. Cells whose receptors acquire useful binding properties may receive survival and differentiation signals; cells that fail to compete effectively may be lost. This process increases the quality of a selected response while also requiring safeguards against harmful self-reactivity.

The products include memory B cells and plasma cells with different survival and migration properties. Long-lived plasma cells can support sustained antibody concentrations without a continuing high count of circulating plasmablasts. Memory B cells can support a later response even when a serum antibody concentration has declined. Consequently, a single serum concentration, a circulating-cell count and the capacity for a recall response are related but distinct observations.

This anatomical sequence clarifies several laboratory puzzles. A lymph-node enlargement can reflect active immune organisation, malignancy or other causes; size alone does not identify the process. A blood specimen may contain only a small fraction of the cells participating in a tissue response. A normal serum immunoglobulin concentration does not demonstrate normal germinal-centre selection against every antigen. The learner should connect each specimen to the compartment and process that it can actually represent.

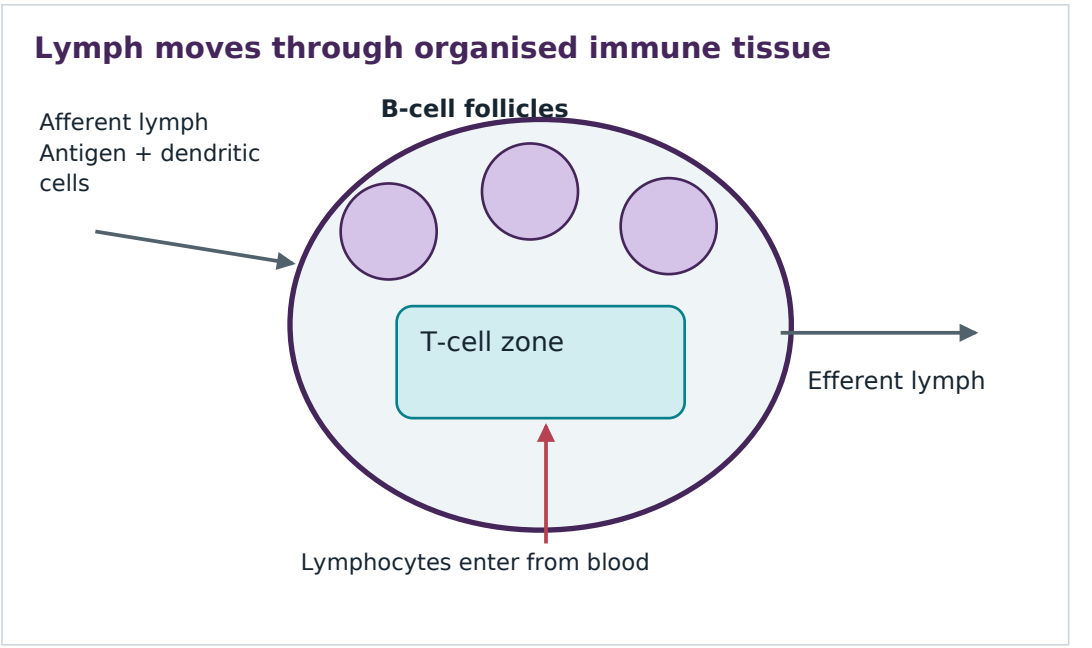


Figure FA01. Lymph-node schematic. Antigen and antigen-presenting cells arrive through afferent lymph; lymphocytes enter through specialised blood vessels. B-cell follicles and the T-cell zone support different interactions. Compartments are simplified, not to anatomical scale. Original CDIL educational schematic; not patient data.

Worked cases 1–4

Case 1 — Normal count after splenectomy

A fictional adult who had a splenectomy after trauma develops fever and rigors. The total leukocyte count is within the laboratory's adult interval. A student argues that this proves the immune system is functioning normally. What is wrong with the argument?

Reasoned answer. The count describes circulating cells, not the filtering and immune functions formerly performed by the spleen. The patient's anatomical history changes the significance of the presentation. Serious infection can occur without an out-of-range leukocyte count, and the normal result must not delay urgent clinical assessment. The laboratory should communicate clinically important results through its escalation process and ensure that the history accompanies relevant testing. This case does not supply a diagnosis or an antibiotic instruction; it demonstrates why anatomical loss cannot be excluded by a numerical test of another compartment.

Case 2 — A percentage that appears to improve

A fictional patient's lymphocyte count changes from 2,000 cells/µL to 1,000 cells/µL. The proportion of CD4-positive cells changes from 30% to 40%. Calculate the two absolute values and explain the apparent contradiction.

Reasoned answer. The initial value is 2,000 × 0.30 = 600 cells/µL. The later value is 1,000 × 0.40 = 400 cells/µL. The fraction is larger because the denominator changed; the actual number is smaller. Before comparing the two reports, also confirm that the same population, gating strategy and reporting units were used. The arithmetic does not establish the cause of the change. It prevents the narrower error of calling an increased percentage an increased cell count.

Case 3 — A node and its drainage territory

A fictional patient has an enlarged axillary node after an inflammatory lesion on the same arm. A learner calls the node a blood filter and assumes that enlargement proves a systemic infection.

Reasoned answer. An axillary node can respond to material arriving through lymph from its drainage territory. Enlargement may reflect a local immune response, although numerous other causes remain possible. The spleen is the more appropriate example of an organ sampling blood. Node enlargement alone neither identifies a microorganism nor proves systemic infection. The useful reasoning

connects the lesion, lymphatic drainage and clinical examination while recognising the limits of anatomical inference.

Case 4 — Delayed cellular specimen

A lymphocyte functional specimen arrives after a transport delay outside the receiving laboratory's validated conditions. A routine blood count from the same patient was processed promptly and was unremarkable. May the delayed functional specimen be treated as acceptable because the count was normal?

Reasoned answer. No. The two tests have different analytical requirements, and the count does not validate the condition of the delayed specimen. The laboratory should apply its acceptance procedure, document the delay and communicate the need for recollection or a qualified interpretation. The central issue is not whether the patient "has lymphocytes" but whether the received cells can support the intended measurement. A technically unsuitable functional test can create a false impression of immune failure.

2 | Innate Recognition, Inflammation and Microbial Killing

Innate immunity provides rapid responses through barriers, soluble proteins and cells equipped to recognise recurring biological patterns. Rapid does not mean nonspecific in the sense of indiscriminate. A receptor may be highly selective for a molecular structure while recognising that structure on many organisms. The distinction from adaptive immunity concerns the organisation and generation of recognition systems, not whether recognition has any specificity. [FA01, FA03]

2.1 Pattern recognition and context

Pattern-recognition receptors detect microbial-associated structures and signals associated with cell damage. Toll-like receptors, cytosolic nucleic-acid sensors and other receptor families occupy different cellular locations. Location helps determine what is encountered. A nucleic acid inside an endosome or cytosol can carry different meaning from the same chemical class in its normal compartment. Recognition activates signalling pathways that alter gene expression, mediator release and cellular behaviour.

A useful distinction is between recognition of a microbe and recognition of damage. Inflammation can occur after sterile trauma, ischaemia or crystal deposition without an invading microorganism. Conversely, some infections produce a muted inflammatory response, especially in an immunocompromised person. An increased inflammatory marker is therefore not a direct assay for "bacteria present." It is evidence about a host response under particular conditions.

The response is also regulated. Uncontrolled inflammatory signalling can injure the host. Excess vascular permeability, widespread endothelial activation and disrupted perfusion are not desirable simply because they began as defence mechanisms. Immune

competence involves both effective activation and appropriate limitation. A patient can simultaneously have damaging inflammation and inadequate control of an infection.

2.2 Recruitment links recognition with a working cell population

Resident cells at a site of injury can release mediators that change local vessels and recruit additional cells. Vasodilation increases local blood flow, while altered permeability allows fluid and proteins to enter tissues. These changes help explain redness, warmth and swelling, but the signs are not sufficiently specific to identify a cause. Their absence does not exclude deep infection or infection in a person unable to mount a typical response.

Neutrophils are prominent in many acute inflammatory responses. Their movement depends on adhesion, chemotaxis and the physical ability to traverse tissue. The blood neutrophil count is influenced by production, release, distribution and removal. A high count can reflect mobilisation, not necessarily a proportional increase in effective killing. A low count can create vulnerability, but its interpretation requires the absolute value, age, history and clinical setting.

In the laboratory, a differential count, an oxidative-burst assay and an adhesion-molecule investigation answer different questions. The first counts or classifies circulating cells. The others evaluate specific properties. A functional defect can exist with a normal or increased count. Understanding the sequence from recruitment to killing prevents the assumption that cell abundance equals cell performance.

2.3 Phagocytosis is a sequence, not a single event

A phagocyte must recognise or attach to a target, engulf it, organise an intracellular compartment and deploy antimicrobial mechanisms. Opsonins improve attachment by coating the target with structures recognised by phagocyte receptors. Antibody and complement fragments are important examples. Opsonisation is not the same as direct lysis: its immediate effect is to make the target easier for a cell to recognise and ingest.

After engulfment, the phagosome interacts with intracellular vesicles and antimicrobial machinery. Reactive oxygen species, enzymes and other mechanisms contribute to killing. The relative importance of each mechanism varies with the organism and cell type. A familiar clinical example is chronic granulomatous disease, in which defects affecting the phagocyte NADPH oxidase impair the oxidative burst. The term "granulomatous" describes a pattern of tissue response; it does not mean that the patient has an excess of useful granulocytes.

An oxidative-burst assay must be interpreted in relation to the stimulus, control sample, cell viability and analytical method. In a dihydrorhodamine-based flow assay, the measured fluorescence pattern reflects oxidation under the specified test conditions. A weak response can result from biological dysfunction or a compromised specimen. Distribution across cells may be as important as the average signal. A report should not reduce every abnormal pattern to the name of one gene without the appropriate clinical and molecular evaluation.

2.4 Natural killer cells and antibody-dependent mechanisms

Natural killer cells can respond to a balance of activating and inhibitory signals on potential targets. Reduced expression of appropriate self MHC class I molecules may remove an inhibitory signal, while stress-associated ligands can provide activation. The phrase "missing self" is a useful shorthand, but it is not a complete description of every NK-cell decision. Many receptors and environmental signals contribute.

NK cells can also participate in antibody-dependent cellular cytotoxicity through receptors that recognise antibody bound to a target. This illustrates cooperation between innate effector cells and adaptive recognition. The antibody identifies a target through its antigen-binding regions; the effector cell recognises the antibody's constant region. The two forms of recognition should not be confused when interpreting an assay.

Counting NK cells is different from testing NK-cell function. A population described by a surface-marker combination may be present while its cytotoxic performance is impaired. Marker expression also varies with activation and disease. Flow-cytometric identification must use a defined panel and gating strategy rather than a single marker interpreted outside its context.

2.5 Acute-phase proteins and the meaning of a trend

The systemic response to inflammation can alter concentrations of proteins made by the liver and other tissues. C-reactive protein is a familiar example. Its concentration may support assessment of inflammation and change over time, but it does not by itself locate the process or name the pathogen. Trends are meaningful only when the time course, treatment, specimen quality and measurement method are considered.

A falling inflammatory marker does not automatically establish recovery. The patient may have a lagging clinical response, a localised process, altered protein production or a complication not captured by that marker. Equally, a persistent increase may not prove treatment failure if another inflammatory process is present. The clinician integrates the pattern; the laboratory contributes reliable measurement and relevant limitations.

A synthetic example shows why units matter. A concentration of 8 mg/dL equals 80 mg/L. Reporting one result in mg/dL and a later result in mg/L without recognising the conversion can create an apparent tenfold change. A correct interpretation begins with the measurand, units and reference or decision framework before discussing biological significance.

2.6 Resolution and trained responses

Inflammation can resolve through coordinated changes in mediators, removal of debris and restoration of tissue function. Resolution is not merely exhaustion of the response. Macrophages participate in clearing dying cells and shaping repair, although their behaviour cannot be represented adequately by a rigid division into only two fixed types. Their state depends on tissue signals and can change over time.

Innate responses can also be modified by earlier exposures through mechanisms often described as trained immunity or tolerance. These changes do not create the same receptor-rearrangement system used by B and T lymphocytes. The existence of such adaptation is a reason to avoid the absolute statement that innate immunity has "no memory of any kind." For foundational learning, the safest distinction is that classical adaptive memory is mediated by selected antigen-specific lymphocyte populations, while innate cells can show other forms of altered responsiveness.

The oxidative burst as a mechanistic example

A phagocyte's oxidative burst begins with assembly and activation of an enzyme system that transfers electrons to oxygen. The resulting reactive oxygen species participate in antimicrobial activity and in downstream chemical reactions. Myeloperoxidase in appropriate cells uses hydrogen peroxide and halide substrates to generate additional antimicrobial products. These pathways operate within a broader killing system that includes granule contents, acidification and nonoxidative mechanisms. "Oxidative burst" is therefore one important mechanism, not a synonym for every function of a neutrophil. [FA02, FA16]

A dihydrorhodamine assay uses a dye whose fluorescence changes after oxidation. Cells are examined before and after a defined stimulus, commonly with a healthy comparator and appropriate analytical controls. The distribution of fluorescence is informative. A uniformly poor response differs from a mixture of responding and poorly responding cells. The latter can arise in particular biological

settings, including some carrier patterns, but it must be interpreted by a laboratory familiar with the assay and the relevant differential.

A stimulus does not test every step of natural host defence. A reagent that directly activates an intracellular pathway may bypass receptor or uptake events that matter during infection. This is why a normal response to one stimulus cannot establish normal chemotaxis, adhesion, phagocytosis and killing of every organism. The assay answers a specific functional question under controlled conditions.

Complement-mediated and antibody-mediated opsonisation also illustrate cooperation. A bacterium coated with C3b-derived fragments can be recognised by complement receptors. A bacterium coated with appropriate IgG can be recognised through Fc receptors. The efficiency of uptake depends on the available opsonin, receptor expression and physical accessibility. An organism's capsule or other surface properties may affect these interactions. A phagocyte can possess intact killing machinery yet encounter difficulty attaching to an inadequately opsonised target.

Inflammasome biology adds another layer. Certain intracellular sensing pathways organise protein complexes that promote activation of inflammatory caspases and processing of cytokines such as IL-1β. In some circumstances, these pathways are associated with inflammatory cell death. The term inflammasome should not be used as a generic name for all inflammation. It refers to particular molecular assemblies and mechanisms that contribute to selected responses.

Neutrophil extracellular traps are another specialised response. They involve extracellular chromatin and associated proteins that can interact with microbes and inflammatory processes. Their presence is not automatically beneficial: extracellular material can also contribute to tissue injury or thrombosis in some settings. Laboratory descriptions should avoid the simplistic claim that every increase in a defensive mechanism represents improved immunity.

The central lesson is causal separation. Recruitment determines whether a cell reaches the site. Opsonisation and receptors influence attachment. Engulfment creates an intracellular compartment. Oxidative and nonoxidative systems influence killing. Inflammatory

regulation affects surrounding tissue. A defect at one stage may produce a similar clinical infection pattern to a defect at another, but the laboratory investigations needed to distinguish them are different.

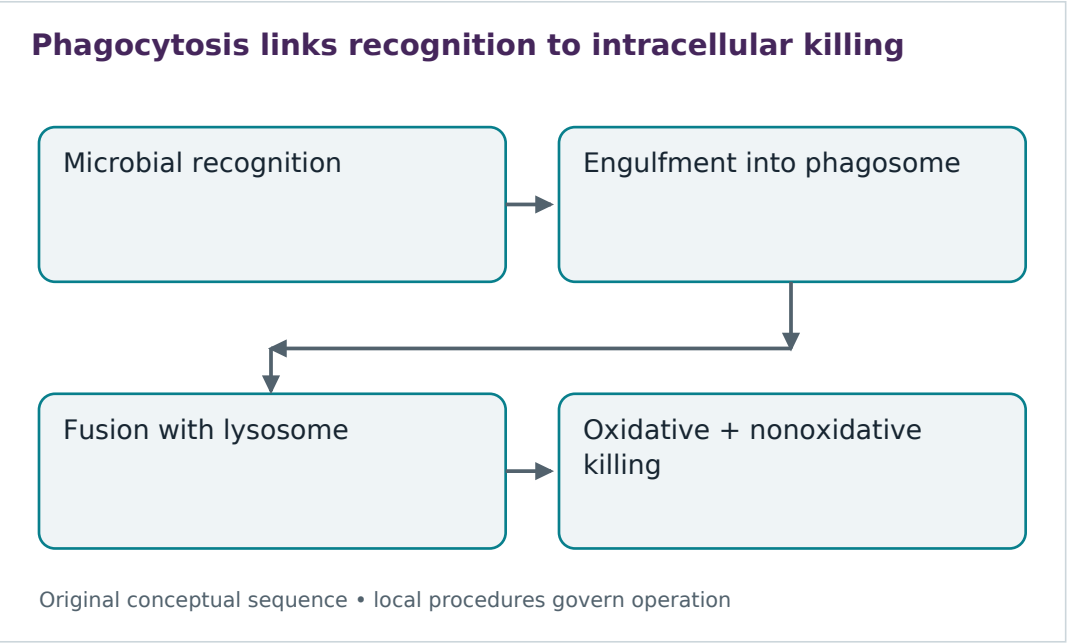


Figure FA02. Recognition, enclosure, phagolysosome formation and killing are separate functions. A failure in oxidative killing can coexist with successful ingestion. Original CDIL educational schematic; not patient data.

Worked cases 5–8

Case 5 — Many neutrophils, poor killing

A fictional child has recurrent deep infections. The neutrophil count is not low, but a properly controlled oxidative-burst assay shows markedly reduced stimulation. A student proposes that the normal count excludes a phagocyte disorder.

Reasoned answer. Abundance and function are different measurements. The result raises concern about a defect in oxidative killing, provided specimen viability, transport and controls are satisfactory. It does not, by itself, identify the causal variant. The clinical team may need confirmatory and molecular investigation. The important inference is that a normal count cannot exclude a functional phagocyte defect; the laboratory should report the

observed pattern and its analytical limitations rather than substituting a broad reassurance.

Case 6 — Sterile inflammation

A patient has an inflammatory response after major tissue injury, with no microorganism yet demonstrated. A learner labels the raised inflammatory marker a positive bacterial test.

Reasoned answer. The marker reflects aspects of the host response, which can be triggered by damage-associated signals as well as infection. The available evidence does not establish a bacterial cause. Absence of microbiological confirmation also does not rule infection out; the appropriate conclusion is narrower. The clinician must evaluate the whole presentation. This case separates a measurement of inflammation from direct detection of an organism or its products.

Case 7 — A tenfold change that is not a biological change

Two fictional reports show CRP values of 6 mg/dL and 60 mg/L. Are the results different by a factor of ten?

Reasoned answer. No. Since 1 dL is one tenth of a litre, 1 mg/dL equals 10 mg/L. Both reports therefore represent the same numerical concentration after conversion. Biological interpretation still depends on the clinical setting, but no increase has been demonstrated by these two values. The laboratory should harmonise display units or make conversions explicit when presenting longitudinal results.

Case 8 — Counting NK cells

A referral asks whether a patient has normal NK-cell function. The available report lists an NK-cell count within an age-appropriate interval but contains no functional assay. What can be concluded?

Reasoned answer. The report supports a statement about the number of cells identified by that laboratory's marker and gating definition. It does not establish normal cytotoxic function. A functional investigation may be considered by the specialist if the clinical question warrants it, with attention to specimen conditions and assay limitations. "Cells are present" and "cells perform every required task normally" are different propositions.

3 | Adaptive Recognition, Lymphocyte Activation and Memory

Adaptive immunity solves a recognition problem by generating a large and diverse repertoire of lymphocytes before most specific encounters occur. An antigen selects cells able to recognise it; the selected population can then expand and differentiate. The antigen does not teach an undifferentiated antibody how to change shape to fit it. This distinction between selection and instruction is essential for understanding vaccination, monoclonal populations, antibody affinity and immune memory. [FA02, FA04]

3.1 Receptor diversity and clonality

B-cell and conventional T-cell receptors acquire diversity through gene rearrangement and associated processes during development. Each clone has a particular recognition potential. The repertoire is not a list of one cell for every possible named disease. Recognition occurs at the level of molecular structures, and one receptor can show cross-reactivity with more than one related structure.

A B-cell receptor can recognise an accessible antigenic structure in its native form. Conventional alpha-beta T-cell receptors usually recognise peptide presented by MHC molecules. This difference explains why an antibody-binding assay and a T-cell stimulation assay are not interchangeable measures of immunity. Nonconventional T-cell populations, including cells recognising nonpeptide ligands through specialised presenting molecules, add complexity beyond the foundational peptide–MHC model.

Clonality is not synonymous with malignancy. Expansion of selected clones is a normal part of an immune response. Conversely, a malignant population may have a characteristic receptor rearrangement or restricted protein product that helps identify it. The biological and clinical context determines whether a restricted pattern is expected, reactive, persistent or concerning. Laboratory

terminology should distinguish a detected restricted population from a final disease diagnosis.

3.2 T-cell activation requires more than recognition alone

A naive conventional T cell needs an appropriate combination of antigen recognition, costimulatory interactions and environmental signals. Dendritic cells are important in initiating many such responses because they can acquire antigen, become activated and present it in a context that supports T-cell activation. A peptide–MHC complex is therefore necessary for many responses but is not the whole activation instruction.

Costimulation provides information about context. If a T cell recognises antigen without the signals needed for productive activation, several outcomes are possible, including forms of nonresponsiveness or regulation. This helps explain why simply exposing a person to an antigen does not guarantee a strong protective response. Route, dose, formulation, tissue environment and host factors affect the resulting response.

Activated CD4-positive T cells can develop different functional programmes, shaped by signals received during activation and subsequent interactions. Some support phagocyte activity, some support mucosal responses, some help B cells, and some suppress excessive activation. These are functional categories with plasticity, not sealed compartments in which a cell can never change its behaviour. Activated CD8-positive T cells can kill appropriate targets, but cytotoxicity is controlled by recognition and effector mechanisms rather than indiscriminate destruction of all nearby cells.

3.3 B-cell activation and linked recognition

When a B cell binds a protein antigen through its receptor, it can internalise the antigen, process it and present peptides to an appropriate helper T cell. The B and T cells need not recognise the same physical epitope. They must recognise linked components of the antigenic material so that help is delivered to the relevant B cell. This is called linked recognition.

CD40–CD40 ligand interactions and cytokine signals are important in many T-dependent antibody responses. They help support proliferation, differentiation and changes in antibody class. Some antigens can activate B cells through pathways that do not require the same form of T-cell help. Responses to repetitive polysaccharide structures are an important teaching example, but age and immune status affect performance. This difference contributes to the rationale for conjugate vaccines, in which a polysaccharide is linked to a protein carrier to recruit T-cell help.

The laboratory consequence is that a normal total immunoglobulin concentration does not guarantee a normal response to every antigen. Quantitative testing asks how much of a class is present. Antigen-specific response assessment asks a narrower functional question. Even that assessment must use a defined timing and interpretation framework. A single antibody concentration obtained at an unspecified time cannot be treated automatically as a complete evaluation of antibody production.

3.4 Germinal centres refine a response

In a germinal-centre response, activated B cells proliferate and undergo processes that can alter receptor affinity and antibody class. Somatic hypermutation introduces changes into variable-region sequences. Selection favours cells whose receptors perform well in the relevant antigen and helper-cell environment. Affinity maturation is therefore a population-level outcome of mutation and selection, not a conscious improvement made by an antibody already circulating in plasma.

Class-switch recombination changes the heavy-chain constant region used by a B cell. It changes the antibody's effector relationships and distribution while generally preserving the rearranged variable-region specificity. Somatic hypermutation may occur in the same overall response and can separately alter binding characteristics. Keeping these two processes distinct avoids the misleading statement that changing from IgM to IgG automatically changes which antigen is recognised.

Some activated B cells become antibody-secreting plasma cells. Others contribute to memory populations. Long-lived plasma cells can maintain antibody production without continuous exposure to the original pathogen, while memory cells support future responses. The persistence of a measurable antibody is not itself proof that the pathogen remains present. The meaning depends on the organism, antigen, assay and clinical question.

3.5 Memory, protection and vaccination

A secondary response may be faster or more effective because selected populations and, in some circumstances, persisting antibody already exist. Protection is not captured by one universal antibody threshold. A correlate of protection established for one infection, vaccine, assay or population cannot be transferred automatically to another. Some protective responses depend strongly on cellular immunity or mucosal compartments that routine serum testing does not fully represent.

Vaccination aims to generate a beneficial response without the risks of the corresponding natural infection. The details differ across vaccine types. The laboratory must distinguish assays detecting vaccine-associated responses from assays directed at antigens not present in a particular vaccine. Hepatitis B provides a familiar example: antibody to surface antigen can follow vaccination, whereas antibody to core antigen ordinarily reflects exposure to viral core material rather than the usual surface-antigen vaccine. Interpretation still requires the full marker pattern and relevant history.

Immune memory also explains why the absence of a currently detectable antibody does not always mean that the person has never responded. Concentrations can fall below an assay's detection limit while memory populations persist. Whether this is reassuring depends on the specific disease and protection framework; it must not become a blanket statement that a negative antibody test proves protection through invisible memory.

3.6 Tolerance is an active achievement

Central selection reduces self-reactivity during development, and peripheral mechanisms restrain potentially harmful responses after lymphocytes enter the circulation and tissues. Regulatory cells, inhibitory receptors, restricted access to antigen and the context of antigen presentation contribute. Tolerance is not the absence of an immune system; it is part of a functioning system.

Failure of regulation can produce autoimmunity, allergy or uncontrolled inflammation. However, detection of an autoreactive antibody does not establish that every regulatory mechanism has failed or that a named autoimmune disease is present. Some antibodies occur at low levels in people without the disease for which the assay is commonly requested. The bridge from basic immunology to diagnostic practice is probability, clinical phenotype and appropriate confirmatory evidence.

Clonal selection, receptor diversity and linked help

The adaptive repertoire is generated before the immune system knows which particular pathogen will be encountered. Gene-segment rearrangement contributes to the diversity of B- and T-cell receptors, while junctional changes and pairing of receptor chains add further variation. The resulting population contains many cells with different recognition properties. Antigen does not instruct a mature lymphocyte to design a completely new receptor from scratch; it selects and activates cells whose receptors can participate in the response. [FA02, FA04]

Activation is not an unlimited permission to proliferate. Conventional naive T-cell activation requires integration of antigen recognition, costimulation and contextual signals. Signals through inhibitory receptors and the absence of appropriate costimulation can alter the outcome. A receptor that binds a relevant complex is therefore necessary in many settings but not sufficient to explain the final cellular behaviour.

CD4 helper populations can support different types of response. Th1-associated activity is important in selected cell-mediated

responses, Th2-associated activity in responses involving type-2 immunity, and Th17-associated activity in particular barrier and inflammatory settings. T follicular helper cells support B-cell responses, while regulatory T cells contribute to restraint. These names are useful functional categories, not a complete catalogue of rigidly separate cell species. Cytokine production and phenotype can change with context, and one surface marker cannot perfectly summarise all helper functions.

CD8 T cells can kill appropriate target cells through mechanisms including release of cytotoxic granules and receptor-mediated death pathways. The target must be recognised in the relevant presentation context. A cell that has lost or altered antigen presentation may evade an otherwise competent T-cell population. Conversely, uncontrolled or misdirected cytotoxicity can damage healthy tissue. Effective defence requires both access to the correct target and limitation of collateral injury.

B-cell activation by protein antigens often involves linked T-cell help. CD40 on the B cell and CD40 ligand on an activated helper cell provide an important interaction, together with cytokines and other signals. Defects in this cooperative system can impair class switching and produce characteristic immunological patterns. A high IgM concentration alone does not diagnose a specific defect; the mechanism must be evaluated with the broader phenotype and appropriate investigations.

Memory is also distributed across compartments and cell types. Memory T cells can differ in migration and residence, while memory B cells and long-lived plasma cells contribute different capabilities. A vaccine-response investigation should therefore not claim to measure "all immune memory" when it measures only a serum antibody concentration. The concentration can be clinically useful, but its scope should remain explicit.

Primary and secondary response curves are teaching models, not fixed clocks. The timing and magnitude depend on the antigen, route, dose, prior exposure, age and immune status. A rapid IgG response can reflect previous exposure, while an IgM result may persist or be nonspecific. The correct clinical interpretation uses organism- or

vaccine-specific evidence rather than a universal diagram applied to every infection.

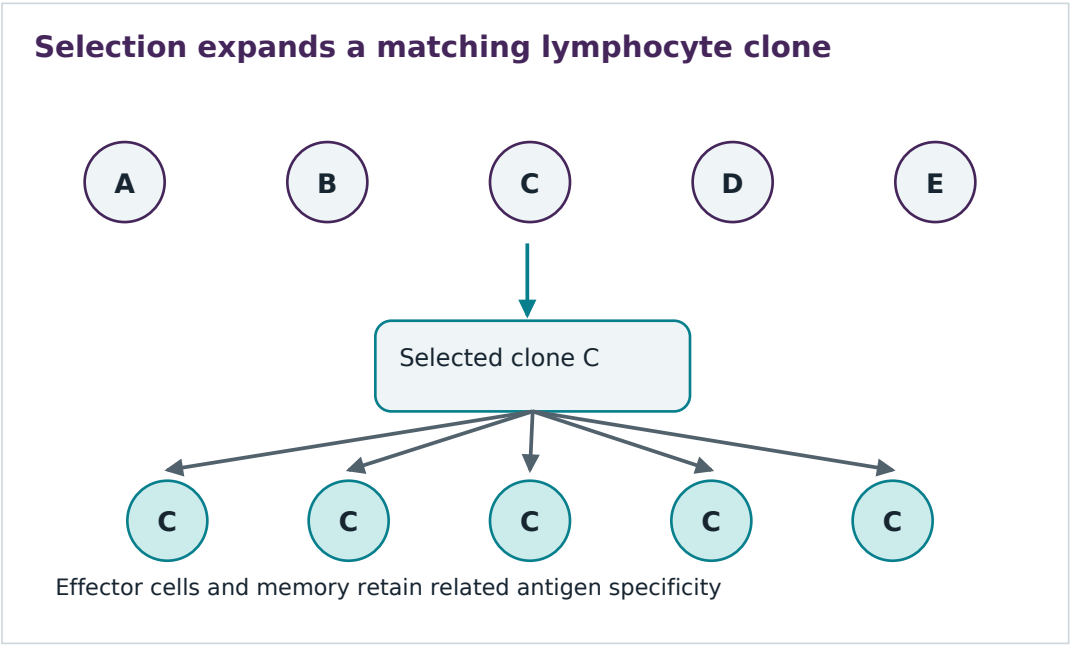


Figure FA03. Different receptor shapes represent a pre-existing repertoire. Antigen selects a matching clone; proliferation produces effectors and memory. The diagram does not imply that antigen instructs a new receptor sequence. Original CDIL educational schematic; not patient data.

Worked cases 9–12

Case 9 — Normal total IgG, recurrent infection

A fictional patient has recurrent bacterial respiratory infections. Total IgG is within the laboratory interval. The referral asks whether specific antibody responses are adequate.

Reasoned answer. Total IgG quantifies a class of proteins; it does not show the range or effectiveness of antigen-specific responses. A specialist may investigate defined responses to selected antigens using an appropriate history and timing framework. The normal quantitative result is useful but does not answer the entire functional question. Other causes of recurrent infection, including structural airway disease, exposure and non-antibody immune defects, must also remain in the differential rather than being forced into an antibody diagnosis.

Case 10 — Vaccine marker or infection marker?

A fictional hepatitis B triple panel shows surface antigen negative, total core antibody negative and surface antibody positive. The history documents vaccination, with no known infection. Explain why the pattern is compatible with vaccination rather than evidence of a current infection.

Reasoned answer. The usual vaccine presents surface antigen, so a surface-antibody response can occur without exposure to core antigen. The negative surface-antigen result does not demonstrate current antigenaemia, and the absent core antibody fits the vaccination history. The pattern must still be interpreted using the assay and patient's circumstances. It is the combination of markers—not the word "positive" beside one antibody—that supports the conclusion. [FA12]

Case 11 — Switching class

An experimental B-cell clone retains recognition of the same antigen after changing from IgM production to IgG production. A learner calls this affinity maturation. Is that sufficient terminology?

Reasoned answer. The described change is class switching. It concerns the heavy-chain constant region and associated effector properties. Affinity maturation refers to selection for improved binding and is associated with variable-region mutation and selection. Both can occur during a response, but one does not define the other. The experiment would need binding measurements and appropriate controls to establish a change in affinity.

Case 12 — Antibody disappears from a report

A fictional patient had a documented positive antibody result years earlier but is now below the assay's detection limit. A student proposes deleting the historical result because memory cannot exist without measurable circulating antibody.

Reasoned answer. Memory cells and circulating antibody are related but different components. Loss of detectability does not erase a documented historical response. The clinical meaning depends on the particular antibody and disease. In transfusion medicine, a

history of a clinically significant red-cell antibody can remain important even when the current screen is negative. Historical evidence should therefore be preserved, not rewritten to match the latest assay's detection limit.

4 | Antigen Presentation, HLA and T-Cell Recognition

Antigen presentation connects a molecule in one compartment with a lymphocyte capable of responding to it. The human major histocompatibility complex is usually discussed as the human leukocyte antigen system, or HLA. HLA molecules bind selected peptides and display them at a cell surface. Their role is not to label every cell as simply "self" or "foreign." They create a molecular display that can be examined by appropriately restricted T cells. [FA02, FA05]

4.1 Class I presentation

HLA class I molecules are expressed on most nucleated cells and also on platelets; mature red cells do not provide the ordinary class I presentation platform described here. In the conventional pathway, intracellular proteins are degraded into peptides, some peptides are transported into the endoplasmic reticulum through TAP, and suitable peptides are loaded into class I molecules. The complex then reaches the cell surface. The alpha chain associates with beta-2 microglobulin, which is not itself encoded within the classical HLA class I region.

The peptide-binding groove of class I molecules generally accommodates relatively short peptides. The exact peptide length and sequence preferences depend on the particular molecule. For introductory learning, approximately 8–10 amino acids is a useful common range, not an absolute law that excludes every longer ligand. The groove and its peptide create the surface recognised by a conventional CD8-positive T cell.

A virus-infected cell may therefore display peptides derived from viral proteins synthesised inside the cell. A cytotoxic T cell recognising an appropriate peptide–HLA complex can deliver a targeted killing response. The cell is not killed because HLA class I is inherently dangerous; it is the recognised complex and the effector context that matter. Tumours may evade immune recognition partly

by changing antigen expression or presentation, but no single presentation defect explains every tumour.

4.2 Class II presentation

HLA class II molecules are expressed prominently on professional antigen-presenting cells, including dendritic cells, B cells and monocytes/macrophages, with expression in other cells under certain conditions. In the conventional pathway, material taken into endosomal compartments is processed and peptides are loaded into class II molecules. The invariant chain and its CLIP fragment help control the loading process, and HLA-DM facilitates appropriate peptide exchange.

The class II groove is open at its ends and commonly accommodates longer peptides than class I. Conventional CD4-positive T cells recognise the resulting complexes. A B cell that has internalised a protein through its antigen receptor can present peptides from that protein to a helper T cell. This provides the cellular basis for linked recognition described in the preceding chapter.

The phrase "class I is endogenous, class II is exogenous" is a useful starting map, but it is not a complete description of every pathway. Cross-presentation allows some antigen-presenting cells to present acquired extracellular material on class I molecules. Other processes can bring intracellular material into class II presentation. The map explains common routes; it should not become a rigid rule that prevents recognition of exceptions.

4.3 HLA diversity and restriction

HLA genes are highly polymorphic. Different alleles encode molecules with different peptide-binding properties. A person expresses a selection inherited from both parents, and codominant expression allows contributions from both parental haplotypes. Population frequencies vary, but a person's HLA type should be measured or inferred through validated testing rather than guessed from appearance or a broad ancestry label.

MHC restriction means that a conventional T-cell receptor recognises antigen in relation to an appropriate presenting molecule. Two

people exposed to the same pathogen may present different peptide sets because their HLA molecules differ. This is one reason a response measured against one peptide cannot be assumed to represent every person's complete response to an organism.

HLA associations with disease alter probability; they usually do not establish a diagnosis by themselves. A common susceptibility allele may be present in many healthy people. The absence of an allele can sometimes be more informative than its presence, depending on the condition and testing purpose. The report must specify whether a result concerns susceptibility, exclusion, pharmacogenetic risk, transplantation or another application.

4.4 HLA typing is not antibody testing

HLA typing describes the patient's or donor's genetic or antigenic characteristics at the loci examined. An HLA antibody test investigates antibodies that bind HLA targets. These are different measurements. A transplant recipient may have antibodies against HLA antigens not present in their own inherited type because of prior sensitising exposure such as pregnancy, transfusion or transplantation.

The laboratory must also distinguish resolution. A broad antigen-level description, a low-resolution allele group and a higher-resolution sequence assignment do not convey identical information. Ambiguity may remain when the tested regions do not distinguish every possible allele. The needed resolution depends on the clinical application and governing transplant programme.

Antibody assays introduce a separate set of limitations. A signal from a bead assay reflects binding under that assay's conditions. It is not a direct concentration of antibody in universal units, and a numerical fluorescence value is not automatically a clinical risk threshold. Donor-specific interpretation requires the actual donor type, recipient antibody history, assay characteristics and the transplant programme's validated framework.

4.5 Crossmatching and the direction of risk

In a transplant crossmatch, recipient material is examined for reactivity against donor cells or relevant donor antigens through a defined method. The direction matters: the concern is not a generic statement that the donor and recipient "have different blood." A physical crossmatch, virtual crossmatch and solid-phase antibody investigation use related information but are not interchangeable procedures.

A virtual crossmatch depends on reliable donor typing, recipient antibody identification and appropriate historical information. Missing or ambiguous donor typing can make the inference unsafe. A negative result in one method does not erase a clinically significant historical antibody or exclude every possible mechanism of graft injury. The appropriate conclusion is bounded by the method and the programme's policy.

4.6 A laboratory reasoning sequence

Before interpreting an HLA-related result, identify the sample owner, clinical purpose, loci or antigen targets, method, resolution and comparison standard. Next ask what the result actually establishes. A genetic result may establish that a particular sequence was detected. An antibody result may establish binding to a reagent antigen. A crossmatch may establish reactivity under the selected conditions. Only after those distinctions are clear should a broader clinical implication be discussed.

This disciplined sequence is especially important when results are moved between institutions. A label such as "HLA positive" is not a meaningful complete report. The reader needs to know positive for what, by which method, in which specimen, for which purpose. Precision in naming the measurand is a safety intervention, not an academic formality.

Two presentation pathways and important exceptions

Class I HLA molecules commonly present peptides derived from intracellular proteins to conventional CD8 T cells. Proteins are degraded, peptides become available for loading through a coordinated intracellular pathway, and peptide–HLA complexes reach the cell surface. The process depends on more than the HLA gene sequence: protein expression, processing, transport, loading and cell viability all influence the displayed repertoire. [FA02, FA05]

Class II HLA molecules commonly present peptides derived from material processed in endosomal compartments to conventional CD4 T cells. Antigen-presenting cells take up material, process it and load selected peptides onto class II molecules. Accessory proteins regulate loading and the removal of placeholder peptides. The simplified contrast of "inside to class I" and "outside to class II" is useful for orientation, but biological exceptions prevent it from being an absolute rule.

Cross-presentation allows some cells to present acquired external material through class I pathways. Autophagy and other processes can contribute intracellular material to class II presentation. Nonclassical molecules also present particular classes of antigen or participate in specialised recognition. A foundational diagram should mark the main routes while acknowledging that it is a model, not an exhaustive account of every antigen-presentation event.

HLA polymorphism changes the repertoire of peptides that can be presented. Individuals inherit sets of alleles from both parents and express multiple class I and class II molecules. A particular allele can be associated with altered susceptibility to disease or a drug reaction, but association is not determinism. The allele does not act independently of other genes, exposures and the relevant biological mechanism.

Histocompatibility typing identifies inherited markers at a specified resolution. Serological, molecular and sequencing approaches do not provide identical information. A low-resolution result can group several alleles that are distinguished by a higher-resolution method. The required resolution depends on the clinical application. A result

should not be described as a complete sequence-level match when the method did not establish that level of detail.

Antibody investigations add another dimension. An anti-HLA antibody result concerns recognition by the recipient's antibodies; an HLA genotype concerns the donor's or recipient's inherited sequence. A virtual crossmatch combines these different data sources under a defined interpretive framework. It is not a physical mixing test, and its reliability depends on current, sufficiently complete antibody and typing information.

Mature red cells do not express HLA class I in the same way as nucleated cells, whereas platelets express class I HLA despite lacking a nucleus. This is a useful warning against turning a broad teaching shortcut into an absolute claim. In transfusion science, the distinction matters when investigating platelet refractoriness and selecting appropriate components. The learner should always ask which cell population is being discussed before applying a general statement about antigen expression.

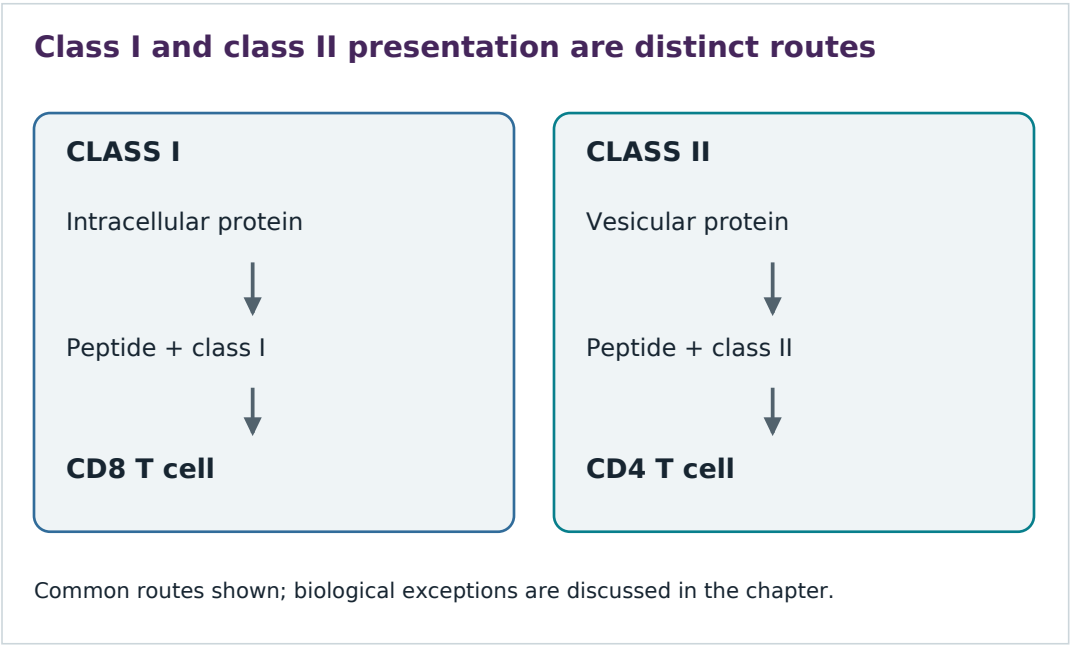


Figure FA04. Class I commonly presents intracellularly derived peptides to CD8 T cells; class II commonly presents vesicular peptides to CD4 T cells. Cross-presentation and other exceptions are explained in the text. Original CDIL educational schematic; not patient data.

Worked cases 13–16

Case 13 — The platelets exception

A student writes, "Only nucleated cells express HLA class I, so platelets cannot carry HLA targets." Identify the error and its transfusion relevance.

Reasoned answer. Platelets are an important exception to the simplified statement about nucleated cells: they express HLA class I. HLA antibodies can therefore be relevant in selected platelet-transfusion investigations, including assessment of immune causes of poor increments. This does not mean that every inadequate platelet increment is immune or HLA-mediated. Fever, consumption, bleeding, splenic sequestration and other factors can contribute. The correction preserves the useful general model without overlooking a clinically important exception.

Case 14 — Susceptibility is not diagnosis

A fictional patient's genetic report identifies an HLA allele associated with a disease. The patient has no compatible symptoms, and the clinician has not established the disease. May the laboratory report be rewritten as a confirmed diagnosis?

Reasoned answer. No. An association changes probability within a defined context. Many carriers of susceptibility alleles remain unaffected. The report should preserve the actual allele finding and its application-specific interpretation. The next clinical steps depend on the reason for testing, phenotype and other evidence. Treating a susceptibility marker as a diagnosis confuses genetic association with the complete clinical criteria for disease.

Case 15 — Antigen outside, class I display

An antigen-presenting cell takes up material from another cell and subsequently stimulates a conventional CD8-positive T cell. A learner says this is impossible because the antigen began outside the presenting cell.

Reasoned answer. Cross-presentation provides a route by which acquired material can be presented on class I molecules. The usual

endogenous/class I and exogenous/class II distinction is therefore a teaching framework rather than an exceptionless rule. The observation should lead to investigation of the presentation pathway, not dismissal of the result solely on the basis of the simplified map.

Case 16 — A fluorescence number without a donor type

A recipient's antibody report contains a high bead fluorescence signal, but the donor's relevant HLA type is incomplete. The team asks whether the antibody is donor-specific.

Reasoned answer. Donor specificity cannot be established merely from the magnitude of the signal. The target recognised by the antibody must be compared with reliable donor typing at the necessary resolution. Assay interference, historical results and the transplant programme's interpretation rules also matter. The immediate laboratory contribution is to identify the missing information and avoid converting an incomplete comparison into a confident compatibility statement.

5 | Antibody Structure, Binding and Effector Function

An antibody can be understood as a molecule with two linked jobs: recognising a target and recruiting or positioning an effect. Its antigen-binding regions provide recognition, while its constant regions influence interactions with receptors, complement and transport systems. A test that measures binding does not automatically measure every downstream effect. This distinction explains many apparent disagreements between immunological assays. [FA02, FA06]

5.1 Heavy chains, light chains and domains

A conventional immunoglobulin monomer contains two heavy chains and two light chains linked through covalent and noncovalent interactions. Each chain contains variable and constant regions. The variable regions of one heavy and one light chain contribute to an antigen-binding site. The complementary determining regions form important parts of the binding surface, but the surrounding framework helps maintain the necessary structure.

Light chains occur as kappa or lambda types. An individual conventional antibody molecule uses one light-chain type, not a mixture of one kappa and one lambda chain. A polyclonal response contains antibodies produced by many clones and ordinarily includes both types across the population. A restricted light-chain pattern may support a clonality investigation, but the clinical meaning depends on the specimen, assay and accompanying findings.

The Fab region includes the antigen-binding portion. The Fc region supports interactions with receptors and other effector systems according to the antibody class and subclass. Proteolytic fragments are useful experimental concepts, but a clinical assay detecting a fragment is not necessarily measuring the same material as one directed at an intact molecule. The assay's epitope requirements determine what it can recognise.

5.2 Epitopes and cross-reactivity

An epitope is the structure recognised by an antibody's binding site. Some epitopes are formed by a sequence of neighbouring residues; others depend on the three-dimensional folding that brings separated regions together. Denaturation can therefore destroy some epitopes and expose others. This helps explain why an antibody can react in one method and not another even when the methods are described as testing "the same protein."

Cross-reactivity occurs when a binding site recognises more than one sufficiently compatible structure. It is not always an analytical failure. Some cross-reactive responses are biologically meaningful; others complicate interpretation. The laboratory question is whether the observed binding is sufficiently specific for the intended clinical use. A manufacturer may choose antigens or epitopes to improve that specificity, but the resulting assay may detect a narrower set of responses than another platform.

A negative result can therefore reflect absence of the relevant antibody, a concentration below detection, poor recognition of the assay's antigen form, unsuitable timing or interference. A positive result can reflect the intended specificity, cross-reactivity or other analytical effects. The correct interpretation depends on the clinical question and the evidence supplied by the method, not on a universal meaning assigned to the words positive and negative.

5.3 Affinity and avidity

Affinity describes the strength of an individual binding interaction. Avidity describes the combined stability produced by multiple interactions in a particular arrangement. A multivalent antibody interacting with a multivalent target can show strong overall binding even when each individual interaction is modest. This is why the number and spacing of binding sites matter in agglutination and other lattice-forming reactions.

These terms should not be replaced by concentration. A large amount of low-affinity antibody and a smaller amount of high-affinity antibody may behave differently in a wash-based assay. Washing removes

unbound material and can also expose differences in binding stability. Changing incubation conditions can alter the observed balance. The result is a property of the biological sample interacting with a defined analytical system.

Avidity assays used in some infectious-disease investigations compare binding under different conditions, often including a dissociating treatment. They provide assay-specific information about binding stability. They do not function as universal clocks that date every infection precisely. Interpretation requires the organism, immunoglobulin class, validation population and clinical timeline.

5.4 Neutralisation, opsonisation and complement recruitment

Neutralising antibodies interfere with a biologically important interaction, such as attachment or entry. Binding to an antigen is not automatically neutralisation. An antibody may recognise a noncritical region and bind strongly without preventing the relevant biological event. A binding assay and a neutralisation assay therefore measure different properties, even when their results are correlated in a particular setting.

Opsonising antibodies can promote recognition by cells bearing appropriate Fc receptors. Complement activation can add another set of effector mechanisms, but different classes and subclasses differ in their capacity to recruit complement. The biological result also depends on antigen density, antibody arrangement, local regulators and target characteristics. A positive antibody test alone does not establish that complement-mediated injury is occurring in the patient.

Antibody-dependent cellular cytotoxicity connects antibody-coated targets with effector cells. Again, an assay must define which step it measures. Detecting antibody binding is not equivalent to demonstrating cell killing. When a therapeutic antibody is evaluated, its target, Fc properties, concentration and biological context all influence performance.

5.5 Monoclonal and polyclonal reagents

A monoclonal reagent originates from a defined clone or engineered sequence and generally recognises a particular epitope or epitope configuration. A polyclonal reagent contains a mixture of antibodies with broader recognition. Neither category is automatically superior for every purpose. A monoclonal reagent may offer reproducibility and specificity but fail to recognise a variant epitope. A polyclonal reagent may recognise more forms of an antigen but introduce different specificity and lot-consistency challenges.

Modern therapeutic antibodies may be engineered in several ways. Their presence can interfere with laboratory tests when the assay detects the drug itself, when the drug binds a reagent target or when it changes the patient's biology. A new band on immunofixation, for example, may require evaluation in relation to therapeutic antibody exposure rather than immediate classification as a new endogenous clone.

For any reagent, the relevant question is not whether its name sounds sophisticated. The question is whether its performance has been established for the intended measurand, specimen and decision. Reagent identity, lot, storage and controls belong in the quality system because a change at the recognition step can alter the meaning of the final signal.

5.6 A practical binding example

Consider two fictional assays for an antibody to protein X. Assay A uses a folded recombinant protein, while assay B uses a denatured antigen preparation. A sample is reactive in A and nonreactive in B. The disagreement does not immediately show that one laboratory made an error. The patient antibody may recognise a conformational epitope retained in A but lost in B. Alternatively, differences in sensitivity, threshold or interference may explain the pattern.

A useful investigation begins with the actual antigen preparations and analytical claims, then considers an independent method or clinically relevant functional assessment where indicated. Repeating the same method without changing any informative condition can

confirm reproducibility, but it may not explain the disagreement. Troubleshooting is strongest when each additional test addresses a specific hypothesis.

Affinity, avidity and the geometry of binding

An antibody's antigen-binding site is formed by variable regions whose three-dimensional structure determines interactions with an epitope. The interaction is noncovalent and depends on complementary shape and chemical forces. Affinity describes the strength of one binding interaction under defined conditions. Avidity describes the combined functional strength of multiple interactions. A multivalent antibody can therefore bind a multivalent target strongly even when each individual interaction has modest affinity. [FA02, FA06]

Geometry affects what the antibody can do. An IgM molecule with multiple binding sites can link particles efficiently when epitopes are appropriately spaced. An IgG molecule may bind strongly to a cell without producing visible agglutination under the same conditions. The absence of a visible clump does not prove absence of antibody binding. Antiglobulin methods exploit this distinction by detecting sensitisation that is not otherwise macroscopically apparent.

The Fab region contains the antigen-binding capability, whereas Fc-mediated interactions connect binding to effector functions. Fc receptors on cells and interactions with complement can translate recognition into uptake, activation or other effects. Different antibody classes and subclasses do not have identical effector properties. The phrase "an antibody was detected" is therefore incomplete when the clinical question concerns complement activation, placental transfer or cellular clearance.

Proteolytic fragments help illustrate structure. A preparation containing antigen-binding fragments can retain recognition without all the effector properties of an intact molecule. Therapeutic antibody fragments and engineered formats use this principle in different ways. The learner should not assume that every reagent labelled "antibody" is a complete, bivalent IgG with an unchanged Fc region.

Class switching changes the heavy-chain constant region while preserving the rearranged antigen-binding specificity of that B-cell lineage. Somatic hypermutation changes variable-region sequences and can alter affinity. These are different processes. A switch from IgM to IgG does not, by itself, explain why binding has become stronger; affinity maturation involves mutation and selection of variable regions.

Cross-reactivity arises when an antibody recognises a sufficiently similar structural feature on another target. It is not necessarily random or evidence that the antibody has lost all specificity. In diagnostic assays, cross-reactivity can be predictable from related antigens or unexpected because of complex molecular forms. Analytical specificity must therefore be evaluated experimentally in relevant specimens rather than inferred from the antibody's label.

Binding also depends on concentration and equilibrium. A weakly reactive specimen near a cutoff may change classification with small analytical variation. Repeating it may provide useful information about reproducibility, but it does not transform a continuous distribution into a perfectly sharp biological boundary. The report should use the assay's validated equivocal or repeat-testing rules where applicable and avoid presenting every borderline change as a new clinical event.

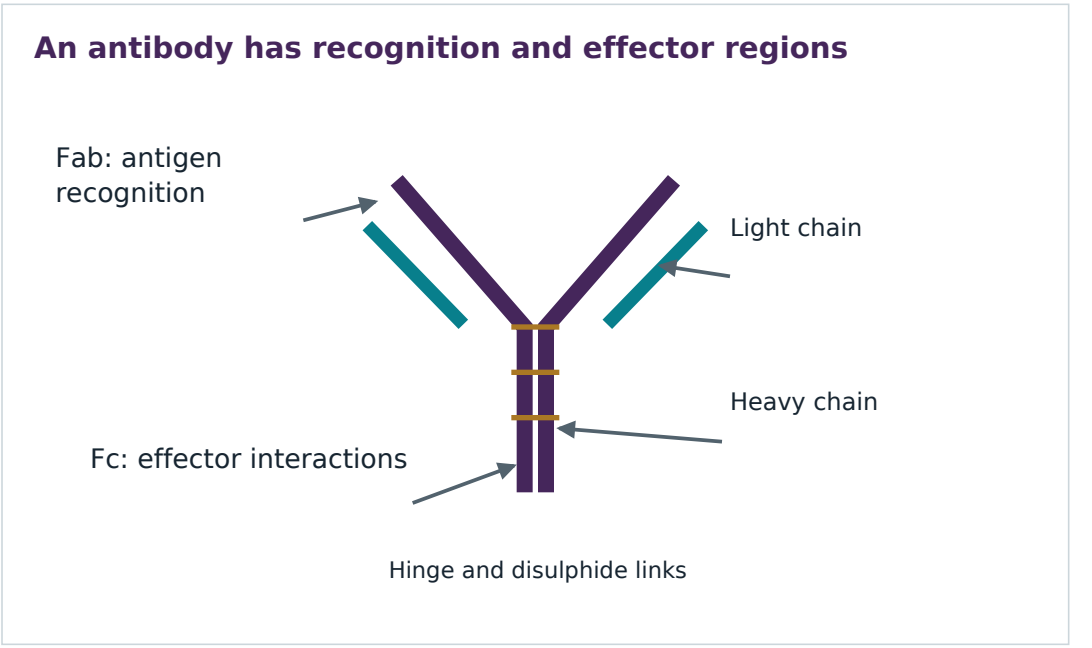


Figure FA05. Original IgG model with two heavy chains and two light chains. Fab regions recognise antigen; the Fc region participates in effector interactions. IgG is not drawn to molecular scale. Original CDIL educational schematic; not patient data.

Worked cases 17–20

Case 17 — Binding without neutralisation

A research sample binds a viral surface protein strongly in an immunoassay but shows little activity in a validated neutralisation experiment. A learner concludes that the neutralisation experiment must be wrong.

Reasoned answer. Binding and interference with infection are different properties. The antibodies may recognise regions that do not block the relevant biological interaction, or the two assays may differ in antigen form and conditions. Technical controls still require review, but disagreement is not itself proof of error. The report should name the measured property accurately rather than calling every binding antibody "protective."

Case 18 — Monoclonal reagent misses a variant

A fictional antigen variant reacts with a broad polyclonal reagent but not with one monoclonal reagent. What mechanism could explain the result?

Reasoned answer. The variant may alter the particular epitope recognised by the monoclonal reagent while leaving other epitopes available to the polyclonal mixture. Differences in sensitivity and technical conditions are alternative explanations that require investigation. The observation does not mean that all monoclonal reagents are unreliable. It demonstrates why reagent specificity and variant recognition must be understood for the intended use.

Case 19 — Affinity is not quantity

Two samples contain the same measured concentration of an antibody. One retains substantially more binding after a dissociating wash. Can the difference be explained solely by the reported concentration?

Reasoned answer. No. Binding stability depends on affinity, avidity, antigen presentation and assay conditions as well as concentration. A controlled avidity investigation may provide additional information, but its interpretation is method- and disease-specific. The result should not be translated into an exact infection date without a validated framework.

Case 20 — A new protein band during therapy

A fictional patient receiving a therapeutic IgG monoclonal antibody develops a small new immunofixation band. No medication history was supplied with the specimen.

Reasoned answer. The laboratory should obtain the treatment history and consider whether the drug can be detected by the method. An exogenous antibody can resemble an endogenous restricted protein. Comparison with prior studies and an appropriate interference-resolution method may be needed. The finding must not be dismissed automatically, but neither should it be called a new malignant clone without investigating the analytical and therapeutic context. [FA23]

6 | Immunoglobulin Classes, Compartments and Clinical Questions

The five major immunoglobulin classes are not five interchangeable quantities of the same substance. Their heavy-chain constant regions help determine structure, distribution and effector relationships. A result becomes useful when the class is connected with the antigen, compartment, time course and clinical question. "IgM positive" or "IgG low" is only the beginning of that interpretation. [FA02, FA06]

6.1 IgG: systemic distribution and persistence

IgG is a major circulating immunoglobulin class. Its subclasses differ in structural and functional properties, including interactions with Fc receptors and complement. IgG can move between vascular and extravascular compartments, and receptor-mediated processes contribute to its persistence. Placental transfer of maternal IgG provides passive antibody to the fetus. The amount and specificity transferred depend on maternal and placental factors; the newborn's antibody profile is therefore not simply a record of the newborn's own immune encounters.

A total IgG measurement pools many specificities. It does not identify which antibodies are present or whether each is protective. A high total concentration can reflect a broad reactive response, a restricted protein population or other clinical circumstances. A low concentration may arise from impaired production, protein loss, treatment effects or another cause. Quantitation should therefore be interpreted alongside the pattern of other immunoglobulins, clinical history and, when indicated, assessment of specific antibody function.

Subclass testing can answer selected questions, but an isolated subclass result is not a universal explanation for recurrent infection. Age, laboratory method, repeatability and functional responses matter. A subclass concentration below an interval in an otherwise

well person does not automatically justify treatment or establish a named immune disorder.

6.2 IgM: early responses and large molecular assemblies

Secreted IgM commonly forms pentameric assemblies, usually incorporating a joining chain, while membrane IgM on a B cell is monomeric. Multivalency gives secreted IgM considerable capacity to form lattices and recruit complement under suitable conditions. Its size influences distribution, with much of the circulating material remaining in the vascular compartment.

IgM often appears early in a primary antibody response, but this is not an exceptionless diagnostic clock. Organism-specific IgM can persist, recur, cross-react or be affected by analytical interference. Some acute infections do not produce a detectable IgM result at the time sampled. The statement "IgM means current infection" is therefore too broad. Interpretation requires the organism, assay performance, timing and other markers.

Elevated total IgM with low concentrations of switched classes can suggest a class-switching problem in an appropriate clinical setting, but several mechanisms can produce related patterns. A total-protein measurement cannot distinguish every defect in T-cell help, B-cell signalling or molecular switching machinery. The investigation must move from phenotype to mechanism rather than assigning a gene from one class pattern.

6.3 IgA: blood and mucosal forms

Serum IgA is predominantly monomeric. Secretory IgA at many mucosal surfaces is commonly dimeric, with a joining chain and secretory component associated with epithelial transport. This arrangement helps support immune exclusion and protection at interfaces repeatedly exposed to environmental material. Serum concentration and mucosal activity are related but are not identical measurements.

An IgA-based antibody assay can be falsely reassuring in a person who cannot produce an adequate IgA response. Coeliac-disease

serology is a practical example. A negative IgA tissue-transglutaminase test must be interpreted in relation to total IgA and the patient's gluten exposure and clinical context. Appropriate IgG-based testing can be useful in IgA deficiency, but indiscriminate addition of IgG assays in every IgA-sufficient person can reduce diagnostic specificity. [FA15]

Low IgA is not automatically the same clinical entity in an infant and an adult. Age-appropriate interpretation is necessary. A laboratory should report the measured concentration, interval and analytical limits without converting a single low value into a complete immunodeficiency diagnosis. The broader evaluation considers persistence, other classes, symptoms and alternative causes.

6.4 IgE and IgD

IgE participates in immediate hypersensitivity and aspects of defence against parasites. IgE bound to appropriate receptors on mast cells and basophils can support rapid mediator release when relevant antigen cross-links receptor-bound antibody. A specific IgE result indicates sensitisation under the assay's definition; it does not by itself establish that exposure causes clinically significant allergy. History and, where appropriate, specialist-supervised assessment are necessary.

Total IgE has even broader limitations. It can be increased in several conditions and can be unremarkable in a person with a clinically important specific allergy. It should not be used as a universal test of "how allergic" a patient is. Cross-reactive components and the relationship between sensitisation and symptoms complicate interpretation of specific tests.

IgD is expressed with IgM on many mature naive B cells and has additional biological roles under investigation. Its presence should not be dismissed as a useless evolutionary remnant. For routine foundational laboratory practice, the important point is that immunoglobulin class includes membrane-receptor roles as well as secreted-protein functions.

6.5 Quantity, specificity, function and timing

Four questions should be kept distinct. How much of a class is present? What antigen does the antibody recognise? What functional effect can it produce? When was the sample obtained relative to exposure, illness, vaccination or treatment? Different tests address different combinations of these questions.

A measurement near the lower analytical limit may be reported as below quantitation rather than as an exact zero. That wording is not evasive: it distinguishes an assay limitation from a biological assertion. Similarly, a positive result above a decision threshold does not imply that the antibody concentration is known accurately far beyond the validated measurement interval. Appropriate dilution or another method may be required.

Passive antibody creates another interpretive problem. Maternal transfer, immunoglobulin replacement and some blood products can introduce antibodies that the recipient did not produce. A newly positive serology after such an exposure may therefore require examination of the source and timing. The laboratory should ask about treatment rather than treating every antibody as evidence of the patient's endogenous response.

6.6 Longitudinal interpretation

Serial results are strongest when the same measurand, units and method are used. A change of platform can introduce a shift unrelated to the patient's biology. Where a method changes, the laboratory may need comparison data and an explanatory comment. Numerical similarity between two assay names is not sufficient evidence of equivalence.

Consider a fictional IgG result of 7.2 g/L followed by 720 mg/dL. These are equivalent concentrations: 1 g/L equals 100 mg/dL. Without checking units, a reader might infer a hundredfold increase. The correction is mathematical; the interpretation of whether 7.2 g/L is appropriate still requires the patient's age and the laboratory's interval.

Transport, half-life and compartment-specific antibody function

IgG is the predominant immunoglobulin class in serum and participates in many systemic responses. Interaction with the neonatal Fc receptor contributes to protection from degradation and to important transport processes, including maternal transfer across the placenta. The receptor's name should not imply that its relevance ends after infancy. These transport and recycling mechanisms help explain why antibody persistence depends on more than the rate of production. [FA02, FA06]

Maternal IgG contributes to early-life protection, but its presence can complicate interpretation of an infant's serology. An infant's positive IgG result may reflect transferred maternal antibody rather than an independently generated response. IgM generally does not cross the placenta in the same manner, but detecting IgM is still not a universal proof of congenital infection. Assay performance, timing and organism-specific diagnostic pathways remain decisive.

Secretory IgA is adapted to mucosal environments. In its polymeric form, it can associate with a joining chain and undergo transport through epithelial cells via the polymeric immunoglobulin receptor. Part of that receptor remains associated as secretory component. This arrangement contributes to function at mucosal surfaces. Serum IgA and secretory IgA are related but are not identical measurements of the same compartment.

IgM is often prominent early in a response and is effective at agglutination and complement activation under suitable conditions. It also exists as membrane immunoglobulin on B cells. A serum assay and a flow-cytometric surface measurement therefore examine different forms and contexts. A high serum concentration does not directly count the B cells bearing surface IgM.

IgE binds strongly to high-affinity receptors on mast cells and basophils. Cross-linking of cell-bound IgE by an appropriate antigen can trigger mediator release in allergic responses. A serum specific-IgE assay demonstrates sensitisation under its analytical definition, not necessarily the severity of a future reaction or proof

that exposure causes the patient's symptoms. The clinical history and, where appropriate, specialist testing remain essential.

IgD is expressed on many naive B cells together with IgM, although its complete biological roles extend beyond a simple marker label. For foundational interpretation, the important point is that immunoglobulin class can describe a soluble molecule, a membrane receptor or a particular effector pathway. Context prevents errors such as assuming that an increased number of IgD-bearing cells must produce a proportionate increase in serum IgD.

Subclass measurements require particular care. An isolated low subclass concentration can be influenced by age, assay, biological variation and the broader immune state. Clinical relevance depends on the pattern, reproducibility and functional antibody response. A textbook should not turn a single numerical result into a universal diagnosis or treatment indication. The laboratory's task is to provide a reliable measurement within an appropriate age- and method-specific framework.

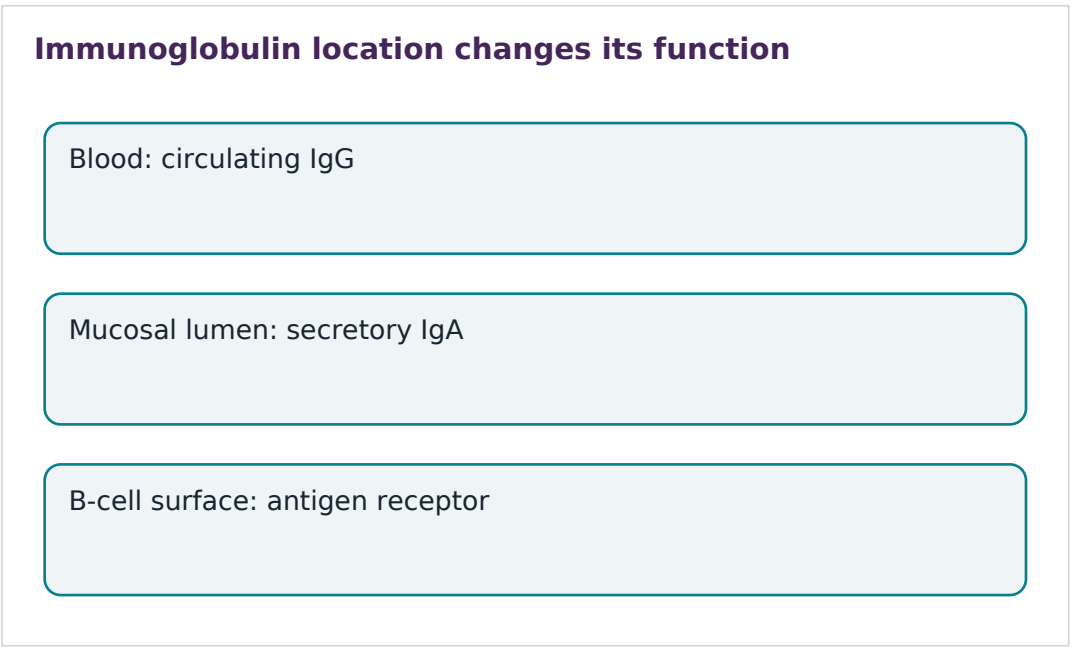


Figure FA06. Serum IgG, mucosal secretory IgA and a membrane B-cell receptor are shown in different compartments. A high concentration in one compartment does not directly quantify protection in another. Original CDIL educational schematic; not patient data.

Worked cases 21–24

Case 21 — Maternal antibody

A newborn has detectable IgG against an organism to which the mother is immune. The infant has no corresponding evidence of active infection. Does the antibody establish that the newborn was infected?

Reasoned answer. Maternal IgG can cross the placenta, so the result may represent passive antibody. The diagnosis of congenital or neonatal infection requires an organism-specific approach using the clinical presentation and suitable direct or serological investigations. The interpretation must not be reduced to "antibody present, therefore infant infected." The class, sample timing and maternal history are essential parts of the evidence.

Case 22 — IgM remains positive

A fictional patient has a persistent organism-specific IgM result several months after an illness. A student interprets each positive result as a new acute infection.

Reasoned answer. Some IgM responses persist, and cross-reactivity or interference can also occur. The result needs the assay's interpretation framework, clinical timeline and relevant additional markers. Repeated positivity is not proof of repeated new infection. A useful report preserves the observed reactivity and explains the limitation rather than inventing an infection date.

Case 23 — Negative IgA serology

A fictional patient with symptoms suggesting coeliac disease has a negative tTG-IgA result and a very low total IgA concentration. What limitation should be recognised?

Reasoned answer. An IgA-based test may not detect the relevant response adequately in IgA deficiency. The clinician and laboratory should use an appropriate diagnostic pathway, which may include suitable IgG-based serology and other assessment. Gluten avoidance before testing can create an additional limitation. The negative IgA

test cannot be interpreted as a stand-alone exclusion of disease. [FA15]

Case 24 — Sensitisation without a demonstrated reaction

A screening panel identifies specific IgE to a food that a fictional patient eats regularly without symptoms. Should the result alone be used to diagnose clinical food allergy?

Reasoned answer. No. Sensitisation is not identical to symptomatic allergy. The history, type of exposure, assay and specialist interpretation determine significance. Unnecessary dietary exclusion can create harm, so a laboratory result should not be converted into a clinical diagnosis without the required context. Any challenge testing belongs in an appropriately supervised clinical setting, not an unsupervised classroom exercise.

7 | Complement: Amplification, Regulation and Functional Testing

Complement is a network of soluble and membrane-associated proteins that can amplify recognition, coat targets, recruit inflammatory cells and contribute to membrane injury. It is not merely "antibody assistance": some activation routes do not require antibody. Complement also needs extensive regulation because the same mechanisms that damage a microbial target can injure host tissue if activated inappropriately. [FA03, FA07]

7.1 Three entry routes, shared downstream work

The classical pathway can be initiated through C1-associated recognition, including recognition of suitably arranged antibody on a target. The lectin pathway begins through pattern-recognition molecules and associated proteases. The alternative pathway involves continual low-level activation and can amplify complement deposition on suitable surfaces. The pathways are often drawn separately for teaching, but they interact in the same biological environment.

All three routes support formation of enzymes that cleave C3. The resulting C3b can attach to nearby surfaces and contribute to opsonisation and further amplification. Smaller cleavage products participate in inflammatory signalling. Additional steps generate C5 convertase activity and initiate assembly of the terminal membrane-attack complex. The sequence is a cascade because an upstream event can produce many downstream active molecules; it is not a simple one-antibody-to-one-cell exchange.

Nomenclature for fragments of C2 has differed between conventions. Many clinical diagrams label the classical/lectin C3 convertase C4b2a, while some texts use revised fragment terminology. The student should identify the components and function rather than assume that a lettering difference necessarily represents a different

enzyme. This book uses the conventional C4b2a label and names the convention explicitly.

7.2 Opsonisation and inflammation

C3 fragments deposited on a target can be recognised by complement receptors. This improves interactions with phagocytes and other cells. Deposition is therefore a way to mark a target for handling; it does not necessarily mean that the target has already been lysed. Complement can also assist clearance of immune complexes and cellular debris.

C5a is a potent inflammatory mediator, and other cleavage products contribute to vascular and cellular responses. The biological outcome depends on local concentration, receptor expression and regulatory conditions. Excessive systemic activation can be harmful. A complement result should therefore not be interpreted through the simplistic rule that "more activation is always better immunity."

The terminal complex contains C5b and later components through C9. It can damage susceptible membranes, but organisms differ in susceptibility and host-cell regulators modify the effect. Terminal-pathway deficiencies are especially relevant to susceptibility to certain Neisseria infections. This association is important but not a complete diagnosis: an infection history should guide appropriate functional and component investigations rather than substitute for them.

7.3 Regulation protects the host

Complement regulation occurs in fluid and on cell surfaces. Regulators can limit convertase formation, promote breakdown of active components or inhibit terminal-complex assembly. CD55 and CD59 illustrate different membrane-protective functions. Loss of appropriate membrane protection contributes to the complement sensitivity of affected blood-cell populations in paroxysmal nocturnal haemoglobinuria, which is investigated through specialised methods rather than inferred from a low serum complement concentration alone.

C1 inhibitor regulates proteases in complement and related contact-system pathways. Deficiency or dysfunction can produce bradykinin-mediated angioedema. This mechanism differs from the histamine-dominant model of many immediate allergic reactions. A patient with swelling therefore needs clinical assessment rather than an assumption that every episode is an IgE-mediated allergy. C1-inhibitor concentration and function are distinct measurements, and normal quantity does not guarantee normal activity.

7.4 Concentration is not pathway performance

C3 and C4 concentrations measure selected proteins. CH50 and AH50 are functional investigations of pathway performance under defined conditions. A normal concentration of a protein does not prove that it functions normally, and a low concentration may reflect consumption, reduced production, loss or an inherited deficiency. The interpretation must connect the pattern with clinical context and specimen integrity.

A simplified teaching pattern is useful. Low CH50 with preserved AH50 suggests an early classical-pathway problem or a related functional limitation. Low AH50 with preserved CH50 suggests an alternative-pathway problem. Reduction of both can occur with a shared-component or terminal-pathway defect, marked consumption or a compromised specimen. These are investigation directions, not final diagnoses. Some assays and clinical circumstances produce patterns that require more nuanced interpretation.

Complement proteins can be labile. Collection, separation, storage and transport requirements depend on the intended assay. A specimen that was acceptable for one chemistry measurement may not be suitable for functional complement testing. Recollection under validated conditions can be more informative than an elaborate disease explanation built on a degraded sample.

7.5 Consumption, deficiency and clinical change

In an immune-complex-associated process, complement may be consumed during activation. Concentrations and functional activity can change over time. However, the pattern is not specific enough to establish a named autoimmune disease without other evidence. An inherited low baseline, acute inflammation, synthesis changes and treatment can alter the interpretation of a trend.

For serial assessment, the laboratory must use the correct method and interval and consider biological variability. A result barely outside an interval may not carry the same meaning as a profound, reproducible functional deficit. The report should describe the actual magnitude and pattern while avoiding unsupported certainty about its cause.

7.6 Therapeutic inhibition changes the test question

Drugs that inhibit complement can deliberately reduce pathway activity. In that setting, an abnormal functional result may reflect the intended pharmacological effect rather than a newly acquired inherited-type deficiency. The interpretation requires the drug, target, timing and assay. Treatment-related infection precautions and monitoring belong to the prescribing team's current guidance, not a generic laboratory value alone.

The laboratory should therefore obtain relevant therapy information before interpreting an unexpected pattern. A result can be analytically correct yet clinically misleading if the report ignores a treatment designed to produce that pattern. This is a recurring theme in immunology: measurements are properties of a patient under particular biological and therapeutic conditions, not timeless labels of the person.

Complement convertases and the logic of regulation

The classical, lectin and alternative pathways provide different routes into a connected complement system. The classical pathway can be initiated by appropriate antibody–antigen arrangements and other recognition events. The lectin pathway uses recognition molecules that bind particular carbohydrate patterns. The alternative pathway includes an amplification mechanism that can increase deposition of complement fragments on a surface. These are not three completely separate systems with unrelated endpoints. [FA03, FA07]

C3 convertases generate C3 fragments that participate in opsonisation and amplification. The conventional classical/lectin convertase notation C4b2a is retained here because it remains widely used; nomenclature for the C2 fragments has not always been presented consistently across sources. The alternative-pathway convertase is commonly represented as C3bBb. These names are structural shorthand, and the learner should connect them to the cleavage and surface-binding events rather than memorise letters without mechanism.

Formation of C5 convertase activity leads toward the terminal pathway. C5b participates in assembly of the membrane attack complex with later components. Small fragments such as C3a and C5a have inflammatory functions, with important differences in potency and receptor biology. Calling every complement fragment a "lytic protein" is incorrect. Some fragments recruit or activate cells; others attach to targets, regulate reactions or contribute to membrane injury.

Regulation protects host surfaces and limits soluble activation. Factor H and factor I contribute to control of C3b-associated processes, while membrane regulators include proteins such as CD55 and CD59. CD55 accelerates decay of selected convertases; CD59 inhibits terminal membrane-attack-complex formation at a later step. Their functions are not interchangeable. Defects affecting surface protection can therefore produce different patterns from deficiency of a component required to initiate activation.

C1 inhibitor regulates proteases in complement and related systems. Its role in contact-system control helps explain why C1-inhibitor deficiency can produce bradykinin-mediated angioedema. This is not simply an IgE allergy with a different name. The distinction has important clinical consequences, although treatment decisions belong to the authorised clinical team and current specialist guidance.

Functional pathway assays test the ability of a specimen to complete a defined reaction. Component concentrations measure abundance. A protein can be present but functionally abnormal, and pathway activity can be low because of consumption, inherited deficiency, treatment or specimen degradation. A normal concentration cannot replace a functional result when the question concerns performance of the pathway.

When interpreting combined results, map the shared and separate components. Low classical-pathway activity with preserved alternative-pathway activity suggests a different starting set of possibilities from low activity in both. The pattern narrows the investigation but does not establish a unique diagnosis. Recollection under appropriate conditions and targeted component or functional testing may be required before assigning a biological explanation.

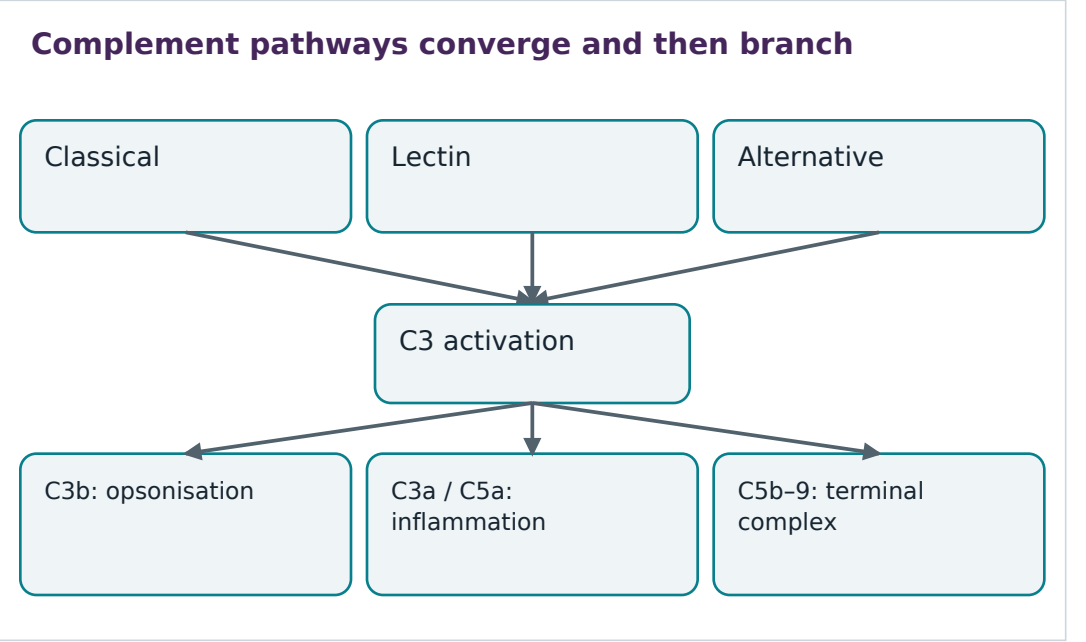


Figure FA07. Classical, lectin and alternative pathways converge on C3 activation. C3b supports opsonisation; C3a/C5a contribute inflammatory signals; C5b–9 forms the terminal complex. Regulatory proteins limit host injury. Original CDIL educational schematic; not patient data.

Worked cases 25–28

Case 25 — Both functional pathways are low

A fictional sample shows very low CH50 and AH50. The transport record reveals a delay under conditions outside the laboratory's validated limits. A learner diagnoses a terminal-component deficiency immediately.

Reasoned answer. A shared or terminal defect is one possible biological explanation, but specimen degradation must be addressed first. The laboratory should evaluate suitability and obtain a properly handled specimen where indicated. A reproducible pattern on a suitable sample can then guide component testing. The original result does not justify a definitive inherited diagnosis.

Case 26 — Normal concentration, abnormal function

C1-inhibitor concentration is within the laboratory interval, but a validated functional assay is reduced in a patient being investigated for recurrent angioedema.

Reasoned answer. Quantity and function are different properties. A normal antigenic concentration does not exclude dysfunctional protein. The clinical team must interpret the result with the phenotype, other investigations and assay limitations. The case demonstrates why a concentration-only test cannot answer every question about a regulator's effectiveness.

Case 27 — A pathway pattern

A properly handled synthetic specimen has markedly reduced CH50 and preserved AH50 on repeat testing. Which part of the system is a reasonable next focus, and what should not be claimed?

Reasoned answer. The pattern directs attention toward an early classical-pathway component or related functional problem. It does not identify one component or gene by itself. Clinical history and appropriate follow-up tests are needed, and the laboratory must consider assay-specific limitations. The result narrows an investigation; it does not complete it.

Case 28 — Complement inhibitor on the medication list

A fictional patient receiving a complement-targeted drug has low pathway activity. The request form omits the medication. Why is the history important?

Reasoned answer. The drug may intentionally inhibit the pathway measured by the assay. Without that information, an expected pharmacological effect could be misreported as an unexplained immune defect. The appropriate interpretation links the result to the drug target, timing and validated monitoring approach. It does not independently decide whether treatment should be changed.

8 | Cytokines, Chemokines and Immune Regulation

Immune cells communicate through soluble mediators, cell-surface interactions and changes in their tissue environment. Cytokines are part of that communication system. They do not act as a set of isolated switches, each producing one effect in every cell. Receptor expression, concentration, timing, other signals and the cell's prior state determine the response. [FA02, FA08]

8.1 Pleiotropy and redundancy

Pleiotropy means that one mediator can have different effects in different settings. Redundancy means that more than one mediator can support a similar effect. These properties make biological sense: host defence requires resilience and coordination. They also make laboratory interpretation difficult. An increased concentration of one cytokine does not reveal the entire network that produced it or all the tissues on which it is acting.

The same mediator can contribute to useful local defence and harmful systemic inflammation. Tumour necrosis factor, interleukin-1 and interleukin-6 provide familiar examples of mediators involved in inflammatory responses. Their actions include effects on cells, vessels and systemic physiology, but none is a unique molecular label for one infection. A result must be interpreted as a measurement within a network.

8.2 Receptors and intracellular signalling

A cytokine acts through a receptor system capable of transmitting a signal. Receptors may differ in affinity, subunit composition and expression across cell populations. Downstream pathways alter gene expression, survival, proliferation or effector function. Some pathways involve Janus kinases and STAT transcription factors; others use different signalling machinery. A defect in a shared signalling component can therefore affect responses to more than one cytokine.

Concentration alone does not measure receptor responsiveness. A patient can have abundant mediator but impaired response if the relevant receptor or signalling machinery is defective or blocked. Conversely, a modest concentration can produce an important effect in a highly responsive compartment. Functional testing may therefore be needed when the question concerns signalling rather than simple abundance.

Soluble receptors, binding proteins and therapeutic antibodies can further change what an assay detects. The measured material may include free and bound forms differently depending on the assay's epitopes. A numerical increase after therapy can sometimes reflect altered clearance or detection of complexes rather than increased biological activity. The laboratory must understand the measurand before inferring pathway activation.

8.3 Functional programmes and their limits

Several cytokine patterns are useful for foundational learning. Interferon-gamma supports important macrophage-related responses. Type I interferons participate in antiviral defence. Interleukin-2 supports lymphocyte growth and also has important regulatory relationships. Interleukin-4 and related pathways contribute to type 2 responses, while interleukin-17-associated responses help recruit and organise defence at selected barriers.

These associations are not a licence to diagnose a complete T-helper phenotype from one serum value. The categories describe programmes observed in particular contexts. Cells can have overlapping or changing states, and tissue measurements may differ from blood measurements. The more specialised the conclusion, the more closely the assay and interpretation must match the intended biological question.

Chemokines are particularly important in directing movement and positioning. A chemokine gradient can help recruit cells or organise lymphoid compartments. Receptor expression determines which cells respond. The presence of a chemokine in blood does not reproduce the spatial information of a local tissue gradient; a circulating concentration is therefore not a complete map of cell trafficking.

8.4 Measuring low concentrations reliably

Cytokine assays may operate near low concentration ranges and can be sensitive to specimen type, processing delay, storage, freeze–thaw history and matrix effects. Cells left in contact with a sample can continue to release or consume mediators. Platelet activation can affect selected analytes. A result may therefore reflect events after collection as well as the state of the patient at collection.

Multiplex assays can measure many targets from a small volume, but more targets also create more opportunities for analytical interaction, out-of-range results and inappropriate multiple comparisons. The laboratory must establish suitable performance for each reported measurand rather than assume that validation of the platform establishes every analyte equally well.

Results from different manufacturers may not be interchangeable. Calibration materials, antibody pairs, detected molecular forms and mathematical models can differ. A trend assembled from different methods without comparison data may be misleading. Research concentrations also should not be imported automatically into clinical decision thresholds.

8.5 Inflammation, cytokine release and urgent communication

Severe systemic inflammatory presentations require clinical assessment and supportive management based on the whole syndrome. A cytokine result may contribute to investigation or monitoring, but emergency care must not wait for a specialised assay when the patient is unstable. The laboratory's role includes reliable turnaround communication, critical-result procedures where applicable and explanation of limitations.

A high cytokine concentration does not by itself distinguish infection, immune-mediated toxicity, malignancy-associated inflammation or another cause. Likewise, a low or unavailable concentration does not exclude a clinically important syndrome. The differential diagnosis is not solved by selecting the highest bar on a multiplex panel.

8.6 Therapeutic targets and feedback

Blocking a cytokine or receptor can change both biological activity and measured concentration. Feedback loops may increase production, and altered clearance may increase circulating material. Assay interference can add another layer. The interpretation must identify whether the question concerns drug exposure, target engagement, downstream response or disease activity.

For example, an assay measuring total cytokine after formation of drug–cytokine complexes may behave differently from one measuring free cytokine. Neither is inherently wrong; they measure different operational definitions. The report must make that distinction clear when it affects clinical interpretation. A textbook cannot provide a universal conversion between such assays because the relation depends on the specific analytical and pharmacological system.

Signal networks, receptors and why cytokine panels are difficult

A cytokine's effect depends on whether the target cell expresses a functional receptor and on the intracellular machinery available after receptor engagement. The same cytokine can therefore have different consequences in different cells. Conversely, several cytokines can produce overlapping effects. This combination of pleiotropy and redundancy explains why a diagram showing one cytokine and one outcome is a teaching simplification rather than a complete physiological rule. [FA02, FA08]

Some receptors use shared subunits. A defect in a shared signalling component can affect responses to several cytokines and produce a broader phenotype than loss of one ligand alone. The common gamma-chain pathway is an important example in immunodeficiency. The laboratory phenotype reflects the particular role of the affected pathway in cell development and function, not simply the serum concentration of one cytokine.

Intracellular pathways such as JAK–STAT signalling connect receptor engagement to changes in gene expression. Feedback mechanisms restrain the response. A sustained signal may result from continued

ligand exposure, altered receptor regulation or changes downstream. Measuring a circulating cytokine concentration does not directly measure all of these processes. A receptor-signalling assay or molecular investigation may answer a different question.

Chemokines influence movement and positioning. A circulating cell population may appear reduced because cells have moved into tissue, while a tissue response may depend on receptor expression and local gradients rather than the total amount of chemokine in serum. A gradient is a spatial relationship; a single blood concentration cannot fully describe it. This is another reason to connect the specimen compartment to the proposed mechanism.

Cytokines can be present at low concentrations, released in bursts and affected by collection and processing. Serum and plasma may not be interchangeable for every measurement because clotting and cell activation can alter the material being measured. Storage, freeze–thaw cycles and matrix effects can also matter. The method's validated specimen requirements are part of the interpretation.

Multiplex panels create additional analytical questions. Each analyte may have a different range, sensitivity and interference profile. A successful calibration for one member of a panel does not prove that every other member is equally reliable. Multiple comparisons also increase the chance of finding an apparently unusual result by chance, particularly when a large panel is ordered without a defined question.

The most useful report connects the measurement to its intended purpose. A research cytokine profile may generate a hypothesis, while a validated clinical assay may support a specified decision. These uses should not be merged. The learner should be able to state whether a result is exploratory, supportive or part of an established diagnostic pathway, and should avoid creating a disease label from a colourful panel of concentrations alone.

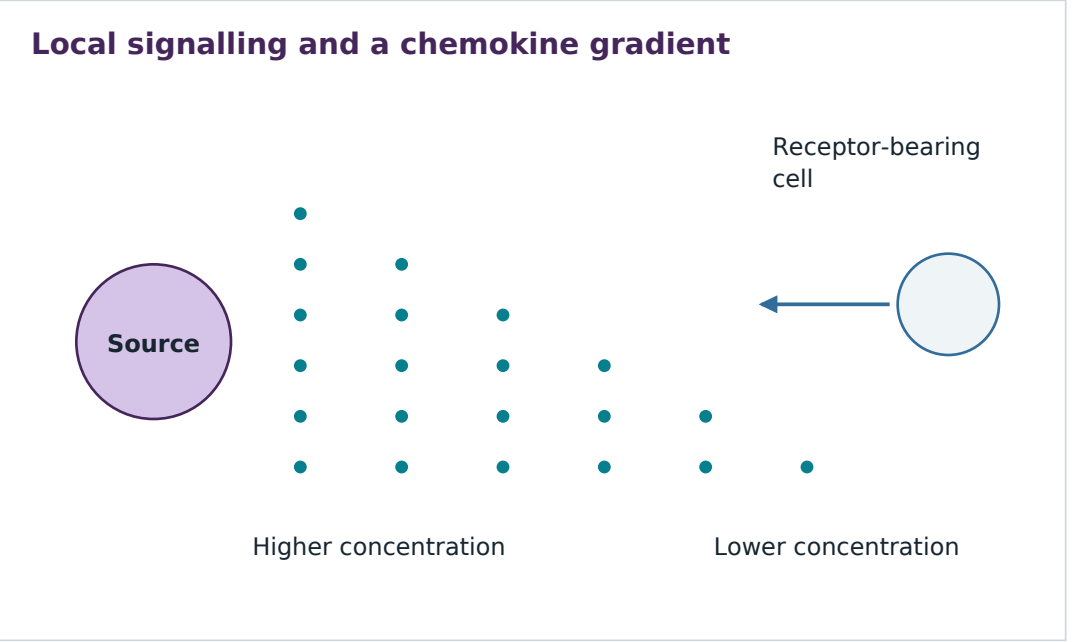


Figure FA08. A local source creates a schematic concentration gradient. Cells respond through appropriate receptors; the arrow represents directed migration, not a universal response to every cytokine. Original CDIL educational schematic; not patient data.

Worked cases 29–32

Case 29 — Delayed processing

A cytokine specimen remains unseparated for a prolonged period outside the receiving laboratory's validated conditions. The result is unexpectedly high, while the patient's clinical state is unchanged.

Reasoned answer. Continued cellular activity and other preanalytical effects can change selected mediator concentrations after collection. The laboratory should investigate handling before attributing the result entirely to the patient. A suitable repeat sample and appropriate method review may be required. The case does not establish that every delayed specimen becomes high; the direction and magnitude depend on the analyte and conditions.

Case 30 — One cytokine, several diagnoses

A fictional patient has a raised interleukin-6 result. A student concludes that a specific bacterial infection has been identified.

Reasoned answer. IL-6 participates in multiple inflammatory settings and is not a pathogen-identification assay. The result may support assessment of a response but does not name the cause. Microbiological, clinical and other evidence are needed. The interpretation should be proportional to the specificity of the measurement.

Case 31 — The method changes

A patient's cytokine monitoring changes from platform A to platform B. The first B result is twice the previous A value. No comparison study is available.

Reasoned answer. A biological increase is possible, but methodological differences must be considered. Calibration, epitopes and detected forms can differ. The laboratory should not present the values as an uninterrupted quantitative trend without an appropriate comparison or explanatory qualification. Repeating B can assess reproducibility, but it does not by itself establish equivalence with A.

Case 32 — A blocked receptor

A patient has measurable cytokine in serum but poor response in a validated receptor-signalling experiment. Why are the results not necessarily contradictory?

Reasoned answer. Abundance of a mediator does not prove that the receiving cells can respond. Receptor expression, signalling defects, inhibitors, specimen quality and assay conditions can affect the functional result. The investigation must identify which step is abnormal. Measuring more of the same circulating cytokine cannot automatically replace a test of cellular responsiveness.

9 | Hypersensitivity: Mechanisms of Immune-Mediated Injury

Hypersensitivity describes immune responses that produce harmful effects in particular circumstances. The traditional four-type classification is a useful map of mechanisms, not a claim that every clinical disorder belongs exclusively to one box. Several mechanisms can coexist, and the patient's presentation must be evaluated rather than inferred from a label alone. [FA02, FA09]

9.1 Immediate reactions and sensitisation

In the classical type I model, a person becomes sensitised to an antigen and produces relevant IgE. IgE binds receptors on mast cells and basophils. On a later suitable exposure, cross-linking of receptor-bound IgE can trigger release of mediators that affect vessels, smooth muscle, mucus production and other tissues. The speed of the response reflects activation of cells already equipped with relevant antibody and preformed mediators.

Clinical severity depends on the exposure, route, dose, distribution of responsive cells and host factors. A local reaction and anaphylaxis are not distinguished simply by the numerical value of one specific IgE result. Anaphylaxis is a clinical emergency; recognition and treatment follow current emergency-care guidance and authorised practice, not delay for laboratory confirmation.

Tryptase measurements can support selected investigations of mast-cell activation, but timing and baseline comparison matter. A result within an interval does not exclude every clinically diagnosed anaphylactic event. A persistently raised baseline also has a different interpretation from a transient event-associated increase. The laboratory should not report either pattern as a complete diagnosis without the appropriate clinical framework.

9.2 Antibody against cells, matrix or receptors

Type II mechanisms involve antibody directed at cell-associated or extracellular-matrix targets, with injury or altered function mediated through several routes. Complement, phagocyte recognition and receptor effects can all be relevant. The category is therefore broader than "antibody always destroys a cell."

Autoimmune haemolytic processes illustrate antibody-associated red-cell injury, but a positive direct antiglobulin test does not by itself establish active haemolysis. Receptor-associated diseases illustrate a different outcome: antibodies can stimulate or block a receptor rather than simply lyse the cell carrying it. The mechanism must be connected with the measured target and clinical evidence.

A laboratory assay detecting an antibody to a receptor may measure binding without directly measuring stimulation or inhibition. A functional assay can answer a different question. This distinction should be explicit when the test is used to support diagnosis or monitoring. The name of the antibody does not guarantee that every assay measures the same biological activity.

9.3 Immune-complex injury

Type III mechanisms involve immune complexes and their interactions with complement, cells and tissues. Formation, size, clearance and deposition depend on antigen–antibody relationships and the local environment. The presence of circulating complexes is not equivalent to demonstrating where injury is occurring or which disease produced them.

Complement consumption can accompany immune-complex-associated disease, but low complement is not specific for that mechanism. Renal, vascular, joint and other manifestations require clinical and, in some circumstances, tissue assessment. An immune-complex model explains a potential pathway of injury; it does not replace differential diagnosis.

The laboratory should also distinguish complexes present in the patient from complexes or aggregates generated during specimen handling. Assay-specific interference and sample quality can alter

results. As with other immunological tests, a surprising finding should be evaluated through a structured analytical and clinical review.

9.4 T-cell-mediated injury

Type IV mechanisms involve T cells and their downstream effects, including recruitment of other cells or direct cytotoxicity. The classical delayed skin response provides one example, but the category includes a range of processes with different time courses and effector patterns. It should not be reduced to a universal rule that every T-cell reaction begins at exactly 48 hours.

Contact dermatitis illustrates how a small chemical can modify proteins and create antigenic structures that participate in a T-cell response. A patch test investigates a different mechanism from a skin-prick test or a specific IgE assay. Substituting one for another simply because all are called "allergy tests" is conceptually wrong.

Immunosuppression, technical conditions and the patient's response capacity can affect cellular testing. A negative test may therefore need interpretation in relation to controls and clinical history. Failure to respond to a positive control is not evidence that the patient lacks exposure to every antigen tested; it raises a problem with response capacity or test validity.

9.5 Matching the test to the mechanism

The most useful first question is what mechanism is being investigated. Specific IgE supports a sensitisation question. A direct antiglobulin test detects selected proteins associated with red cells. Autoantibody assays investigate defined targets. Complement tests assess selected proteins or pathway performance. Cellular assays evaluate responses under specific stimulation conditions. No single test covers all hypersensitivity mechanisms.

Panels can create misleading certainty when requested without a focused question. Multiple low-probability tests increase the chance of incidental findings. A positive result must be interpreted with the pretest context, analytical specificity and possible alternative explanations. The laboratory contributes by making the measurand

and limitations clear and by supporting appropriate reflex or confirmatory pathways.

9.6 A mechanism-based report

A useful report separates the observed result, the supported inference and the unresolved question. For example, "Specific IgE to component X was detected" is an observation. "This supports sensitisation to the tested component" is a bounded interpretation. "The patient will develop anaphylaxis if exposed" is an unsupported prediction unless a much broader clinical assessment establishes risk.

The same discipline applies to antibody-mediated cell injury. Detection of red-cell-bound IgG is not the same as proof that haemolysis is occurring, and evidence of haemolysis is not the same as proof that one identified antibody caused it. Each link in the reasoning chain requires appropriate evidence.

Four mechanisms of hypersensitivity, with overlap

The traditional four-type classification is a framework for organising mechanisms, not a rule that every patient belongs to one exclusive category. Type I reactions involve IgE-associated mast-cell or basophil activation in appropriate circumstances. Type II mechanisms involve antibodies directed against cell-surface or matrix-associated targets and can produce injury or altered function. Type III mechanisms involve immune-complex-associated injury. Type IV mechanisms are predominantly T-cell mediated. [FA02, FA09]

In a type I response, prior sensitisation can lead to cell-bound specific IgE. Later exposure may cross-link receptors and trigger mediator release. The clinical presentation depends on the route, distribution and intensity of the response. A positive specific-IgE result demonstrates an analytical finding that must be linked to exposure and symptoms. It does not establish a universal reaction threshold or guarantee that every subsequent exposure will have the same severity.

Type II mechanisms are broader than direct destruction of a target cell. Antibodies can activate complement, promote

Fc-receptor-mediated clearance or alter receptor function. An antibody that stimulates a receptor and one that blocks it can both fit within antibody-mediated functional disturbance, despite producing opposite physiological effects. The laboratory should identify the target and functional question rather than assume that every pathogenic antibody works by lysis.

Immune complexes can form when antibodies interact with soluble antigens. Their size, clearance and tissue deposition depend on concentration, valency and other factors. Complement activation and inflammatory recruitment can contribute to injury. Detecting an antibody in serum does not prove that pathogenic complexes have deposited in a particular organ. Tissue findings and clinical evidence may be needed to establish the relevant mechanism.

T-cell-mediated responses include several effector patterns. Cytokine-driven inflammation, macrophage activation and direct cytotoxicity can all contribute. The familiar description of "delayed hypersensitivity" reflects the timing of certain responses, but timing alone is not enough to identify a mechanism. An immediate-looking symptom does not automatically prove IgE, and a later symptom does not automatically prove a pure type IV reaction.

Drug reactions demonstrate the limitations of simple classification. A drug can act through different immunological and nonimmunological mechanisms, and different patients may respond differently. Some reactions involve metabolites, altered antigen presentation or direct interactions with immune recognition systems. Laboratory testing is available and useful for selected questions, but there is no universal blood test that safely confirms or excludes every drug allergy.

Clinical urgency takes priority when a severe reaction is suspected. The laboratory's role is to support the authorised pathway, preserve appropriate specimens when indicated and communicate reliable findings. A textbook discussion of mechanisms must not encourage delaying emergency treatment while waiting for an immunological classification. Mechanistic explanation is valuable because it informs subsequent investigation and prevention, not because it replaces immediate clinical assessment.

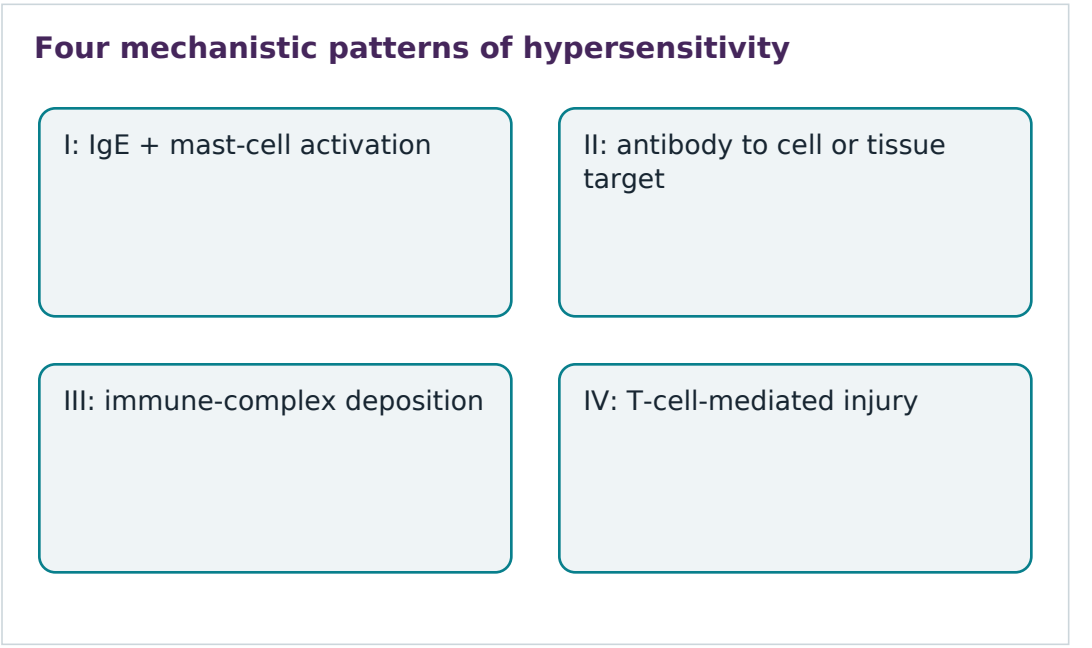


Figure FA09. These four models organise mechanisms, not a complete diagnostic classification for every disease. One clinical condition can involve more than one pathway. Original CDIL educational schematic; not patient data.

Worked cases 33–36

Case 33 — Emergency first, laboratory support second

A fictional patient develops a rapidly progressive reaction with airway and circulatory concerns after an exposure. Someone proposes waiting for a tryptase result before initiating the emergency response.

Reasoned answer. Anaphylaxis is assessed and managed clinically under current emergency protocols. Laboratory testing can support later investigation but must not delay urgent care. A normal or unavailable tryptase result does not make the initial clinical concern disappear. The case tests the distinction between a time-critical clinical decision and a supportive laboratory investigation.

Case 34 — Positive DAT without haemolysis

A fictional patient has a positive direct antiglobulin test but no current laboratory or clinical evidence of red-cell destruction. A student writes "autoimmune haemolytic anaemia confirmed."

Reasoned answer. The DAT identifies selected proteins associated with red cells under the test conditions. It does not establish haemolysis by itself. The clinical team needs evidence of anaemia and destruction, treatment and transfusion history, and appropriate investigation of the antibody pattern. The report should not collapse these separate questions into one diagnosis.

Case 35 — The wrong allergy test

A patient has a delayed eczematous reaction after skin contact with a material. A learner argues that a negative specific IgE result excludes the problem.

Reasoned answer. A delayed contact reaction can involve T-cell-mediated mechanisms rather than the IgE mechanism assessed by that test. The negative result does not exclude every form of hypersensitivity. Appropriate specialist assessment may use a different testing approach. Matching the method to the suspected mechanism is more informative than ordering a broad collection of unrelated tests.

Case 36 — Receptor binding versus receptor function

Two assays for an autoantibody disagree: one detects binding to a receptor, while another evaluates a functional effect in a validated system.

Reasoned answer. The assays do not measure identical properties. Some antibodies may bind without producing the particular functional effect measured, and technical differences or interference may also contribute. The investigation should compare the actual assay claims and clinical question. It is not enough to decide that the assay with the larger number is correct.

10 | Autoimmunity, Autoantibodies and Diagnostic Probability

Autoimmunity concerns responses to self structures, but a laboratory autoantibody result is not a complete clinical diagnosis. The same antibody can have different significance in a symptomatic patient, an asymptomatic person, a treated patient or someone with an alternative inflammatory process. Good interpretation begins with the phenotype and asks what the test adds to the existing evidence. [FA09, FA10]

10.1 Loss of tolerance is multifactorial

Genetic susceptibility, antigen handling, tissue injury, environmental exposures and regulatory pathways can contribute to autoimmune disease. No single mechanism explains every disorder. Some diseases are strongly associated with particular antibodies, while others are driven through processes not fully captured by routine serology.

An autoantibody can be directly involved in injury, can mark an associated immune response, or can occur without the corresponding clinical disease. These possibilities should be kept separate. The laboratory may detect the antibody accurately while the clinical inference remains uncertain. Analytical truth and diagnostic certainty are not the same thing.

10.2 ANA is a family of observations

Antinuclear antibody testing encompasses methods with different antigen presentations and detection characteristics. HEp-2 indirect immunofluorescence displays patterns across cellular structures; solid-phase assays present selected antigen targets. Differences in substrate, target composition, threshold and interpretation can produce discordant results without either assay being a perfect universal reference for every clinical question.

In an IFA report, titre and pattern provide information beyond a simple positive label. A titre records reactivity through a defined dilution endpoint; it is not a direct mass concentration. Pattern terminology should follow a recognised system such as the International Consensus on ANA Patterns when applicable. A schematic teaching drawing can explain a pattern concept, but it cannot replace validated image training and competency assessment for clinical interpretation. [FA10]

Low-level ANA reactivity can occur without a systemic autoimmune rheumatic disease. The probability of disease after a positive result depends on the patient's presentation and the assay's performance. Testing people with very low pretest probability can produce more incidental positives than clinically useful discoveries. A positive ANA is therefore an invitation to context-specific reasoning, not a stand-alone label of lupus.

10.3 Specific antibodies and focused questions

Antibodies to double-stranded DNA, extractable nuclear antigens and other targets can support particular diagnostic questions. Their performance depends on the method and population. A result from one platform may not be numerically interchangeable with another. The assay's specificity for the intended target and its susceptibility to interference matter especially when a result is unexpected.

Clinical features should guide further investigation. Muscle weakness, skin changes, inflammatory arthritis, renal findings, thrombosis and mucosal symptoms do not all justify the same antibody panel. A focused approach reduces the chance that an incidental result drives an inappropriate diagnosis. Reflex testing can be useful when designed around a validated clinical and laboratory pathway rather than an automatic search for any possible positivity.

Classification criteria serve research and other defined purposes and are not identical to a complete diagnostic process. Meeting or failing a classification threshold should not be presented as the only determinant of clinical diagnosis. The laboratory should describe the role of a test accurately within the framework being used.

10.4 Antiphospholipid testing and persistence

Antiphospholipid investigations include clot-based lupus-anticoagulant testing and selected solid-phase antibody assays. These are not the same analytical measurement. Anticoagulant therapy, acute illness and other factors can complicate clot-based testing. The name "lupus anticoagulant" can mislead beginners: the in-vitro prolongation of selected clotting tests does not mean that the patient is protected from thrombosis.

Persistence is important in established antiphospholipid-syndrome assessment frameworks, with repeat testing at an appropriate interval, commonly at least 12 weeks, used to distinguish persistent from transient findings. Clinical events and the exact testing framework remain essential. A single positive antibody result during an acute illness does not by itself establish the syndrome. [FA11]

10.5 Organ-specific autoimmunity and assay context

Organ-specific investigations may target thyroid, pancreatic, adrenal, neuromuscular or other antigens. The result should be linked with the organ's functional assessment and clinical picture. An antibody can precede overt dysfunction, persist after treatment or fail to capture the entire disease mechanism. Serial antibody testing is not automatically a useful substitute for the functional measurement that actually guides care.

Coeliac testing demonstrates several interacting limitations: immunoglobulin class, total IgA status, gluten exposure, age and method. A negative result obtained after dietary exclusion may not have the same sensitivity as testing during gluten exposure. The laboratory should identify the limitation rather than treat the result as a universal exclusion. Dietary changes or challenge decisions belong with the treating clinician. [FA15]

10.6 Reporting uncertainty constructively

Uncertainty is useful when it is specific. "Clinical correlation required" alone often adds little. A better comment states the particular limitation: for example, that an isolated low-titre ANA is not diagnostic, that an assay measures a selected antigen set, or that anticoagulant interference limits interpretation of a clot-based result.

A responsible report also avoids implying that a negative panel excludes every autoimmune disease. The panel covers particular targets with particular sensitivities. The clinician may still have strong evidence for a disease not captured by those tests or may need a different investigation. Laboratory interpretation should refine the clinical question rather than close it prematurely.

Tolerance failure and the interpretation of disease-associated antibodies

Central tolerance reduces the survival of some strongly self-reactive lymphocytes during development. Peripheral tolerance includes additional controls such as anergy, deletion, regulatory-cell activity and inhibitory signalling. These mechanisms overlap, and no single checkpoint removes every potentially self-reactive receptor. Some self-reactive antibodies can occur without a systemic autoimmune disease. The clinical question concerns whether a detected response participates in, predicts or merely accompanies pathology. [FA02, FA09]

Loss of tolerance can involve genetic susceptibility, tissue injury, infection-associated signals, altered antigen availability and regulatory defects. The presence of a risk allele does not make disease inevitable. Likewise, an exposure that precedes symptoms is not automatically the cause. Mechanistic plausibility should be distinguished from evidence that a particular pathway explains an individual patient's illness.

Autoantibodies can serve different roles. Some are directly pathogenic, some support classification or diagnosis, and some are associated with a disease without being its main cause. The analytical method may detect binding but not demonstrate functional effect. A

receptor-binding assay and a functional stimulation assay can therefore answer different questions even when both concern antibodies to the same receptor.

Antinuclear antibody testing illustrates the need for layered interpretation. A fluorescence pattern on an appropriate substrate provides morphological information about binding. Antigen-specific assays identify selected molecular targets. Neither replaces the clinical assessment. A pattern may suggest useful follow-up investigations, but it should not be converted automatically into a particular antigen specificity without the relevant evidence. [FA10]

A negative panel has defined coverage. It means that the selected assays did not demonstrate the included targets under their analytical conditions. It does not establish the absence of every autoimmune process. The term "autoimmune screen negative" can become misleading when readers assume it covers diseases or targets that were never tested.

Disease activity and antibody concentration are also not universally coupled. Some markers may be useful for monitoring selected conditions, while others persist without tracking current organ injury. The relationship depends on the disease, target, assay and individual course. Serial testing should be ordered for a defined purpose, not because repeating every positive antibody seems inherently informative.

The practical discipline is to write an interpretation in three parts: the observation, its supported association and its limitation. For example, the laboratory may report detection of a specified antibody, note the clinical contexts in which it is relevant and state that diagnosis requires integration with the clinical findings. This approach is neither evasive nor overconfident. It gives the treating team the most useful information that the assay can actually provide.

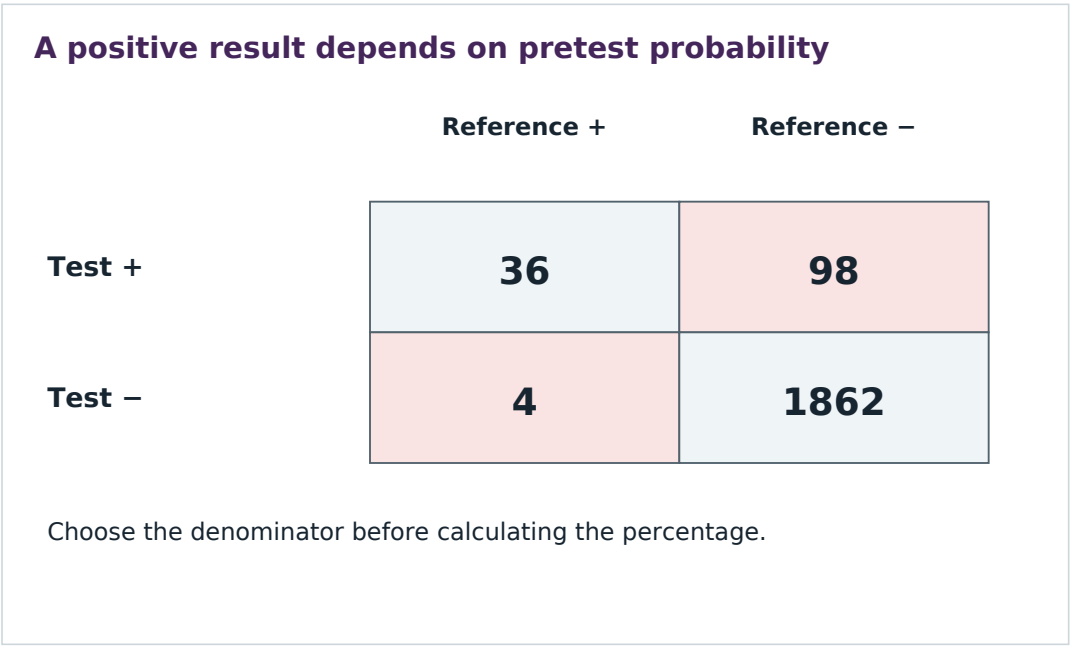


Figure FA10. Synthetic cohort: 2,000 people, prevalence 2%, sensitivity 90%, specificity 95%. There are 36 true positives and 98 false positives; positive predictive value is 26.9%. Original CDIL educational schematic; not patient data.

Worked cases 37–40

Case 37 — An incidental positive ANA

A fictional asymptomatic person has a low-titre ANA after broad screening. No compatible clinical features are documented. A learner diagnoses systemic lupus erythematosus.

Reasoned answer. The result does not establish that diagnosis. ANA reactivity must be interpreted with the phenotype, method, titre, pattern and relevant additional evidence. In a low-pretest-probability setting, incidental positivity is an important possibility. The laboratory should report the finding accurately without converting it into a disease label.

Case 38 — A single antiphospholipid result

A fictional patient has one positive antiphospholipid antibody result during an acute illness. The result is immediately labelled a persistent antiphospholipid syndrome.

Reasoned answer. Persistence has not been demonstrated, and a syndrome diagnosis requires the appropriate clinical and laboratory framework. Repeat testing at the specified interval and evaluation of relevant clinical events may be needed. The acute setting and possible assay interference must be considered. A single result cannot supply evidence that requires observations over time.

Case 39 — Negative panel, strong phenotype

A patient has clinical findings concerning for an autoimmune process, but a limited solid-phase panel is negative. The report lists only six antigen targets.

Reasoned answer. The negative result applies to those targets under that method's conditions. It does not exclude every autoimmune disorder or every autoantibody. The clinician and laboratory should determine whether another method or a differently focused investigation is appropriate. The first step is to recognise the panel's actual scope rather than expand its negative claim beyond what was tested.

Case 40 — Anticoagulant interference

A lupus-anticoagulant investigation is requested from a fictional patient receiving an anticoagulant known to affect the laboratory's clot-based method. The medication is omitted from the request.

Reasoned answer. The treatment can alter the analytical interpretation. The laboratory must obtain the history and apply a validated strategy for evaluating or limiting interference; an unqualified result may be misleading. The book does not direct interruption of anticoagulation. Any medication decision requires the treating team's assessment of risk and the relevant testing plan.

11 | Immunodeficiency: From Clinical Pattern to a Testable Mechanism

Immunodeficiency is not defined by one universally low laboratory value. A defect can affect cell number, migration, recognition, signalling, killing, antibody production, regulation or another function. Inborn errors of immunity can also present with autoimmunity, autoinflammation, allergy, lymphoproliferation or malignancy rather than only recurrent infection. The IUIS classification provides an evolving framework; a short list of childhood infections is not an adequate substitute for that broader understanding. [FA16]

11.1 Start with the pattern, not a panel

The type, severity, location and timing of infections help direct investigation. Repeated infections with an anatomical explanation may require a different approach from invasive infections with unusual organisms. A history of persistent fungal or opportunistic infection raises different questions from recurrent uncomplicated infections in a person with high exposure. Family history, growth, medication, protein loss and acquired disease all matter.

The laboratory should not interpret a request for an "immune screen" as permission to make every possible measurement without a defined question. A staged investigation can be more informative. Initial tests may identify broad abnormalities, while subsequent tests examine a specific mechanism. The choice and urgency belong to the clinical team in consultation with the laboratory; an unstable patient needs care rather than waiting for a textbook sequence to be completed.

11.2 Quantitative and functional antibody defects

Quantitative immunoglobulin testing describes selected classes in circulation. Age-appropriate intervals are essential because the developing immune system and maternal antibody transfer influence early-life values. A concentration can be reduced by impaired production, protein loss or treatment, among other causes. A low result should therefore be interpreted with the clinical history and other measurements.

A patient can have immunoglobulin concentrations within an interval yet respond inadequately to selected antigens. Functional response assessment uses a defined exposure or vaccination history, appropriate pre- and post-exposure timing when required, and validated interpretation. It is not legitimate to invent a universal "protective titre" for every antigen. Different pathogens, vaccines and assays have different evidence frameworks.

B-cell enumeration adds another dimension. Very few circulating B cells can support one set of hypotheses, while preserved B-cell numbers with poor antibody function suggest others. The count does not reveal every stage of maturation or every plasma-cell compartment. The investigation is strongest when it combines quantity, phenotype and function without treating them as interchangeable.

11.3 T-cell and combined defects

T-cell defects can affect development, numbers, repertoire, activation or effector function. Combined defects can impair both cellular and antibody responses because effective B-cell responses often depend on appropriate T-cell help. A low absolute lymphocyte count can be an important clue, but a normal total count does not exclude every T-cell disorder.

Newborn screening that measures T-cell receptor excision circles investigates a marker associated with new T-cell production. It is a screening approach, not a complete direct count of every functional T cell or a definitive molecular diagnosis. An abnormal screen requires the programme's follow-up pathway. The consequences of delayed

evaluation can be serious, so a screening result should be communicated according to the relevant urgent procedure rather than filed as an ordinary minor abnormality. [FA17]

Functional assays may examine proliferation or signalling after defined stimulation. Their interpretation depends on cell viability, specimen age, medication and technical controls. A weak response in a degraded specimen is not proof of an inherited signalling defect. Conversely, a reassuring result with one strong stimulus does not establish normal performance against every physiological antigen.

11.4 Phagocyte, complement and regulatory disorders

Phagocyte investigation may require separate assessment of number, adhesion, oxidative killing or another function. Complement assessment combines selected concentrations and functional pathways. The clinical pattern determines which questions deserve priority. Repeating a total white-cell count cannot replace a functional investigation when the suspected problem lies downstream of cell production.

Regulatory defects can produce excessive inflammation, autoimmunity or uncontrolled lymphocyte activation. The word deficiency can therefore be misleading if interpreted as "too little inflammation." A defect in a restraining pathway may produce too much immune activity. The modern concept of inborn errors of immunity accommodates these apparently opposite presentations.

The same clinical phenotype can arise through different molecular mechanisms, and one gene can produce different phenotypes depending on the variant and its functional effect. Gain-of-function and loss-of-function variants are not interchangeable. A report describing a variant of uncertain significance must not be treated as a confirmed explanation without the appropriate evidence and specialist interpretation.

11.5 Secondary causes and the treatment history

Acquired conditions and treatments can alter immunity. Relevant possibilities include infection, malignancy, immunosuppressive therapy, protein loss, nutritional problems and other systemic disease. The exact contribution depends on the patient. The laboratory should avoid describing an abnormal result as "primary" simply because no history was provided.

Therapies targeting B cells, T cells, cytokines or complement can create characteristic changes in cell populations and functional assays. Replacement immunoglobulin can also alter serology through passive antibody. The timing of treatment relative to collection is therefore part of the specimen's clinical identity. A technically correct measurement can be interpreted incorrectly when this information is missing.

11.6 Interpreting a staged investigation

A useful staged record identifies the initial question, the observation, the hypothesis supported, the alternatives remaining and the next test capable of distinguishing them. For example, low IgG does not automatically imply defective B-cell development. If B cells are present and there is substantial protein loss, the interpretation differs from a pattern of nearly absent B cells with a compatible early-life history.

The final report should state what has been established. "Reduced oxidative burst in a suitable specimen" is different from "a pathogenic variant in a specified gene was identified," and both differ from a complete clinical diagnosis. Preserving these distinctions allows specialists to combine evidence correctly and prevents the laboratory from claiming more than the assay can show.

Recognising immune dysregulation as well as deficiency

An inborn error of immunity can produce excessive inflammation or autoimmunity as well as susceptibility to infection. A defect in regulation may allow persistent activation, while a defect in pathogen control may create continuing inflammatory stimulation. These mechanisms can coexist. The term immunodeficiency should therefore not encourage the learner to imagine that every affected patient has a uniformly quiet or absent immune response. [FA16]

Some disorders affect development of a cell population; others affect signalling, metabolism, trafficking or effector function. A classification based on a visible laboratory phenotype is useful for organising the investigation, but it may not map to one unique gene. For example, similar T-, B- and NK-cell patterns can arise through different molecular mechanisms. The laboratory phenotype narrows the question without completing the diagnosis.

Secondary immune impairment is equally important. Protein loss can lower circulating immunoglobulin concentrations without a primary failure of B-cell development. Malignancy, infection, malnutrition and immunosuppressive treatment can affect different immune compartments. The correct investigation distinguishes decreased production, increased loss, redistribution and altered function rather than treating every low concentration as the same biological problem.

Newborn screening for severe combined immunodeficiency commonly uses T-cell receptor excision circles as a marker related to newly produced T cells. A low screening result requires prompt follow-up but is not a complete diagnosis of one genetic disorder. Prematurity and other causes of T-cell lymphopenia can affect the result. Conversely, the screen is not designed to detect every possible immune disorder. Its value depends on an organised follow-up pathway, not merely on producing a laboratory number. [FA17]

Clinical communication should reflect the degree of urgency. A result suggesting a severe early-life immune problem should be handled through the programme's escalation process, even if confirmatory

investigations are still underway. The laboratory should not delay appropriate notification until a molecular explanation is available. At the same time, the report should distinguish a screening finding from a confirmed diagnosis and should not create unsupported certainty for the family.

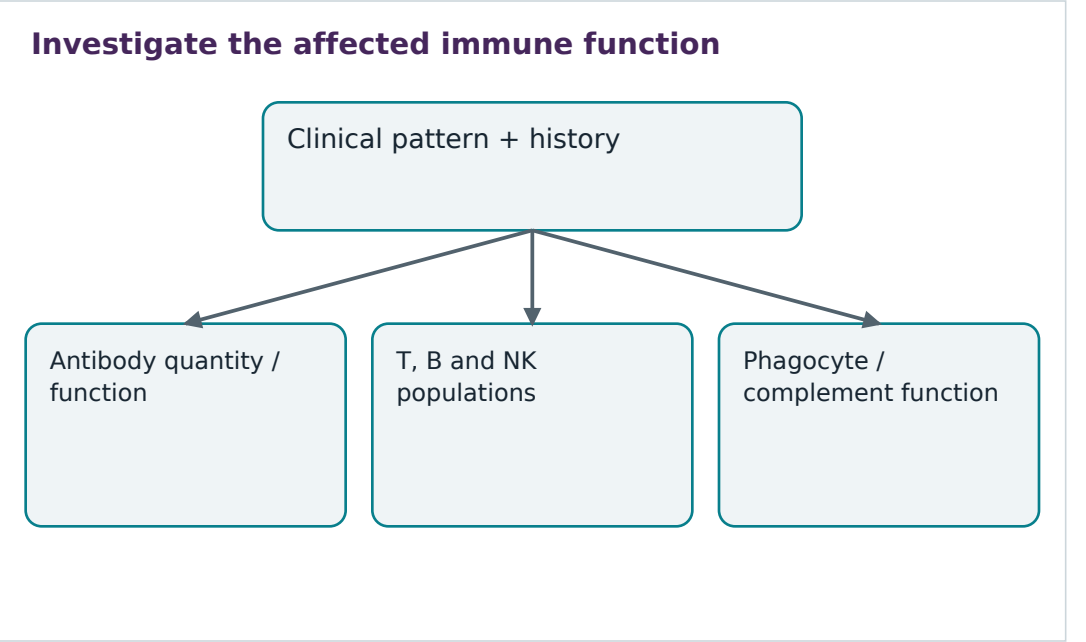


Figure FA11. Clinical infection patterns guide a staged investigation. Cell numbers, immunoglobulin quantities and functional tests answer complementary questions; this is not a standalone diagnostic algorithm. Original CDIL educational schematic; not patient data.

Worked cases 41–44

Case 41 — Protein loss and low IgG

A fictional adult has low serum IgG and a history of substantial protein loss. B-cell enumeration is preserved. A student diagnoses failure of B-cell development from the IgG result alone.

Reasoned answer. The conclusion ignores an important alternative mechanism. The measured concentration reflects production, distribution and loss. Preserved circulating B cells do not prove normal antibody function, but the protein-loss history must be incorporated before assigning an inherited developmental defect.

Further investigation should be directed by the clinical question rather than by the isolated number.

Case 42 — An abnormal newborn screen

A newborn screening programme reports a low TREC result and requests urgent follow-up. A learner proposes waiting several months to see whether infections develop.

Reasoned answer. Screening is intended to identify risk before preventable harm occurs. The result is not a definitive diagnosis, but it requires the programme's timely confirmatory pathway. The laboratory and clinical team should follow the applicable urgent communication and assessment process. Waiting for illness would defeat the purpose of screening. [FA17]

Case 43 — A variant with uncertain significance

A genetic panel identifies a variant of uncertain significance in an immune-related gene. The phenotype and functional studies do not yet establish its effect.

Reasoned answer. The variant cannot be treated as a confirmed causal diagnosis solely because it occurs in a relevant gene. Interpretation requires the variant evidence, inheritance, phenotype and, where appropriate, functional and family information. The uncertainty category must be preserved in communication. A molecular result can generate a hypothesis without completing the causal argument.

Case 44 — Treatment changes the count

A fictional patient's circulating B-cell count falls after B-cell-targeted therapy. The laboratory receives no treatment history and is asked to interpret the finding as a new unexplained immune disorder.

Reasoned answer. The medication history is essential. The change may reflect the intended pharmacological effect or another treatment-associated process. The result still requires correct measurement and clinical interpretation, but it must not be described as unexplained until relevant treatment information is obtained. The assay observes a treated patient, not an untreated theoretical baseline.

12 | Flow Cytometry: Cells, Signals, Gates and Denominators

Flow cytometry measures properties of particles as they pass through an optical system. In immunology, fluorescently labelled reagents help identify selected cellular features, while scatter and viability information help define the population being analysed. The final plot is not a photograph of cells arranged inside the patient. It is a representation of measured signals after a series of analytical decisions. [FA18]

12.1 From specimen to event

A suitable specimen must contain particles that can support the intended measurement. For cellular immunophenotyping, anticoagulant, collection volume, processing, transport and delay can affect viability and marker expression. The accepted conditions are assay-specific. A sample that yields many events is not necessarily suitable; debris, aggregates and dead cells can generate events too.

The instrument records signals from particles that pass the interrogation point. Forward and side scatter are influenced by size, internal complexity and optical properties, but they do not identify lineage independently. A region with "lymphocyte-like scatter" is a starting hypothesis, not proof that every event in it is a viable lymphocyte.

Doublets and aggregates can create misleading combinations of signals. Singlet selection uses relationships among pulse measurements to reduce this problem. It is a separate task from viability selection and lineage identification. Omitting one step cannot be justified merely because another gate looks visually neat.

12.2 Fluorescence and marker combinations

A fluorochrome absorbs and emits light over ranges of wavelengths. Multiple fluorochromes can have overlapping emission, so signal measured in one detector may include contributions from more than the intended label. Conventional compensation estimates and corrects selected spillover relationships. Spectral approaches use reference signatures and unmixing methods. Neither process can rescue an experiment whose controls do not represent the actual fluorochromes and conditions adequately.

A single marker rarely answers every lineage question. CD3 supports identification of conventional T-lineage populations in an appropriate panel; CD19 and other markers can help identify B-lineage populations; CD16 and CD56 contribute to selected NK-cell definitions. Activation, disease and targeted therapy can change expression. The panel and interpretation must be appropriate for the specimen and purpose rather than assembled as a universal list of "one marker, one cell."

Dim expression near background requires particular care. A fluorescence-minus-one control can help assess the distribution produced by the other reagents in a multicolour panel when setting a boundary. It does not replace the single-colour controls used to determine compensation. An unstained control answers another question about background. The controls are complementary, not interchangeable.

12.3 Gating is an explicit reasoning chain

A gate selects events meeting defined conditions. Each selection changes the denominator for the next result. A defensible analysis records the sequence: for example, stable acquisition, suitable scatter, singlets, viable cells and then the population of interest. The exact sequence depends on the assay. The student should be able to explain why each exclusion is made and what could be lost if the gate is too narrow.

Overly aggressive gating can remove a clinically relevant abnormal population. Overly broad gating can include debris or unrelated cells.

A tidy plot is not the objective; faithful representation of the intended population is. Where rare events are important, acquisition size and event-count uncertainty become especially relevant.

A count of ten events is much less stable proportionally than a count of ten thousand under otherwise comparable conditions. Increasing total acquisition can improve precision for rare populations, but it does not remove systematic errors in staining, gating or specimen quality. More data from a biased selection remain biased data.

12.4 Absolute counts and percentages

A percentage must name its denominator. "CD4-positive cells: 35%" could mean a fraction of lymphocytes, T cells or another selected population. Those values are not equivalent. A report should make the population definition explicit and include absolute counts when required for the clinical use.

In a dual-platform approach, a percentage may be combined with a count obtained from another analyser. The compatibility of timing, specimen and population definitions matters. In a single-platform counting-bead approach, event ratios and the known bead concentration or number contribute to the calculation. The formula must match the validated assay, including volumes and dilution factors.

Consider a synthetic teaching tube containing 50,000 beads and 1,250 target-cell events among 10,000 acquired bead events. If the validated calculation uses the ratio of target events to bead events, the estimated target-cell number in the tube is $1{,}250/10{,}000 \times 50{,}000 = 6{,}250$. If 50 µL of original specimen contributed to that tube and no additional correction is required by the defined model, the estimated concentration is 125 cells/µL. These invented values illustrate the calculation; a real assay must use its actual preparation and validated formula.

12.5 Quality control and comparison

Instrument performance checks, reagent control, compensation or unmixing control and biological control answer different questions. A stable instrument does not prove that the right antibody was added. A correct reagent label does not prove that the specimen remained viable. Quality assessment must cover the whole process.

Changes in instrument settings, fluorochrome lots or panel composition can alter distributions. Longitudinal comparisons require appropriate standardisation and documentation. A fluorescence intensity measured on one platform is not automatically a universal quantity that can be compared numerically with another platform. Calibration can support specified comparisons, but the intended measurand and validation must be clear.

Quality problems should be investigated before a biological story is built around them. An abrupt shift affecting every sample after a reagent change is different from an isolated patient change with stable controls. The pattern of the problem helps locate its source. Repeating a failed control until one acceptable result appears is not a valid substitute for understanding and resolving the failure.

12.6 Interpreting and reporting a phenotype

A phenotype is a measured pattern, not a complete identity card for the cell. The report should state the markers, population, relevant intensity or distribution and the limits of the panel. For specialised disease investigations, morphology, molecular findings and clinical context may be required. The laboratory must not assign a disease from a single attractive cluster without the appropriate evidence.

A good teaching exercise therefore asks students to trace the events from specimen to report. Which events were included? Which were excluded? What controls support the measurement? What is the denominator? What alternative explanations remain? Answering those questions makes the plot interpretable and reduces the risk of treating software output as an unquestionable diagnosis.

Compensation, unmixing and the purpose of controls

Fluorochromes emit light across a range of wavelengths. A detector intended to measure one fluorochrome may therefore receive signal from another. In conventional multicolour flow cytometry, compensation uses appropriate controls to estimate and correct this spillover. It is a mathematical correction for signal overlap, not a method for removing biologically positive cells. Incorrect compensation can create misleading population boundaries and apparent co-expression. [FA18]

Single-stained controls must be suitable for the fluorochrome and analytical setup. They should provide adequate positive and negative populations and behave appropriately under the acquisition conditions. A fluorescence-minus-one control contains the other relevant reagents but omits one marker; it helps evaluate the distribution of background and spread when placing a gate. It does not replace the single-stained controls required to calculate compensation.

Spectral flow systems use the broader emission signature to separate signals through unmixing. Reference controls and the quality of those signatures remain essential. Calling a system spectral does not eliminate the need for panel design, control selection or careful interpretation. Autofluorescence and changes in specimen condition can affect the separation of signals.

A gate is an analytical decision with biological consequences. If debris, dead cells or doublets are included, the resulting percentages may not describe the intended population. Viability exclusion and singlet identification can therefore be important parts of the strategy. The denominator should be stated: a marker-positive percentage of all events differs from a percentage of viable lymphocytes or of a defined T-cell subset.

A valid result also depends on sufficient events. Detecting a small population requires enough relevant cells and an appropriate background estimate. A handful of events can be affected substantially by counting variation or contamination. The limit of detection and the limit of quantification are not identical concepts. A

laboratory may be able to detect a population without being able to estimate its proportion with the precision required for a clinical decision.

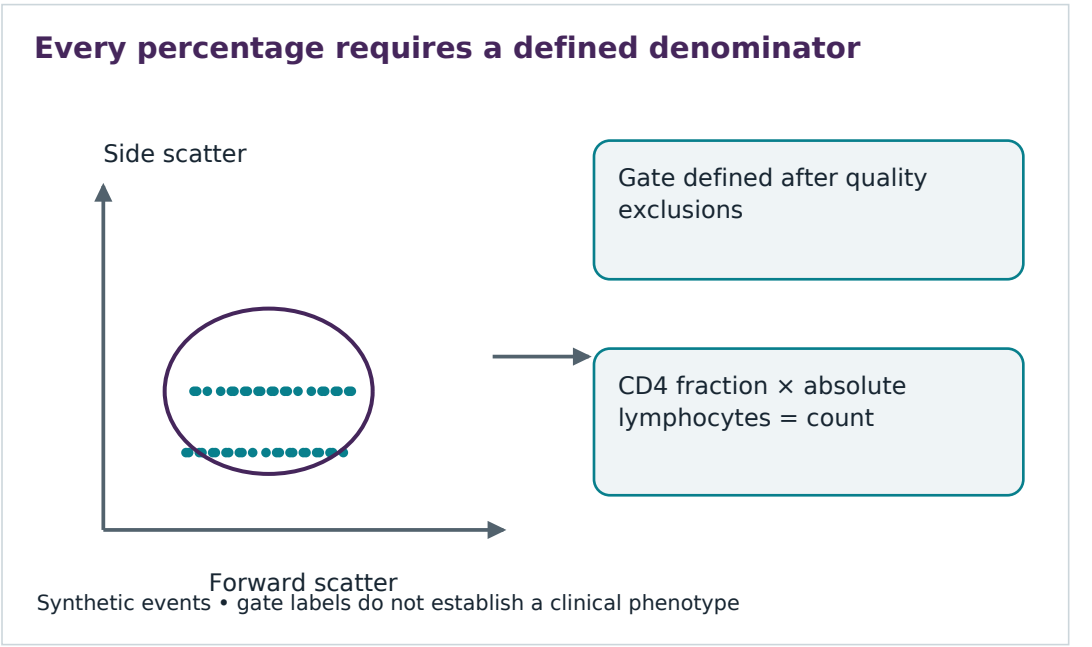


Figure FA12. Synthetic flow-cytometry model. Debris, doublets and nonviable events are excluded before population analysis. Gate placement and controls affect the reported fraction; the dots are not patient events. Original CDIL educational schematic; not patient data.

Worked cases 45–48

Case 45 — Two different denominators

A fictional report states that CD4-positive cells are 40% of lymphocytes. Another states that they are 60% of CD3-positive T cells. A learner calls the reports contradictory without examining the T-cell fraction.

Reasoned answer. The denominators differ. If T cells constitute about two thirds of lymphocytes in the relevant analysis, 60% of that T-cell population can correspond to about 40% of lymphocytes. The exact comparison requires the actual gates and counts. A percentage cannot be interpreted safely when its denominator is omitted.

Case 46 — FMO is not a compensation control

A student uses a fluorescence-minus-one tube as the only control for calculating spillover from a missing fluorochrome. Explain the problem.

Reasoned answer. The fluorochrome whose spillover needs characterisation is absent from that tube. An FMO control helps evaluate the background distribution created by the other reagents when defining a boundary; it does not supply the required single-colour signal for the missing label. Appropriate compensation or unmixing controls must represent the actual labels and conditions. [FA18]

Case 47 — A bead calculation

A synthetic tube contains a known 20,000 beads. Acquisition records 5,000 bead events and 800 target-cell events. The tube was prepared from 40 µL of original specimen, with no further correction in the stated teaching model. Calculate the target concentration.

Reasoned answer. The estimated number of target cells in the tube is 800/5,000 × 20,000 = 3,200. Dividing by 40 µL gives 80 cells/µL. The calculation assumes representative sampling and the defined preparation. A real assay must also meet its requirements for mixing, bead integrity, minimum acquisition and validated volume handling.

Case 48 — More events do not fix a wrong gate

A learner acquires a million events but excludes a dim abnormal population by using a gate copied from a healthy control. They argue that the large event count guarantees accuracy.

Reasoned answer. A large acquisition can reduce random uncertainty but does not correct systematic exclusion. The gate must be appropriate to the clinical question and specimen. Review of the distribution, controls, alternative markers and relevant morphology may be necessary. Precision in counting the wrong selected population is not accuracy in identifying the intended one.

13 | Immunoassays: Turning Molecular Recognition into a Measured Signal

An immunoassay uses antigen–antibody recognition within an analytical design. The design determines how binding is converted into a signal and what that signal means. The same patient sample can behave differently in two assays because the targets, antibodies, labels, separation steps and calibration models differ. Understanding the architecture is the foundation of troubleshooting. [FA19, FA20]

13.1 The measurand comes first

A measurand is the quantity or property intended to be measured. "Antibody level" is incomplete unless the target, class and operational definition are specified. A qualitative assay may classify reactivity against a threshold. A quantitative assay estimates a concentration within a validated interval. A semiquantitative index may express signal relative to a calibrator without representing a universally comparable concentration.

Units can appear similar while the assays remain noninterchangeable. Arbitrary units, international units and signal-to-cutoff ratios have different meanings. A result of 20 on one platform is not necessarily twice the biological amount represented by a result of 10 on another. The calibration and standardisation framework must support any such comparison.

13.2 Indirect antibody assays

In a common indirect design, a reagent antigen captures patient antibody, and a labelled reagent detects bound human immunoglobulin of a specified class or range of classes. Washing removes unbound material before signal development in many formats. The assay therefore depends on antigen presentation, the patient's binding characteristics and the detecting reagent.

The antigen may be a purified protein, recombinant product, extract, peptide or another preparation. Different preparations expose different epitopes. A detecting reagent directed at IgG will not answer the same question as one directed at IgM or total antibody. Class-specific interpretation must therefore follow the actual assay, not the clinical shorthand used on a request form.

Nonspecific binding, cross-reactivity and interference can create reactivity unrelated to the intended clinical inference. Appropriate controls and confirmatory pathways reduce risk, but a valid run does not eliminate every patient-specific interference. Controls demonstrate selected aspects of system performance; they are not miniature versions of every possible patient matrix.

13.3 Sandwich designs

A sandwich assay commonly uses one reagent to capture an analyte and another to detect a different accessible part of the captured material. Over the validated interval, signal often increases with analyte concentration. This relationship is an analytical property, not a guarantee that every possible sample concentration produces a larger signal.

At very high concentrations, some designs can show a high-dose hook effect when the binding relationships needed to form the measurable complex are disrupted by antigen excess. Whether this occurs, and at what concentration, depends on the assay. A unexpectedly low result in a clinically discordant sample may require dilution or another investigation specified by the method. The word "sandwich" alone does not establish immunity from this problem.

A heterophile antibody can sometimes bridge capture and detection reagents or interfere in other ways. The resulting signal may mimic analyte detection. An alternative platform, blocking strategy or dilution investigation can help, but no single troubleshooting manoeuvre is universally decisive. The investigation should test a specific hypothesis and use validated methods.

13.4 Competitive designs

In a competitive assay, patient analyte competes with a labelled or otherwise detected reagent for limited binding opportunities. In many common designs, more patient analyte produces less measured signal. The direction must be learned from the actual architecture. Assuming that "higher light always means more analyte" can reverse the interpretation of a competitive method.

Competition is useful for some small molecules that cannot support the two-site binding arrangement required by a typical sandwich assay. The result still depends on specificity, calibration and matrix effects. Structurally related compounds may cross-react to varying degrees, and a manufacturer's interference information applies to the tested conditions rather than every imaginable exposure.

13.5 Calibration and reportable intervals

Calibration relates signal to assigned values. The relationship may be linear over a restricted interval or require a nonlinear model. A straight line drawn through a few points cannot be assumed appropriate for every immunoassay. The chosen model must fit the assay design and validation evidence.

For a simple synthetic linear example, suppose standards of 0, 10, 20 and 40 units/mL produce signals of 0.05, 0.30, 0.55 and 1.05. The relationship is signal = 0.05 + 0.025 × concentration. A signal of 0.80 corresponds to (0.80 − 0.05)/0.025 = 30 units/mL. If the measured tube contains a validated fivefold dilution of the original sample, the original concentration is 150 units/mL. This calculation is valid only within the stated teaching model and the accepted dilution procedure.

Results beyond the measurement interval require appropriate handling. Extrapolating a nonlinear curve far beyond its validated range can produce a precise-looking but unsupported number. Reporting "above the validated range" with an appropriate dilution or follow-up pathway is more accurate than inventing a value.

13.6 Controls, blanks and patient-specific effects

A blank helps assess background under defined conditions. Calibrators establish the relationship between signal and assigned values. Controls test whether the system performs acceptably at selected levels. These materials have different purposes. A control cannot replace calibration, and a successful calibration does not prove that every patient's sample is free of interference.

Quality-control failure requires investigation according to the laboratory's procedure. The impact assessment may include previously tested samples if evidence suggests the problem existed before detection. A laboratory should not release patient results merely because they seem clinically plausible when the run's required control conditions were not met.

13.7 Results that travel between laboratories

When a patient transfers care, the method name, units, interval and relevant interpretive information should accompany the result. A change in assay can explain a discontinuity in a trend. Method comparison may support a transition, but a general conversion formula should not be invented from one paired result.

The report should also distinguish screening reactivity from a completed diagnostic algorithm. Some infections and autoimmune questions require linked testing or confirmation. Releasing the first reactive result without its required follow-up context can misclassify the patient's status even when the initial analytical measurement is correct.

A worked sandwich-assay signal chain

In a simplified sandwich immunoassay, a capture antibody binds a target, and a detection antibody recognises another accessible epitope. A label associated with the detection system produces a measurable signal. The ability to form the sandwich depends on the target's structure and the accessibility of both binding sites. Fragments, complexes or modified molecular forms may behave differently from the material used in calibration. [FA19]

Washing separates bound from unbound material in many assay formats. Inadequate washing can leave excess label and raise background. Excessive or inappropriate washing may disturb the intended reaction or remove material in a way that affects recovery. Automated systems control these steps, but automation does not make them conceptually irrelevant. Understanding the signal chain helps an operator interpret quality-control failures and instrument flags.

Calibration relates measured signal to assigned concentration using an appropriate model. Many immunoassay curves are nonlinear. A straight-line equation is useful in selected teaching exercises but should not be imposed on a platform whose validated calibration uses a different function. Interpolation within a validated range differs from extrapolation beyond it. A number calculated outside the supported range can look precise while having no validated analytical meaning.

Controls are independent materials used to monitor performance at relevant levels. A calibrator establishes the relationship between signal and assigned value; a control tests whether the measurement process behaves acceptably after that relationship has been established. Using the same material and assigned values for both purposes can weaken the independence of the check. Quality control should challenge clinically important parts of the measuring range, not only the easiest central point.

A result near a clinical cutoff requires special care. Small bias or imprecision can change categorical classification. The laboratory should apply the method's repeat, equivocal or reflex rules and understand how quality-control performance relates to the decision point. The report should not imply that the biological state changes abruptly at the last decimal place printed by the analyser.

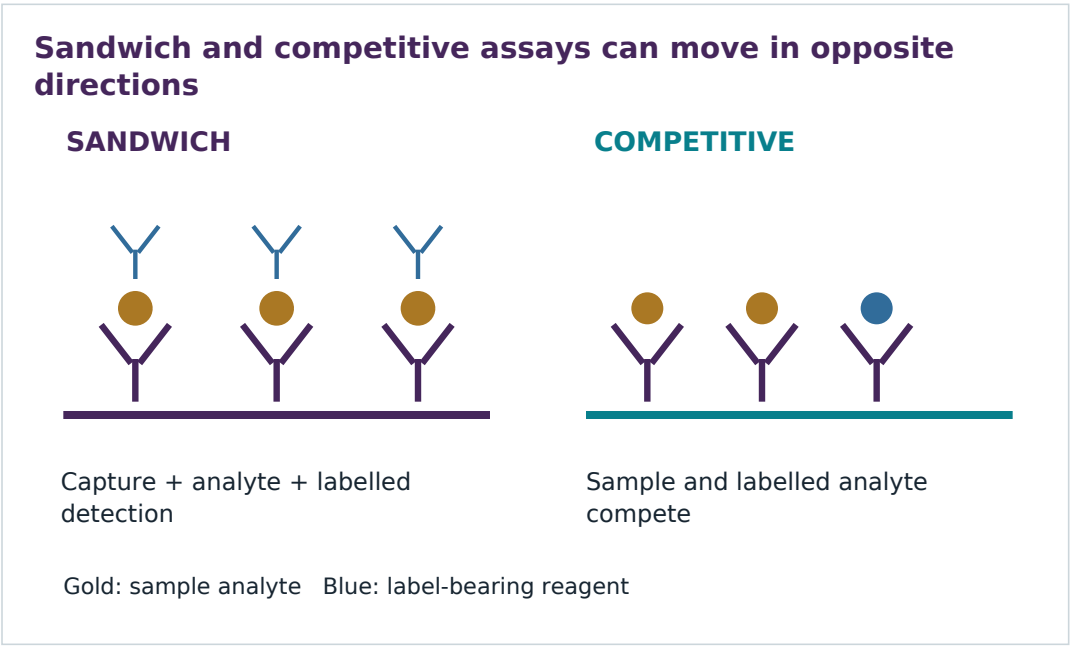


Figure FA13. In the simple sandwich model, more analyte can generate more bound label within the validated range. In the competitive model, more sample analyte can reduce labelled binding. Real assay architecture determines interference direction. Original CDIL educational schematic; not patient data.

Worked cases 49–52

Case 49 — A competitive signal

In a fictional competitive assay, increasing analyte reduces the detected signal. A sample with a low signal is labelled "low analyte" by a student using sandwich-assay logic.

Reasoned answer. The interpretation reverses the stated relationship. The sample's signal must be converted through the competitive assay's validated calibration model. The result cannot be inferred from brightness alone. Before interpreting any immunoassay, identify what is labelled, what competes or is captured, and how the measured signal changes with the measurand.

Case 50 — Dilution correction

Using the synthetic calibration described above, a diluted sample gives a signal of 0.55. It was diluted one part sample to four parts diluent. What is the original concentration?

Reasoned answer. The measured concentration is (0.55 − 0.05)/0.025 = 20 units/mL. One part sample plus four parts diluent produces a total dilution factor of five, so the original concentration is 100 units/mL. Confusing "one plus four" with a fourfold dilution would produce a wrong result. The preparation must also be validated for the real assay.

Case 51 — Valid controls, implausible patient result

A fictional patient's immunoassay result is clinically implausible, but both routine controls are acceptable. A learner says interference is impossible because the run passed.

Reasoned answer. Controls demonstrate selected system performance; they do not reproduce every patient-specific matrix or antibody interaction. The laboratory should investigate the discordance through an appropriate strategy, potentially including an alternative method or validated interference studies. Passing controls support run validity but do not eliminate all causes of an incorrect patient result.

Case 52 — Beyond the curve

A student extrapolates a nonlinear immunoassay curve far beyond the highest validated calibrator and reports a concentration to three decimal places.

Reasoned answer. The apparent precision is unsupported. The assay's validated range and dilution procedure govern reporting. A qualified result or properly performed dilution may be appropriate, but an unvalidated extrapolation is not made reliable by adding decimal places. Precision of display must not be confused with accuracy of measurement.

14 | Diagnostic Performance, Probability and Measurement Uncertainty

A test result changes a clinical probability; it does not remove the need for reasoning. Sensitivity and specificity describe performance in relation to an agreed reference classification, while predictive values describe the meaning of positive and negative results in a particular setting. The same assay can produce very different proportions of true positives when used in populations with different disease prevalence or pretest probability. [FA21]

14.1 Build the two-by-two table

The basic table compares test classification with reference classification. A true positive is positive by the test and positive by the reference. A false negative is negative by the test despite a positive reference. A false positive is positive by the test despite a negative reference. A true negative is negative by both. The quality and limitations of the reference classification matter; it is not automatically an infallible biological truth.

Sensitivity is TP/(TP + FN). Specificity is TN/(TN + FP). Positive predictive value is TP/(TP + FP), while negative predictive value is TN/(TN + FN). The denominators answer different questions. Sensitivity begins with people who have the reference condition; positive predictive value begins with people whose tests are positive. Confusing these populations is one of the most common interpretation errors.

A synthetic study of 2,000 people illustrates the difference. Assume prevalence is 2%, sensitivity 90% and specificity 95%. Forty people have the reference condition: 36 test positive and four negative. Of 1,960 without the condition, 98 test positive and 1,862 negative. The positive predictive value is 36/134, or approximately 26.9%, not 90%. The negative predictive value is 1,862/1,866, approximately 99.8%.

14.2 Why prevalence changes predictive values

Now keep sensitivity and specificity the same but use a population with 20% prevalence. Among 2,000 people, 400 have the condition: 360 test positive and 40 negative. Of 1,600 without it, 80 test positive and 1,520 negative. Positive predictive value becomes 360/440, approximately 81.8%. Negative predictive value becomes 1,520/1,560, approximately 97.4%.

The assay did not become analytically more specific. The composition of the tested population changed. This is why indiscriminate low-probability screening can generate many positive results that do not represent the target condition. It is also why a negative result in a high-probability patient may be less reassuring than the same result in a low-probability setting.

Real sensitivity and specificity can also vary across clinical spectra, stages of disease and study designs. The calculation above holds them fixed to isolate one principle. It must not be interpreted as proof that performance is immutable across every population.

14.3 Likelihood ratios and odds

A positive likelihood ratio is sensitivity/(1 − specificity). A negative likelihood ratio is (1 − sensitivity)/specificity. For the synthetic test above, LR+ is 0.90/0.05 = 18, and LR− is 0.10/0.95, approximately 0.105. These ratios describe how the result changes odds under the assumed performance model.

Probability and odds are related but not identical. Odds = probability/(1 − probability). Posterior odds can be calculated as prior odds multiplied by the applicable likelihood ratio. The resulting odds are converted back to probability using odds/(1 + odds). At a 2% prior probability, prior odds are 0.02/0.98. Multiplying by 18 gives posterior odds of about 0.367, corresponding to a probability of approximately 26.9%, matching the table calculation.

These calculations are educational models, not a replacement for clinical judgement or an excuse to select a convenient prior probability after seeing the result. The assumptions must be defensible. Repeated tests are often correlated, especially when they

use the same antigen or platform, so likelihood ratios cannot be multiplied as if every result were independent without justification.

14.4 Thresholds and trade-offs

Changing a decision threshold can alter sensitivity and specificity. A lower threshold may detect more affected people while classifying more unaffected people as positive. The appropriate balance depends on the purpose of testing and the consequences of errors. Screening, confirmation, monitoring and emergency decisions may require different strategies.

A receiver-operating-characteristic curve summarises relationships across thresholds under a study's conditions. Its area is not a complete statement of clinical usefulness. The consequences of false results, the relevant range of thresholds, prevalence, turnaround time and downstream decisions still matter. A test with an attractive overall statistic can be poorly suited to a particular decision.

14.5 Precision, bias and uncertainty

Precision concerns the dispersion of repeated measurements under defined conditions. Bias concerns systematic difference from an appropriate target or reference. A method can be precise but biased: repeated results cluster tightly around the wrong value. Conversely, an unbiased average can coexist with unacceptable scatter for individual results.

For five synthetic replicates of 9.8, 10.1, 10.0, 9.9 and 10.2 units, the mean is 10.0. The sample standard deviation is approximately 0.158, and the coefficient of variation is approximately 1.58%. These values describe the small teaching dataset. They are not sufficient on their own to establish full method precision across days, operators, reagent lots and concentrations.

Measurement uncertainty is broader than a display of decimal places. It considers relevant sources of variation within a defined measurement process. An uncertainty estimate does not excuse poor quality; it makes the limits of a measurement more transparent. Biological variability and clinical decision uncertainty add further layers beyond the analytical estimate.

14.6 Reference intervals and decision limits

A reference interval describes a distribution in a defined reference population under specified conditions. A decision limit is linked to a particular clinical or operational decision. The two should not be used interchangeably. A result outside an interval is not automatically a diagnosis, and a result inside it does not exclude every disease.

Age, pregnancy, physiological state and method can influence the appropriate comparison. A laboratory must establish or verify applicable intervals and decision frameworks rather than copying a number from a textbook without validation. The textbook's role is to explain interpretation and arithmetic, not to create universal clinical thresholds for every laboratory.

14.7 Communicating a result near a boundary

A value just above a cutoff should not be described with more certainty than the method supports. The assay may have a defined equivocal category or repeat strategy. Small changes around a threshold can reflect analytical and biological variation rather than a true transition from "disease absent" to "disease present."

A useful report names the category, the relevant method and any required next step in the validated pathway. It avoids claims such as "100% certain" unless such certainty is genuinely justified, which routine diagnostic testing rarely permits. Honest limits make the result more useful, not less scientific.

Precision, bias and uncertainty are different descriptions

Precision concerns the closeness of repeated measurements under stated conditions. Bias concerns a systematic difference from an appropriate reference or assigned value. A method can be precise but biased, or relatively unbiased on average while showing substantial scatter. The distinction is important because repeating a specimen can reduce the influence of some random variation but does not necessarily correct systematic error. [FA21, FA22]

The coefficient of variation expresses standard deviation relative to the mean, usually as a percentage. It is useful for comparing relative imprecision at different concentrations, but it becomes unstable or difficult to interpret near zero. A small absolute standard deviation can produce a large coefficient of variation when the mean is very low. The selected performance measure should match the concentration and clinical purpose.

Measurement uncertainty describes a range associated with a measurement under specified assumptions and sources of variation. It is not a statement that the laboratory does not know anything useful. Rather, it makes the limits of numerical knowledge explicit. The appropriate estimation and reporting approach depends on the measurand, method and regulatory or accreditation framework. A textbook calculation using a few repeated results is not a complete laboratory uncertainty budget.

Diagnostic accuracy introduces additional uncertainty beyond analytical measurement. Sensitivity and specificity are estimated from study samples and have confidence intervals. The estimates may change with disease spectrum, stage, reference standard and specimen handling. A large sample can reduce random sampling uncertainty while leaving systematic bias unresolved. Adding more patients to a poorly designed study does not automatically repair its design.

When the comparator is not an adequate reference standard, agreement measures may be more appropriate than calling the results sensitivity and specificity. Positive and negative percent agreement describe concordance with that comparator, not unquestionable truth about disease status. The terminology protects readers from overinterpreting an analytical comparison as a definitive clinical validation. [FA21]

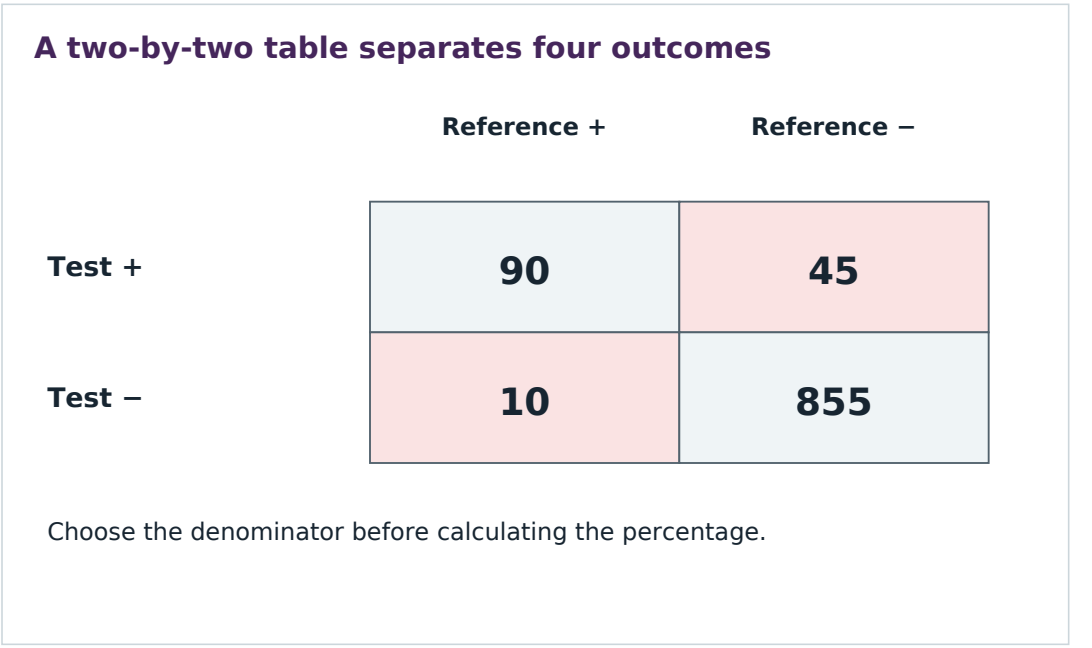


Figure FA14. Synthetic reference-standard study. Sensitivity uses the disease-positive column; specificity uses the disease-negative column. Predictive values instead condition on the test result. Original CDIL educational schematic; not patient data.

Worked cases 53–56

Case 53 — A screening population

Use the 2,000-person, 2%-prevalence synthetic model with 90% sensitivity and 95% specificity. How many positive tests are expected, and what proportion are true positives?

Reasoned answer. There are 36 true positives and 98 false positives, giving 134 positive tests. The true-positive proportion among positives is 36/134 = 26.9%. The 90% sensitivity answers a different question: among the 40 people with the reference condition, 36 test positive. The exercise demonstrates why a positive screening result may require an appropriate diagnostic pathway.

Case 54 — Precision without accuracy

A control with an assigned target of 100 units repeatedly measures 112, 111, 112, 113 and 112. A learner praises the method because the values agree closely.

Reasoned answer. The results are tightly clustered, suggesting good repeatability under these conditions, but their mean is 112, showing a positive difference of 12 units from the assigned target. The example therefore raises a bias concern. The target's uncertainty and suitability also need consideration. Reproducibility alone does not establish accuracy.

Case 55 — Correlated confirmation

A laboratory repeats a reactive antibody test using the same platform and reagent lot. A student multiplies two likelihood ratios as if the results were independent confirmations.

Reasoned answer. Repetition can demonstrate reproducibility but may reproduce the same cross-reactivity or interference. Independence has not been established. A confirmatory strategy should use the validated diagnostic algorithm and, when appropriate, a method that contributes different information. Two correlated observations cannot automatically be treated as two independent pieces of evidence.

Case 56 — A value near a cutoff

A fictional assay classifies 0.98 as negative and 1.02 as reactive, with a defined repeat policy around the cutoff. Two samples from one patient produce those values on adjacent days.

Reasoned answer. The small difference may reflect analytical or biological variation near the decision boundary. The laboratory should follow the assay's validated repeat and interpretation procedure and consider the clinical timeline. It should not announce a definite biological conversion solely from crossing the displayed numerical line by a small amount.

15 | Agglutination, Precipitation, Titres and Classical Serology

Classical serological methods make antigen–antibody interactions visible through lattice formation, altered turbidity, movement in a matrix or other measurable effects. Their apparent simplicity can be deceptive. A visible clump depends on target density, antibody properties, concentrations, temperature, time and the physical conditions of the method. Absence of visible clumping does not always mean absence of binding. [FA02, FA19]

15.1 Sensitisation and lattice formation

Sensitisation occurs when antibody binds its target. Agglutination occurs when particles become linked into visible aggregates under suitable conditions. The two stages are related but not identical. An antibody can coat a cell without producing obvious direct agglutination. Antiglobulin methods exploit this distinction by detecting immunoglobulin or complement associated with cells through an additional reagent system.

Particle size and antigen arrangement influence lattice formation. Large multivalent antibodies can be effective at bridging suitable particles, while other antibodies may require conditions or additional reagents that reveal their binding. The method must therefore be understood as a complete system rather than as a universal visual test for all antibodies.

Reaction grading describes the observed appearance under a defined method. A grade such as 1+ or 3+ is not a universal concentration and should not be compared across unrelated methods as if it were an exact amount. Training must use the laboratory's standardised reading criteria and appropriate competency materials.

15.2 Antibody excess and antigen excess

Lattice formation is affected by the relative amounts of antigen and antibody. At an appropriate relationship, cross-linking can produce a strong visible reaction. Antibody excess can interfere with the formation of an effective lattice, a phenomenon commonly called prozone. Antigen excess can also reduce lattice formation and is often described as postzone in classical precipitation models.

These terms should not be applied indiscriminately to every false-negative immunoassay. A high-dose hook effect in a particular sandwich assay has an architecture-dependent mechanism. It shares the broader principle that extreme concentrations can disrupt an expected signal relationship, but the investigation must follow the actual method rather than a generic dilution ritual.

A dilution series can sometimes reveal a masked reaction. However, dilution also lowers weak antibodies and can change matrix effects. A negative diluted sample does not exclude every interference, and an apparently improved result does not identify one mechanism without further evidence. The procedure and interpretation must be validated for the intended assay.

15.3 Titres are endpoints, not mass concentrations

A titre is commonly reported as the reciprocal of the highest dilution meeting a defined reaction endpoint. In a twofold series of 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64, if 1:32 is the last reactive dilution and 1:64 is nonreactive, the endpoint titre is 32 under that method's criterion. The exact reaction strength required for the endpoint must be specified.

A change from 1:8 to 1:32 is a fourfold increase, corresponding to two twofold dilution steps. A change from 1:32 to 1:64 is twofold, not a doubling of an independently measured mass concentration. Method variability and the clinical framework determine whether a change is meaningful. Titres from different methods or laboratories should not automatically be compared as a continuous trend.

Preparation notation must be unambiguous. A 1:10 dilution commonly means one part sample in ten total parts, such as one part

sample plus nine parts diluent. The phrase "add ten parts diluent" would create a different dilution. Every practical calculation should state the volumes and total factor rather than rely on informal language.

15.4 Precipitation and diffusion

Soluble antigen and antibody can form complexes that precipitate under suitable conditions. In diffusion methods, movement through a gel creates spatial relationships between reactants. The pattern can provide information about identity, partial identity or nonidentity in a controlled experimental setting. Interpretation requires knowledge of which wells contain which materials and whether the reagents are suitable.

In radial immunodiffusion, an antigen diffuses into an antibody-containing matrix. Under a validated endpoint method, the relationship between ring size and concentration is established through calibrators, often using a squared-diameter relationship over the relevant range. The student should not memorise an unqualified rule that every ring diameter is directly proportional to concentration in every assay.

Electrophoretic methods add movement in an electric field. Protein migration depends on properties and conditions of the method, including charge, matrix and buffer. Immunofixation combines separation with immunological detection to characterise selected proteins. A visible band must be interpreted in relation to the lanes, controls and clinical context rather than named solely by its darkness.

15.5 Turbidimetry and nephelometry

Turbidimetry measures reduction of transmitted light associated with particles in suspension, whereas nephelometry measures light scattered at defined angles. Immunocomplex formation can provide the particles used for measurement. The analytical system must distinguish the intended reaction from background turbidity, lipaemia, precipitation unrelated to the target and other matrix effects.

Calibration and reaction kinetics matter. A method measuring an early rate does not necessarily use the same relationship as a method measuring an endpoint. An antigen-excess check may be built into the assay, but its effectiveness is limited to the validated conditions. Extremely unusual samples still require investigation when the result conflicts with other evidence.

15.6 Controls and records in a practical exercise

A safe teaching exercise uses simulated or appropriately authorised materials and a defined procedure. Students should record sample identity, dilution preparation, reagent identity, incubation conditions, observations and endpoint rules. A completed worksheet should allow another person to reconstruct the calculation without guessing which tube was which.

The most important errors are often mundane: a missed mixing step, an incorrect total volume, a skipped tube or a transcribed endpoint. A beautiful dilution curve cannot rescue an unidentified specimen. Technical skill includes traceability and arithmetic as well as recognition of a reaction pattern.

15.7 Interpreting disagreement

If a titre changes unexpectedly, first check whether the same method and endpoint were used. Then review specimen timing, reagent changes, preparation and controls. Only after those questions are addressed should the change be attributed entirely to biology. The aim is not to explain every discrepancy away as laboratory error; it is to distinguish analytical and biological possibilities systematically.

A result should be reported in the form supported by the method. A titre is a titre. A semiquantitative grade is a grade. A calibrated concentration is a concentration. Converting one into another without validation creates false precision and can mislead clinical decisions.

Lattice formation, zones and endpoint reading

Visible agglutination or precipitation requires more than the existence of antigen–antibody binding. The participants must form a sufficiently large network under the test conditions. Valency, concentration, temperature, ionic environment and reaction time influence the result. A molecule can be bound without forming a visible lattice, and a visible aggregate can occasionally arise through nonimmune mechanisms. [FA02, FA19]

The classical zone phenomenon describes how relative concentrations affect lattice formation. Antibody excess can reduce visible network formation in some reactions, while antigen excess can also produce weak or absent visible precipitation. The equivalence region supports more extensive lattice formation. These concepts explain why appropriate dilution can sometimes reveal a reaction that was weak in an undiluted specimen, but they do not justify diluting every assay outside its validated instructions.

A titre is an endpoint defined by the method: commonly the reciprocal of the highest dilution that still meets the specified positive criterion. It is not an exact molar concentration. The starting dilution, dilution factor, incubation, reagents and reading threshold influence the number. Titres from different methods should not be compared as if they were calibrated concentrations in identical units.

Reading variability matters. A weak endpoint can be difficult to distinguish from background, particularly when visual grading is used. Training, reference examples, appropriate controls and standardised reading conditions improve consistency. Photographs or synthetic diagrams in a textbook demonstrate principles; they do not replace the laboratory's validated grading system or competency assessment with actual materials.

For serial clinical monitoring, specimens may need to be tested using the same method and, where the validated protocol permits, compared in a way that limits run-to-run variation. A twofold change represents one dilution step in a twofold series; a fourfold change represents two steps. Whether that change has clinical meaning

depends on the particular infection or antibody system and the relevant guideline, not on the arithmetic alone.

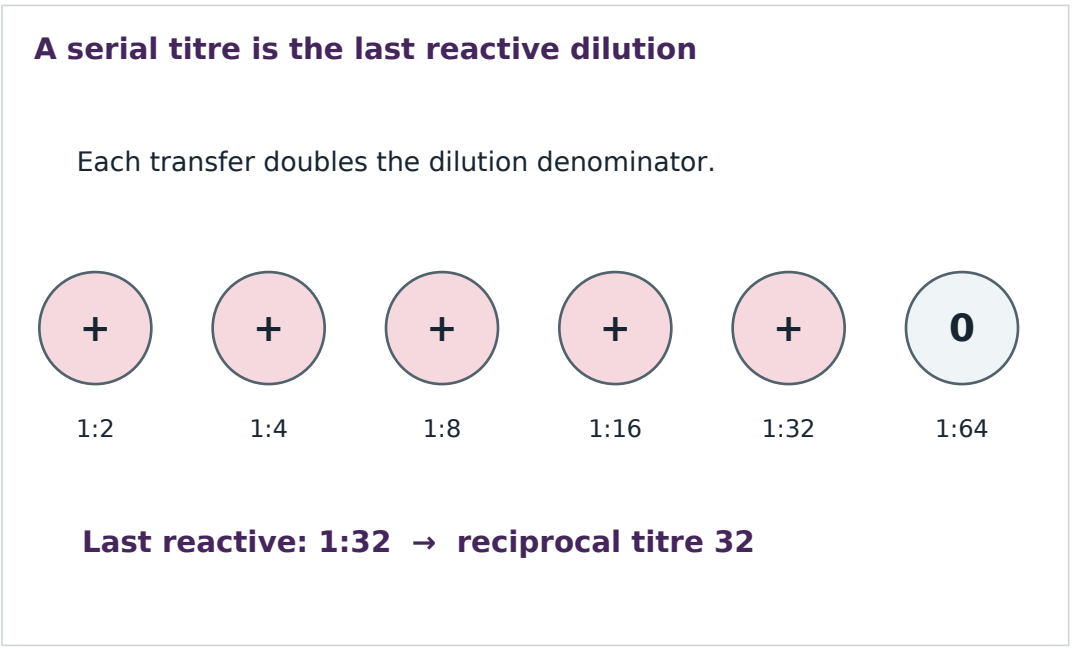


Figure FA15. Synthetic twofold series with reactions through 1:32. The reciprocal titre is 32. One further twofold step is 64; a fourfold change spans two such steps. Original CDIL educational schematic; not patient data.

Worked cases 57–60

Case 57 — The endpoint

A synthetic twofold dilution series gives reactions of 3+, 2+, 2+, 1+, 1+ and 0 at 1:2 through 1:64. The defined endpoint is the highest dilution with at least 1+ reactivity. Report the titre.

Reasoned answer. The last qualifying reaction is at 1:32, so the titre is 32. The nonreactive 1:64 tube confirms that the endpoint was reached within the tested series. Reporting 64 would confuse the first negative dilution with the last positive one. The endpoint definition must accompany the training exercise because another method may use a different criterion.

Case 58 — A fourfold change

A patient's result changes from 1:16 to 1:64 using the same validated method. How many twofold steps and what fold change are represented?

Reasoned answer. The sequence is 1:16 → 1:32 → 1:64: two steps and a fourfold increase in the reported endpoint titre. Whether that change has clinical significance depends on the disease, timing and method. It is not a direct statement that the antibody mass concentration increased exactly fourfold.

Case 59 — Preparing a dilution

A student mixes 100 µL sample with 900 µL diluent. Another mixes 100 µL sample with 1,000 µL diluent. Are both 1:10 dilutions?

Reasoned answer. No. The first contains 100 µL sample in 1,000 µL total, giving a tenfold dilution. The second contains 100 µL in 1,100 µL total, giving an elevenfold dilution. The difference can propagate through a serial calculation. Recording actual volumes is safer than relying on ambiguous shorthand.

Case 60 — A weak undiluted reaction becomes stronger

An unexpectedly weak synthetic agglutination reaction becomes clearer after a validated dilution. A learner declares that prozone is proved.

Reasoned answer. Antibody excess is one possible explanation, but dilution can also alter matrix effects and other interactions. The result supports further investigation; it does not identify the mechanism uniquely. The laboratory should use the method's validated troubleshooting approach, review controls and consider relevant alternatives before issuing a definitive explanation.

16 | Infectious-Disease Serology: Exposure, Timing and Linked Testing

Serology measures a host response or selected antigen targets; it is not a universal direct count of living organisms. A positive antibody can represent current infection, past infection, vaccination, passive transfer, cross-reactivity or interference, depending on the assay. A negative antibody can occur before a response becomes detectable or when the host cannot produce the expected response. The central task is to place the result on the correct biological and diagnostic timeline. [FA12–FA14, FA24–FA27]

16.1 The timeline has several clocks

Exposure, onset of symptoms, appearance of antigen or nucleic acid, antibody production and treatment do not occur simultaneously. A specimen collected early after exposure may be negative by antibody testing while a suitable direct-detection method is positive. Later, antibody may persist after the relevant direct marker is no longer detected. The meaning of a result therefore depends on which clock the assay observes.

Seroconversion describes a change from a previously nonreactive to a reactive antibody result under an appropriate testing framework. It requires reliable identification, comparable methods and meaningful timing. A small fluctuation around a cutoff is not automatically a true new infection. A change of platform, specimen quality or interference can produce an apparent conversion that needs investigation.

Paired samples can contribute useful information when the disease and method support that approach. They should be labelled with actual collection dates, and comparison should follow the laboratory's validated process. The student must not invent a universal interval for all organisms. Some clinical decisions require direct testing or urgent action rather than waiting for a convalescent sample.

16.2 Hepatitis B as a marker-pattern problem

The CDC adult screening framework uses a triple panel: HBsAg, anti-HBs and total anti-HBc. The components answer different questions. Surface antigen supports assessment of current antigenaemia; surface antibody can follow recovery or vaccination; core antibody generally indicates exposure to viral core material rather than the usual surface-antigen vaccine. The combination is more informative than a single positive label. [FA12]

A pattern of HBsAg negative, total anti-HBc negative and anti-HBs positive is compatible with vaccine-associated immunity in an appropriate history. HBsAg negative with total anti-HBc positive and anti-HBs positive commonly supports resolved infection. HBsAg positivity requires the relevant clinical and confirmatory interpretation. Isolated core-antibody reactivity has several possible explanations and should not be forced into one category without context. Recent vaccination, passive antibody and immunosuppression can complicate selected patterns.

IgM anti-HBc can contribute to investigation of recent infection, but an IgM result must still be interpreted through the full clinical framework. A textbook table cannot replace the current assay instructions, diagnostic pathway and clinical assessment. Where reactivation risk is relevant before immunosuppressive treatment, historical infection markers may matter even when surface antigen is not detected.

16.3 Hepatitis C: antibody does not establish current viraemia

A reactive HCV antibody result can reflect current infection, resolved infection or biological false positivity. RNA testing provides the next key information in the CDC diagnostic sequence. Antibody reactive with RNA detected supports current infection; antibody reactive with RNA not detected usually indicates no current infection at that time, while recent exposure, clinical concerns or specimen issues can require follow-up. [FA13]

Automatic linked testing reduces the chance that a patient receives an incomplete interpretation and is lost before a second specimen is collected. The laboratory should connect the initial antibody and subsequent RNA findings in the final report. A patient should not be labelled currently infected solely from an incomplete antibody result, nor should a negative antibody shortly after a relevant exposure be treated as a universal exclusion.

16.4 HIV testing uses an algorithm, not an isolated reaction

Laboratory HIV testing combines methods according to a validated diagnostic algorithm. In the widely used US laboratory framework, a reactive antigen/antibody screening assay is followed by an HIV-1/HIV-2 differentiation assay, with nucleic-acid testing used to resolve specified negative or indeterminate differentiation results. The exact pathway must match the setting, specimen and authorised tests. [FA24]

Screening reactivity is not the same as a completed diagnosis. The report should identify where the sample stands in the algorithm and link required follow-up results. Recent exposure, antiretroviral use and other clinical circumstances can complicate interpretation. These situations require the relevant current specialist and public-health guidance rather than a simplified classroom rule.

16.5 Syphilis: complementary test families

Treponemal and nontreponemal tests contribute different information. Traditional and reverse screening sequences are both used, with appropriate linked testing to resolve results. A single reactive test is insufficient to describe every patient's infection status. Nontreponemal endpoint titres can contribute to follow-up, but comparison requires the appropriate method and clinical framework. [FA14]

A treponemal result can remain reactive after treatment and should not automatically be used as a treatment-response measurement. Conversely, a nontreponemal reaction is not specific enough to establish syphilis without the required context and linked testing.

The laboratory's report should keep the sequence visible so that a partially completed investigation is not mistaken for a final clinical classification.

16.6 EBV, CMV and the limits of IgM

For Epstein–Barr virus, the relationship among antibodies to viral capsid antigen and EBV nuclear antigen contributes to interpretation. The pattern and timing are more informative than an isolated statement that "EBV antibody is positive." Heterophile tests have limitations and are not equivalent to a complete EBV-specific antibody profile. [FA25]

CMV IgG indicates prior exposure or passively acquired antibody in an appropriate context, while IgM alone does not establish a primary recent infection. Avidity and other investigations can contribute in selected circumstances. Congenital CMV cannot be diagnosed from newborn antibody testing alone; direct testing within the required early-life window is important for distinguishing congenital from later acquisition. [FA26, FA27]

In immunocompromised patients, direct detection and quantitative monitoring may answer questions that serology cannot. The laboratory should identify whether the purpose is exposure assessment, diagnosis, risk stratification or monitoring. Using the wrong test for the question can produce a technically valid but clinically unhelpful result.

16.7 Interpreting the final report

A complete serology report should preserve the organism, antigen target, immunoglobulin class where relevant, method category, result, collection date and diagnostic status. Where a reflex sequence is required, the linked results should be visible. Uncertainty should be specific: early sampling, passive antibody, equivocal reactivity or an incomplete algorithm each means something different.

The laboratory should avoid stigma-producing certainty that the evidence does not support. An unconfirmed reactive screen is not a licence to disclose a definitive diagnosis to an employer or other unauthorised recipient. Confidentiality and appropriate clinical

communication are part of quality, especially when infection-related results can affect a person's relationships, work or access to care.

Three linked-testing examples that prevent overdiagnosis

Hepatitis B investigation illustrates the value of combining markers with different biological meanings. Surface antigen, surface antibody and total core antibody contribute complementary information. A pattern can support susceptibility, vaccination-associated immunity, previous infection or current infection, but unusual or discordant combinations require attention to timing, immune status and additional testing. An isolated marker should not be given a universal interpretation detached from the panel and clinical question. [FA12]

For hepatitis C, a reactive antibody test indicates exposure or reactivity that requires interpretation; RNA testing determines whether viraemia is demonstrated at the time of testing. A reactive antibody with RNA not detected usually means no current infection is demonstrated, while recent exposure, specimen concerns and selected clinical circumstances can alter follow-up. The antibody result does not by itself distinguish resolved from current infection. Reflex testing improves completion of the diagnostic pathway when implemented appropriately. [FA13]

Syphilis testing uses treponemal and nontreponemal methods in linked algorithms. The order can differ between traditional and reverse-sequence approaches. Discordance requires the relevant next step, not an improvised majority vote among assays. Nontreponemal titres can support monitoring in appropriate circumstances, but results should be compared with attention to method and timing. A treponemal result may remain reactive after treatment and is not a simple test of cure. [FA14]

Timing changes every serological interpretation. Early infection may precede a detectable antibody response. Immunosuppression can alter response patterns. Passive antibody, cross-reactivity and previous exposure can produce reactivity without the current disease being investigated. The appropriate organism-specific algorithm

addresses these possibilities more reliably than a general rule that "IgM means new and IgG means old."

Congenital cytomegalovirus provides a particularly important exception to antibody-based assumptions. Maternal antibodies complicate infant serology, and diagnosis relies on appropriate direct testing within the early postnatal window under the clinical pathway. The timing of collection matters because later detection may not distinguish congenital from postnatal acquisition. This is a chronological diagnostic problem as well as an analytical one. [FA26, FA27]

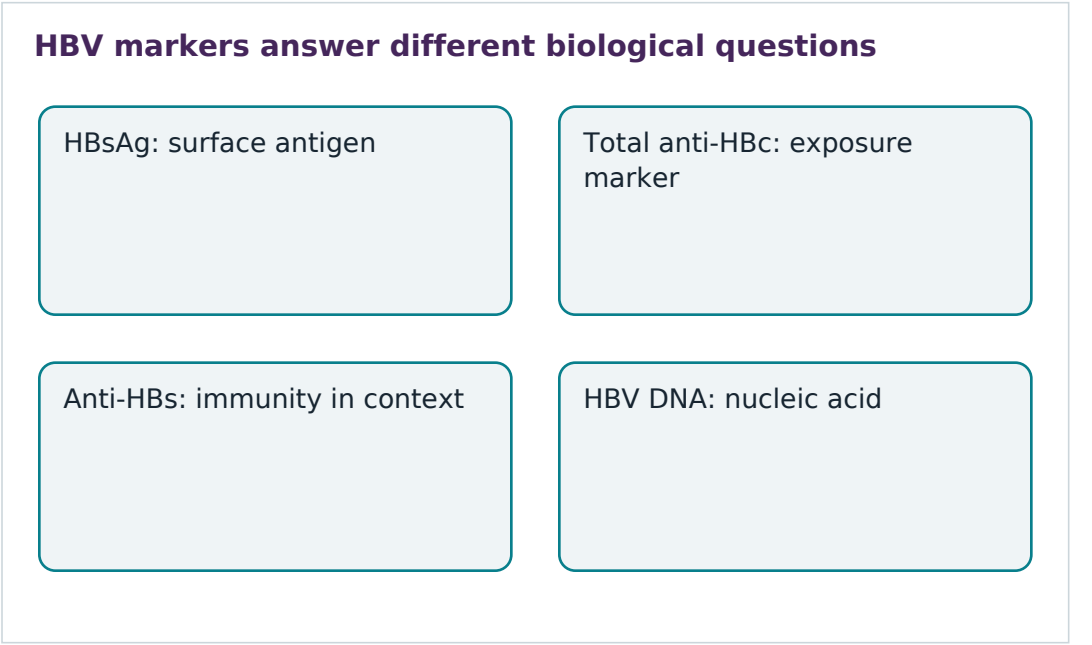


Figure FA16. HBV marker roles are distinct: HBsAg indicates current antigen detection, total anti-HBc indicates exposure to core antigen, anti-HBs supports immunity in context, and HBV DNA detects viral nucleic acid. Interpret the combined pattern. Original CDIL educational schematic; not patient data.

Worked cases 61–64

Case 61 — Reactive HCV antibody, RNA not detected

A fictional patient's HCV antibody is reactive and the linked RNA test is not detected. There is no recent exposure or other concern documented. A learner reports "active hepatitis C proved."

Reasoned answer. The linked findings do not support that statement. The RNA result indicates no current infection in the usual interpretation of this pattern, while the antibody may reflect resolved infection or biological false positivity. Recent exposure, clinical evidence or specimen concerns could change the follow-up plan, so the context remains important. The report must include both results rather than letting the initial reactive screen stand alone. [FA13]

Case 62 — A historical treponemal result

A fictional patient previously treated for syphilis remains reactive on a treponemal assay. A student calls the persistent result proof of treatment failure.

Reasoned answer. Treponemal reactivity can persist after treatment. Assessment of treatment response and possible reinfection uses the appropriate clinical history and nontreponemal testing framework, not an isolated persistent treponemal result. The result must be interpreted in relation to the assay family and purpose. [FA14]

Case 63 — An incomplete HIV sequence

An antigen/antibody screen is reactive, but the required follow-up testing has not been completed. The draft report says "confirmed HIV infection."

Reasoned answer. The report overstates the evidence. It should identify screening reactivity and the pending or required diagnostic steps in the applicable algorithm. The laboratory must complete or arrange the linked investigation and communicate through the appropriate confidential pathway. A reactive first step is not equivalent to a completed diagnostic classification. [FA24]

Case 64 — Newborn CMV IgG

A newborn has CMV IgG detected. A learner diagnoses congenital infection from that antibody alone.

Reasoned answer. Maternal IgG can be transferred to the infant, so antibody alone cannot establish congenital CMV. Appropriate direct testing within the early-life diagnostic window is required. The exact specimen and confirmation pathway should follow current guidance

and the receiving laboratory's procedure. Delayed testing can make congenital and postnatal acquisition harder to distinguish. [FA26, FA27]

17 | Autoimmune Serology: Patterns, Targets and Clinical Use

Autoimmune serology becomes useful when the result answers a defined clinical question. The same numerical result can have different implications in a patient with a characteristic phenotype and in someone tested without a relevant indication. This chapter develops a practical approach to pattern recognition, targeted testing and longitudinal interpretation without converting laboratory associations into automatic diagnoses. [FA09–FA11, FA15]

17.1 HEp-2 pattern recognition is a disciplined observation

HEp-2 indirect immunofluorescence can show nuclear, cytoplasmic and mitotic patterns. The observed pattern depends on substrate preparation, conjugate, dilution, microscope conditions and reader interpretation. A training image should identify its method and limitations. A stylised drawing can explain distribution, but clinical competency requires validated images and appropriate assessment, not memorisation of an idealised cartoon.

The International Consensus on ANA Patterns provides standardised terminology and descriptions. Pattern recognition can guide consideration of antigen-specific tests, but a pattern is not a unique disease diagnosis. Similar appearances can arise through different antibody combinations, and mixed patterns may be difficult to interpret. The laboratory must use its validated reporting and referral process for uncertain or unusual findings.

Titre adds another dimension. A high titre may carry different weight from a low titre in a particular setting, but no titre is a universal disease certificate. The endpoint must be read using the defined method, and longitudinal comparison should account for method changes and reader variability. A one-dilution difference may not support the same inference as a large reproducible change.

17.2 Antigen-specific testing refines, but does not replace, context

Tests for antibodies to double-stranded DNA, Sm, Ro, La, RNP, centromere, topoisomerase and other targets can help answer specific questions. Their sensitivity, specificity and numerical scales depend on the method. A name shared by two assays does not guarantee equivalent antigen presentation or clinical performance.

A clinically unexpected isolated positive should trigger consideration of pretest probability, cross-reactivity and assay limitations. An alternative method may contribute useful information, but simply ordering every available platform can create additional discordance without a clear plan. The investigation should state what hypothesis each additional test is intended to examine.

A negative antigen-specific result can also be meaningful without being absolute. The disease may occur without that antibody, the antibody may be below detection, or the assay may not capture the relevant response. The report should not expand a narrow negative into "no autoimmune disease."

17.3 ANCA and antigen specificity

Antineutrophil cytoplasmic antibody investigations include antigen-specific assays, commonly directed at proteinase 3 and myeloperoxidase, and immunofluorescence methods used in appropriate settings. Pattern terminology and antigen specificity are related but not identical. The clinical question may concern a small-vessel vasculitic syndrome, but positive ANCA-related findings can occur in other circumstances.

The laboratory should interpret the result with the clinical phenotype and the applicable diagnostic pathway. A low-level unexpected result has different significance from a strong finding in a patient with a compatible presentation, but neither should be treated as a substitute for the complete clinical evaluation. Medication, infection and other factors can complicate interpretation. The book does not direct treatment from an isolated ANCA result.

17.4 Neuromuscular and endocrine questions

Autoantibodies can interfere with receptors, mark tissue-directed immune responses or identify susceptibility. In neuromuscular investigations, assays may detect binding, blocking or modulating effects through different designs. In endocrine disease, a receptor antibody may be stimulating or inhibitory, while other antibodies may primarily support identification of an autoimmune process.

The functional state of the organ must be assessed through the appropriate clinical and biochemical measurements. Repeating an antibody titre is not automatically the best way to monitor disease activity. The relationship between antibody concentration and clinical response varies by condition and method. The testing plan should be justified by evidence for the specific use.

17.5 Antiphospholipid investigations require method awareness

A lupus-anticoagulant investigation examines clotting behaviour through a defined sequence, while anticardiolipin and anti-beta-2-glycoprotein I assays measure selected antibody binding. The tests contribute different evidence. Anticoagulants and other factors can interfere with clot-based methods, and solid-phase assays have their own analytical limitations.

Persistent laboratory findings and relevant clinical events are evaluated within an established diagnostic or classification framework. A positive result obtained during acute illness may be transient. The laboratory must not erase the need for a time-separated observation by repeating the same specimen several times on one day. Analytical reproducibility and biological persistence answer different questions.

17.6 Trends and meaningful change

A trend is interpretable only when the compared results are sufficiently comparable. Changes in platform, calibration, antigen preparation or reporting units can create discontinuities. Where monitoring is clinically useful, the laboratory should support continuity or explain the transition. The patient should not be told that disease activity doubled simply because the numerical scale changed.

The interval between samples also matters. Some markers change more slowly than symptoms or organ function. A stable antibody result does not prove that the patient is clinically stable, and a change does not automatically justify treatment adjustment. Laboratory information belongs within a broader decision, not above it.

17.7 Writing a useful interpretive comment

A good comment is specific enough to influence safe interpretation. It may explain that an isolated low-level result has limited specificity, that the panel covers selected antigens, that anticoagulant interference limits a clot-based result, or that an observed pattern suggests a defined follow-up question. It should not become a long list of every possible disease associated with the antibody.

The report must also preserve the difference between observation and recommendation. The laboratory can explain the evidence and suggest appropriate specialist correlation within its role. It should not issue an unsupported diagnosis or prescribe therapy from a pattern alone. Professional restraint is part of analytical quality.

Reading fluorescence patterns without turning them into diagnoses

Indirect immunofluorescence on an appropriate cellular substrate reveals the distribution of antibody binding. Nuclear, cytoplasmic and mitotic structures can produce recognisable patterns. The appearance depends on substrate preparation, fixation, dilution, microscope settings and reader experience. A pattern is therefore a method-dependent observation, not an unmediated photograph of the patient's disease. [FA10]

International pattern terminology improves communication, but it does not make every pattern uniquely specific for one antigen or disorder. A pattern can suggest useful follow-up testing, while antigen-specific assays evaluate selected targets. Mixed patterns may be present, and a dominant signal can obscure another component. The laboratory should avoid forcing every complex image into one convenient category.

A homogeneous nuclear pattern, for example, directs attention to a set of possible antigen associations but does not establish a single specificity by itself. Speckled patterns encompass several appearances and associations. Centromere-associated patterns can be recognisable, but the clinical interpretation still requires the relevant context. Synthetic teaching illustrations in this book are labelled as pattern models; they are not diagnostic reference micrographs and should not be used to validate a laboratory's competency programme.

Antiphospholipid investigations also require distinction between classification frameworks and clinical diagnosis. Persistence of selected antibodies over an appropriate interval is important in established frameworks, but a single positive result during an acute illness does not automatically establish a persistent syndrome. Functional coagulation testing and immunoassays answer different questions, and treatment or preanalytical factors can affect some components of the investigation. [FA11]

Coeliac serology provides another example of method and context. IgA-based testing must be interpreted with awareness of IgA

deficiency and gluten exposure. IgG-based approaches have a role in specified circumstances; they are not simply interchangeable alternatives for every patient. Testing after substantial dietary restriction can reduce the information available. The laboratory should communicate these conditions clearly so that a negative result is not overinterpreted. [FA15]

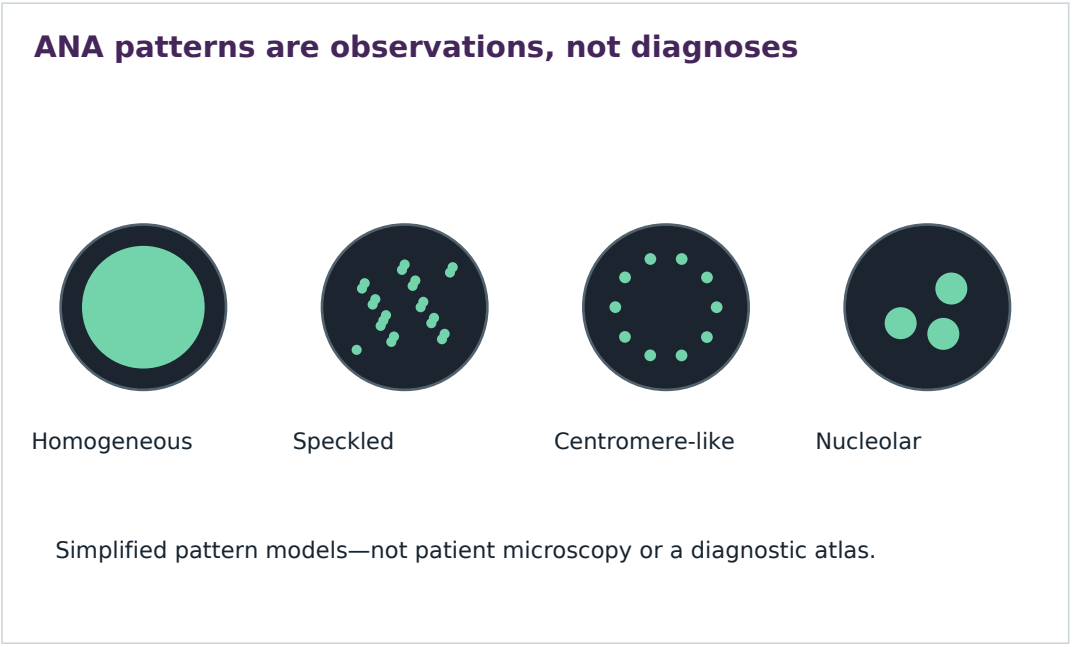


Figure FA17. Synthetic nuclear-pattern models: homogeneous, speckled, centromere-like and nucleolar. These simplified drawings are not diagnostic HEp-2 reference images. Pattern, titre, antigen-specific tests and clinical context must be integrated. Original CDIL educational schematic; not patient data.

Worked cases 65–68

Case 65 — Pattern is not a unique diagnosis

A fictional HEp-2 study shows a speckled nuclear pattern. A learner selects one disease from a memorised list and reports it as confirmed.

Reasoned answer. A pattern can guide a focused investigation but is not uniquely diagnostic. The titre, antigen-specific testing, clinical phenotype and method limitations matter. Mixed or uncertain patterns may require further review. The report should state the observed pattern using the laboratory's terminology and provide a

bounded interpretation rather than a disease label unsupported by the full evidence.

Case 66 — Repeating one sample does not show persistence

A single antiphospholipid-positive specimen is tested three times on the same day. All repeats are reactive. A student says the required persistence has now been proved.

Reasoned answer. The repeats demonstrate analytical reproducibility of that specimen under those conditions. They do not show that the finding persists over the required clinical interval. A time-separated specimen and the relevant clinical framework are still necessary. The distinction between repeatability and persistence must remain visible in the report.

Case 67 — A changed platform

A fictional anti-dsDNA monitoring programme changes methods. The new assay uses a different numerical scale and gives a higher value than the previous platform.

Reasoned answer. The numbers cannot be interpreted as a biological increase without appropriate comparability evidence. Antigen presentation, calibration and reporting scale can differ. The laboratory should explain the method transition and support a new baseline where appropriate rather than invent a conversion from one paired sample.

Case 68 — A negative selected panel

A myositis-related panel is negative, but it does not include every known target and the patient has a concerning clinical phenotype.

Reasoned answer. The negative result applies to the included targets and method. It does not exclude all inflammatory muscle disease or all relevant antibodies. Further assessment should be directed by the clinical team and laboratory expertise. Expanding the negative claim beyond the panel's scope would be an interpretive error.

18 | Complement Testing, Consumption and Angioedema Investigation

The complement system can be investigated through concentrations, functional pathways, activation products and selected regulatory proteins. These tests overlap, but they are not interchangeable. The analytical plan should distinguish a suspected inherited defect, active consumption, treatment effect or regulatory problem. A result without that question can be difficult to interpret and easy to overstate. [FA07, FA28]

18.1 Choosing the first measurement

A clinical history of recurrent invasive infection with particular organisms may lead to functional pathway testing. A question about immune-complex activity may involve selected concentrations and clinical trends. Recurrent angioedema without the expected features of a typical histamine-mediated reaction can raise a different regulatory question. The appropriate selection depends on the phenotype and current clinical guidance.

A panel containing only C3 and C4 cannot assess every complement component. Normal concentrations do not exclude a terminal-pathway defect, and low values do not name the underlying cause. Functional assays provide additional information by testing whether a sequence can perform under the assay's conditions. They still require suitable specimens and controls.

18.2 Reading combined patterns

The simplified CH50/AH50 pattern described earlier is a useful starting point. A selective reduction can direct attention toward a pathway-specific component, while reduction of both can suggest a shared component, terminal sequence, consumption or preanalytical loss of function. The exact interpretation must consider the assay's design and the patient's circumstances.

Component concentrations can refine the picture but should not be treated as perfect substitutes for function. A protein can be present but defective, and a concentration can remain within an interval despite a clinically relevant change from the patient's usual value. Repeat testing under appropriate conditions can help establish reproducibility, while targeted investigations examine the suspected mechanism.

18.3 Preanalytical control is central

Complement activation and degradation can continue outside the patient. Different tests require different specimen types and handling conditions. A specimen intended to measure activation products may need conditions that prevent ex-vivo activation, while a functional assay needs preservation of the components required for its reaction. The receiving laboratory's validated instructions govern collection and transport.

The report should distinguish a biological abnormality from an uninterpretable specimen. When handling was unsuitable, an honest limitation and timely recollection may prevent a false diagnosis. A low functional result from an unvalidated sample should not become a permanent label of inherited deficiency in the patient's record.

18.4 Consumption and synthesis occur together

A measured concentration reflects several processes: production, distribution, activation, consumption and loss. During inflammation, synthesis of some proteins can increase while consumption also occurs. The final concentration is the net result, not a direct meter of one process. A normal result therefore does not prove that no activation is taking place.

Serial results can be useful when the clinical question and method support monitoring. They should be compared with the patient's prior state, disease activity, treatment and other laboratory findings. The laboratory should avoid a universal statement that a specified percentage fall proves a flare or that a normal C3 excludes immune-complex disease. Such claims require condition-specific evidence.

18.5 C1 inhibitor: concentration and function

C1-inhibitor assessment illustrates why two different tests may be needed. An antigenic assay measures the amount recognised by the reagent. A functional assay measures activity in a defined system. A normal amount with reduced function can be clinically important. The pattern must be interpreted with the patient's presentation and relevant additional investigations.

Bradykinin-mediated angioedema differs mechanistically from many histamine-mediated reactions. The distinction has clinical implications, but laboratory testing should not delay emergency management of airway compromise. The treating team determines immediate care and the appropriate diagnostic pathway. The laboratory supports the mechanism-based investigation and communicates relevant limitations.

18.6 Complement-targeted treatment and assay effects

A drug that blocks a complement component can alter functional tests intentionally. The assay may detect target inhibition, residual activity or another property depending on its design. The medication history and timing are necessary to interpret the result. A value obtained during treatment should not be compared with an untreated reference model without recognising the intervention.

Some tests may also be affected analytically by therapeutic material or sample processing. The laboratory must distinguish a pharmacodynamic effect from assay interference where the distinction matters. The investigation should use the drug's current information and the assay's validated performance rather than a generic statement that all complement inhibitors produce the same laboratory pattern.

18.7 A worked pattern comparison

Consider three synthetic profiles. Profile A has markedly low CH50, preserved AH50 and reproducible findings in properly handled samples. Profile B has both functional results low, with a documented transport problem. Profile C has low activity after a known complement-blocking therapy. The numerical pattern alone is insufficient to treat all three patients as having the same inherited defect.

Profile A directs a pathway-focused investigation. Profile B requires resolution of specimen suitability before confident biological interpretation. Profile C must be interpreted in relation to therapy. The example shows why a laboratory algorithm needs clinical and preanalytical branches, not merely a set of arrows from numbers to disease names.

A pathway-pattern matrix with an explicit uncertainty branch

Imagine three fictional specimens. Specimen A has low classical-pathway activity with preserved alternative-pathway activity. Specimen B has low activity in both. Specimen C has normal pathway activity despite a clinical question about intermittent consumption. The patterns guide different investigations, but none is a final diagnosis. [FA07, FA28]

For specimen A, possibilities include defects or consumption affecting components more specific to the classical pathway, depending on the complete profile and specimen quality. For specimen B, shared components, terminal-pathway problems, substantial consumption, treatment effects or degradation may be relevant. For specimen C, a normal result at one time point does not exclude every intermittent or tissue-localised process. The clinical timing and the assay's scope remain important.

C3 and C4 concentrations add information but do not make the pattern self-explanatory. Low concentrations can reflect consumption or reduced production, while normal concentrations may occur despite particular functional defects or compensating production. A

pathway assay and a concentration assay should be interpreted as complementary evidence. The laboratory should avoid converting a simple two-test pattern into a unique genetic diagnosis.

Angioedema investigation may include C4, C1-inhibitor concentration and C1-inhibitor function, with additional tests according to the clinical setting. A normal concentration does not exclude functional impairment. Acquired and hereditary mechanisms can require different consideration, and other forms of angioedema may occur with different laboratory patterns. The appropriate specialist framework is essential. [FA28]

Specimen handling can mimic disease. Complement components are labile, and ex vivo activation or degradation can alter functional results. A poorly handled specimen should not be rescued by attaching a disease label. Where quality is uncertain, recollection and controlled processing may provide more useful information than adding many expensive component assays to an unreliable starting sample.

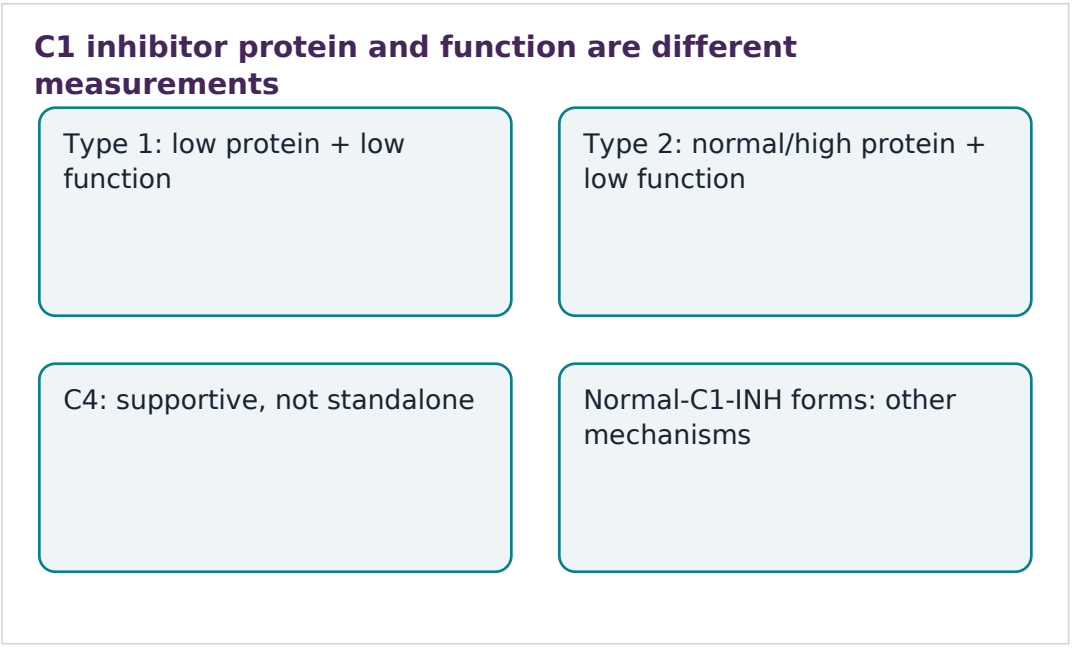


Figure FA18. Type 1 and type 2 C1-inhibitor deficiency patterns are simplified. C4 alone is insufficient, and normal-C1-inhibitor forms require a different investigation. Confirm abnormal results in the appropriate clinical context. Original CDIL educational schematic; not patient data.

Worked cases 69–72

Case 69 — Normal C3 and C4 do not test everything

A fictional patient has repeated invasive Neisseria infections. C3 and C4 concentrations are within the laboratory intervals. A learner concludes that every complement defect has been excluded.

Reasoned answer. Those concentrations do not evaluate every component or the complete functional pathways. The clinical history may justify further specialist-directed functional and component testing. The normal results are valid observations within their scope, not a comprehensive exclusion of complement-related susceptibility.

Case 70 — A transport-related low result

Two specimens from the same fictional patient differ markedly in functional complement activity. The first experienced unsuitable transport; the second met the validated conditions and was tested with acceptable controls.

Reasoned answer. The handling difference is a plausible explanation that must be investigated. The suitable specimen provides a stronger basis for biological interpretation, but the laboratory should document the discrepancy and resolution rather than silently deleting the first result. The case teaches traceability as well as preanalytical reasoning.

Case 71 — Normal amount, low activity

A C1-inhibitor antigenic concentration is normal while function is reduced in a suitable specimen. A student reports the investigation as entirely normal because the protein is present.

Reasoned answer. Presence does not guarantee effective function. The combined pattern may be important in the appropriate clinical setting and requires the relevant confirmatory and specialist assessment. The report should preserve both measurements and their different meanings rather than allowing one to cancel the other.

Case 72 — A treatment-associated pattern

A functional complement result is suppressed after a documented complement-targeted therapy. The laboratory is asked to compare it directly with an untreated population interval.

Reasoned answer. The intervention changes the question. Interpretation must consider the drug target, timing and validated monitoring method. Suppression can reflect intended activity, although the clinical significance and adequacy of treatment require the prescribing team's assessment. The report should not present the treated result as an unexplained spontaneous defect.

19 | Biologics, Therapeutic Antibodies and Laboratory Interference

Immune-targeted treatments can change the patient, the assay or both. A falling cell population may be an intended pharmacological effect. A new protein band may be the therapeutic antibody itself. A broadly reactive compatibility test may reflect drug binding to reagent cells. Distinguishing these possibilities requires a treatment history and an understanding of the measurement system. [FA20, FA23, FA29]

19.1 Three different questions about a drug

Drug concentration, target engagement and clinical response are not the same measurement. A concentration assay estimates exposure under its validated definition. A functional assay may assess inhibition or another downstream effect. Clinical response involves the patient's condition and cannot be reduced automatically to either laboratory result.

Anti-drug antibody testing adds another question: whether the patient has a detectable immune response against the therapeutic product under the assay's conditions. Such antibodies may be binding or neutralising, transient or persistent, and clinically important or not. A positive result does not have one universal implication across all drugs and methods.

19.2 Drug tolerance and sample timing

Circulating drug can interfere with detection of anti-drug antibodies by occupying binding sites or forming complexes. An assay's drug tolerance describes an aspect of its ability to detect antibodies in the presence of drug under specified conditions. It is not an unlimited guarantee. Timing relative to dosing can therefore influence interpretability.

A result obtained at an unspecified point in the dosing interval may not be comparable with a properly timed monitoring specimen. The laboratory should obtain the required timing and preparation information. It should not invent a universal trough schedule or treatment target for every biologic. Those details are drug- and indication-specific and must come from current clinical guidance and validated laboratory procedures.

19.3 Therapeutic antibodies can be measured as proteins

A therapeutic monoclonal antibody is a protein that may be detected by methods used to investigate endogenous immunoglobulins. In serum electrophoresis or immunofixation, it can produce a restricted feature that complicates assessment of the patient's own protein. The effect depends on the drug, concentration, migration and assay.

The laboratory may use a validated drug-specific shift, removal or alternative analytical strategy where available. The aim is to distinguish exogenous therapy from endogenous material without concealing a genuine patient abnormality. A treatment history and comparison with earlier patterns are essential. The correct conclusion is based on the evidence, not on the assumption that every new band during therapy is harmless.

19.4 Anti-CD38 interference in blood-bank testing

Daratumumab and related anti-CD38 therapies can interact with CD38 on reagent red cells and produce broad indirect antiglobulin reactivity. This can obscure clinically significant red-cell antibodies. The phenomenon does not mean that every donor unit is biologically incompatible through a new alloantibody, nor does it mean that the laboratory can ignore the reactivity. A validated interference-resolution pathway is needed. [FA29]

Dithiothreitol treatment is one established approach in appropriate laboratories, but it changes other red-cell antigens as well. Kell-related limitations are especially important. A method that removes the drug's interference can also remove information needed to exclude another antibody. The laboratory must account for what

the treatment destroys, not celebrate a negative result without considering the new blind spots.

19.5 Biotin and architecture-dependent effects

Biotin can interfere with some assays that use biotin–streptavidin interactions. The direction of effect depends on architecture. In many susceptible sandwich designs, excess biotin can cause falsely low results; in many susceptible competitive designs, it can cause falsely high results. These are common patterns, not universal predictions for every assay sold under those categories. [FA20]

The medication and supplement history should include products used for hair, skin or nails as well as prescribed high-dose biotin. A laboratory must use the actual assay's interference information and an appropriate clinical plan. It should not advise every patient to stop all supplements for a universal number of hours, especially when doses, renal function and method susceptibility differ.

19.6 Biological effects versus analytical artefacts

A therapy can change immune-cell counts, immunoglobulin production, complement activity or inflammatory mediators through its intended or adverse biological actions. These are real changes in the patient. An analytical interference, by contrast, changes the relationship between the specimen and reported result without necessarily representing the inferred biological change.

The two can coexist. A drug may suppress a cell population while also interfering with an antibody assay. A structured investigation asks what changed biologically, what the assay detects and whether the result is consistent across methods measuring different properties. The answer may require collaboration between the laboratory, pharmacist and treating specialist.

19.7 Reporting and continuity

A report should identify clinically relevant interference or treatment effects clearly enough to prevent repeated confusion. Historical drug exposure can remain relevant after the last dose for a period that depends on the drug and assay. The laboratory's information system should retain important alerts according to policy rather than relying on the next clinician to remember every prior treatment.

The report must also avoid prescribing from an isolated laboratory result. Therapeutic targets, dosing changes and decisions to stop treatment require the current clinical framework and authorised professional judgement. The laboratory provides measured evidence, limitations and timely communication; it does not replace the prescribing decision.

A therapeutic antibody can be both treatment and analyte-like material

A therapeutic monoclonal antibody has a defined molecular target, but its presence can influence tests beyond the intended pharmacological effect. It may bind a structure on reagent cells, contribute to an electrophoretic pattern or interfere with an immunoassay through the architecture of the measurement. The laboratory should distinguish a biological treatment effect from direct analytical interference. [FA23, FA29]

Daratumumab targets CD38 and can interfere with indirect antiglobulin testing because reagent red cells express CD38 to a degree sufficient for the interaction. The resulting broad reactivity can conceal clinically significant alloantibodies. Treatment history and a pretreatment baseline are therefore important. The interference does not mean that ABO and RhD grouping are generally invalid in the same way; each test must be considered according to its mechanism.

Dithiothreitol treatment can reduce this interference by altering susceptible surface structures, but it also destroys or weakens other relevant antigens, notably Kell-system antigens. A negative result with treated cells cannot exclude antibodies to antigens removed by

the treatment. Component selection must account for this blind spot through the authorised transfusion-laboratory pathway. The treatment is not a universal cleansing step that leaves all other antigen information unchanged. [FA29]

Therapeutic antibodies can also be measured as part of pharmacokinetic monitoring, and anti-drug antibodies may be investigated. Drug-tolerant and drug-sensitive assay designs differ in their ability to detect anti-drug antibodies in the presence of circulating treatment. A negative anti-drug-antibody result may therefore depend on the timing of collection and assay design. It should not be interpreted without knowing whether the drug itself can mask the response.

The practical safeguard is structured treatment metadata. The name of the drug, last administration, start date and relevant pretreatment results should be available to the laboratory. A generic note saying "on immunotherapy" may be insufficient because different products produce different analytical problems. Accurate medication history can prevent unnecessary investigation and, in transfusion settings, reduce dangerous delays.

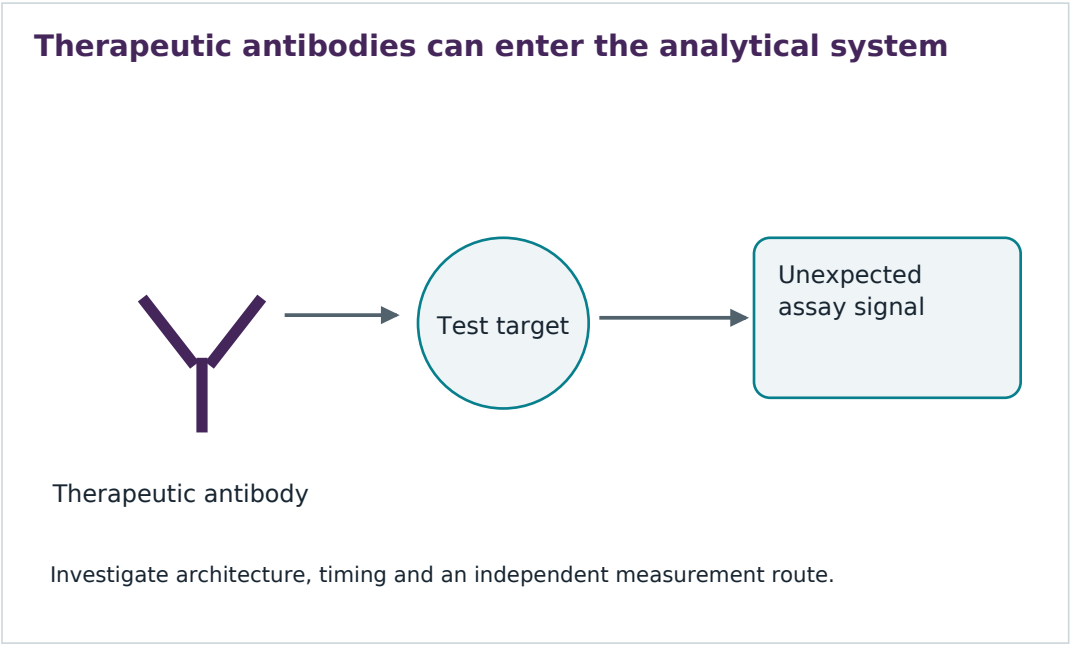


Figure FA19. A therapeutic antibody can bind a test-system target and create a signal unrelated to the intended patient analyte. The mechanism and corrective experiment are assay-specific. Original CDIL educational schematic; not patient data.

Worked cases 73–76

Case 73 — Negative anti-drug antibodies during high drug exposure

An anti-drug antibody assay is negative in a specimen containing drug above the method's validated tolerance. A learner concludes that the patient has never formed anti-drug antibodies.

Reasoned answer. The assay may not be able to detect relevant antibodies reliably under those conditions. The report must reflect the limitation and the applicable monitoring pathway. A properly timed or differently investigated specimen may be needed. "Not detected under these conditions" is narrower than "biologically absent at all times."

Case 74 — Panreactivity after anti-CD38 therapy

A fictional patient begins anti-CD38 treatment and subsequently has broad indirect antiglobulin reactivity. No such pattern was present before therapy.

Reasoned answer. The drug is a plausible source of interference, but clinically significant alloantibodies must still be considered. The laboratory should use its validated resolution pathway and relevant historical phenotype or genotype information. If a treatment such as DTT is used, its antigen-destroying effects and corresponding component-selection implications must be addressed. The negative appearance after treatment is not an unrestricted all-clear.

Case 75 — Opposite biotin effects

Two susceptible assays use different architectures. One is a sandwich assay and one competitive. A learner expects excess biotin to move both reported values in the same direction.

Reasoned answer. The direction can differ because biotin disrupts different signal relationships. The actual manufacturer's design and interference data govern interpretation. An architecture-based hypothesis is useful, but it must be verified with method-specific information rather than treated as a universal rule. [FA20]

Case 76 — A therapeutic band

A fictional patient's immunofixation shows a small IgG-kappa feature after treatment with an IgG-kappa monoclonal antibody. What evidence is needed before calling it residual or recurrent endogenous disease?

Reasoned answer. The treatment identity, timing, expected analytical behaviour, prior patient pattern and an appropriate interference-resolution method may be needed. The band could be exogenous, endogenous or a combination. Its presence alone does not establish the source. The laboratory should make the uncertainty specific and investigate it rather than issue an unsupported disease conclusion.

20 | Transplant Immunology and Histocompatibility Reasoning

Transplantation brings tissues with one set of antigens into a recipient whose immune system has its own history and repertoire. Compatibility assessment therefore involves donor characteristics, recipient antibodies and cells, prior sensitisation, the organ or tissue involved and the transplant programme's clinical framework. A single result labelled "match" cannot describe all these dimensions. [FA05, FA30]

20.1 The direction of recognition

The recipient can recognise donor antigens through several mechanisms. In solid-organ transplantation, recipient responses against the graft are central concerns. In haematopoietic cell transplantation, donor-derived immune cells can also react against recipient tissues, contributing to graft-versus-host disease. The direction of immune recognition matters and changes the meaning of compatibility discussions.

Direct, indirect and related routes of allorecognition describe how donor-associated material is encountered by recipient T cells. In a direct route, recipient T cells recognise donor MHC-associated structures on donor cells. In an indirect route, recipient antigen-presenting cells process donor material and present peptides through recipient MHC. These models explain why graft immune risk cannot be reduced to one immediate crossmatch result.

20.2 Sensitisation and antibody history

Pregnancy, transfusion and previous transplantation can expose a person to nonself HLA or other antigens and support sensitisation. The resulting antibody repertoire may change over time. A currently weak or undetectable antibody does not erase a clinically relevant historical response. The transplant programme must decide how current and historical evidence are incorporated into risk assessment.

The laboratory should distinguish a broad measure of sensitisation from identification of antibodies directed at a particular donor. A sensitisation percentage or similar summary does not prove that every possible donor is incompatible. Donor-specific assessment requires the donor's actual antigen information and the recipient's relevant antibody findings.

20.3 Antibody assays and MFI

Solid-phase assays can provide detailed information about binding to HLA targets. Mean fluorescence intensity is often used in interpretation, but it is not a universal antibody concentration or a universally transferable risk threshold. Antigen density, bead characteristics, interference, complement effects and assay conditions can influence the signal.

A high signal may require investigation, but magnitude alone cannot determine every clinical consequence. A low signal may also be important in a specific historical or donor-related context. The laboratory should use the programme's validated interpretation framework, including any dilution or interference-resolution procedures. A number without its analytical context is an incomplete description.

20.4 Physical and virtual crossmatches

A physical crossmatch tests recipient material against donor cells through a specified method. Complement-dependent cytotoxicity and flow-cytometric approaches measure different aspects of reactivity. A virtual crossmatch uses donor typing and recipient antibody information to infer compatibility under the programme's rules. It depends on the completeness and reliability of those inputs.

Discordance between methods can be informative. It may reflect differences in sensitivity, antibody class, target expression, interference or the biological property measured. The correct response is not automatically to select the method with the strongest reaction. The team must understand what each result adds and what it cannot establish.

20.5 ABO and non-HLA considerations

ABO compatibility can be relevant, but its role differs across organ types and transplant strategies. A laboratory should not transfer a red-cell transfusion compatibility table directly into every transplantation decision. Programmes may use specialised approaches for selected ABO-incompatible transplants, with defined risk assessment and monitoring.

Non-HLA antibodies and nonimmune factors can also contribute to graft outcomes. A reassuring HLA investigation does not exclude infection, ischaemic injury, drug toxicity or every immune mechanism. The laboratory report should remain bounded by the tests performed rather than claim that the entire graft is "safe" or "normal."

20.6 Monitoring after transplantation

Post-transplant testing may assess donor-specific antibodies, drug exposure, infection, organ function or other questions. Each measurement has its own time course and limitations. A new antibody finding is not automatically identical to clinically established rejection; integration with the graft's condition and the programme's diagnostic criteria is necessary.

Serial results should be compared using suitable methods and clinical timing. A platform change can alter apparent strength. Treatment can modify antibody concentrations or assay behaviour. The report should identify these limitations and support timely specialist communication when the pattern is clinically important.

20.7 A decision record that can be reconstructed

A defensible compatibility record includes donor and recipient identity, specimen dates, typing resolution, antibody methods, relevant history, crossmatch approach, interpretation and authorisation. If a decision is made under urgent or exceptional circumstances, the rationale and limitations should be recorded through the programme's procedure.

This record is not merely administrative. Transplant decisions can depend on information assembled from different laboratories and times. A missing donor locus, an outdated recipient specimen or an unrecognised historical antibody can change the interpretation. Traceability is therefore part of the scientific validity of the conclusion.

Virtual, cellular and solid-phase crossmatches answer different questions

A virtual crossmatch combines donor antigen information with the recipient's known antibody profile. It can support timely decisions, but its reliability depends on the completeness, resolution and currency of both datasets. A recipient's antibody profile can change after sensitising events, and donor typing may not resolve every relevant allele. The word virtual does not mean speculative; it means that the interpretation is constructed from data rather than a direct donor-cell mixing test. [FA05, FA30]

A cellular crossmatch examines interaction with donor cells under a defined method. Complement-dependent cytotoxicity and flow-cytometric approaches have different analytical properties and detect different aspects of the interaction. Cell viability, target populations, treatment effects and non-HLA antibodies can influence results. A positive result should be interpreted within the full

histocompatibility investigation, not treated as an unexplained binary prohibition without context.

Solid-phase antibody assays use selected antigen preparations. Their signal can be influenced by antigen density, conformation, interference and assay conditions. Mean fluorescence intensity is not a universally calibrated antibody concentration. A threshold adopted by one programme cannot automatically be transferred to another platform, population or clinical decision. Dilution and other validated investigations may help when interference or saturation is suspected.

Donor-specific antibody means that the detected specificity corresponds to an antigen carried by the donor under the interpretation used. It is not a synonym for every anti-HLA antibody in the recipient. The distinction depends on adequate donor typing and correct assignment of antibody specificity. A broad antibody screen without donor information cannot establish donor specificity by itself.

Transplant direction also matters. Recipient immune responses against a graft differ from graft-derived immune responses against the recipient after haematopoietic transplantation. Graft-versus-host disease is not simply organ rejection with the names reversed; the participating cell populations and tissues differ. A clear diagram should show which immune system is acting on which target so that the learner does not confuse the two mechanisms.

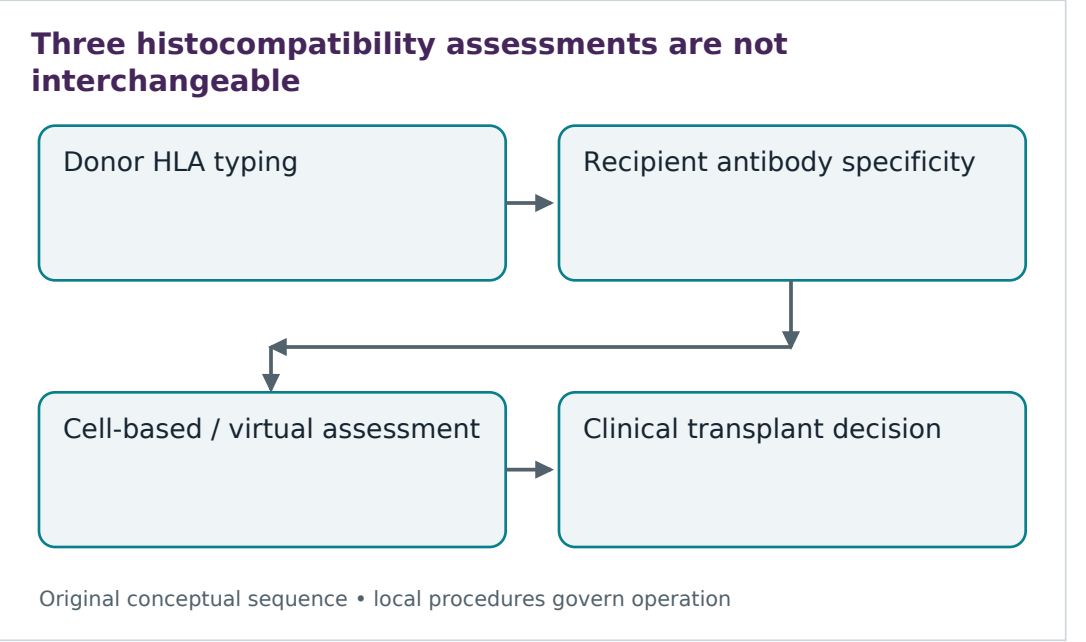


Figure FA20. Cell-based cytotoxicity, flow crossmatch and virtual crossmatch use different evidence. The transplant team integrates donor typing, antibody specificity, method limitations and the clinical plan. Original CDIL educational schematic; not patient data.

Worked cases 77–80

Case 77 — High sensitisation is not donor specificity

A fictional recipient has a high sensitisation summary, but the proposed donor lacks the antigens targeted by the documented antibodies. A student declares incompatibility solely from the summary percentage.

Reasoned answer. The summary describes the breadth of sensitisation under a defined framework, not the specific relationship with every donor. Donor-specific assessment requires the actual donor type, recipient antibody history and programme rules. The case does not declare the transplant acceptable; it shows why a population summary cannot replace an individual comparison.

Case 78 — Missing donor information

A virtual crossmatch is proposed, but the donor's relevant HLA typing is incomplete at a locus needed to assess a recipient antibody.

Reasoned answer. The missing information limits the inference. The laboratory should obtain the necessary typing or use the programme's appropriate alternative pathway. A virtual crossmatch is not an independent physical test that can compensate for absent inputs. Its reliability depends on the data and rules used.

Case 79 — MFI is not a universal concentration

Two laboratories report different fluorescence values for a recipient antibody using different systems. A learner converts the ratio directly into a fold change in antibody concentration.

Reasoned answer. The values are not necessarily quantitatively interchangeable. Calibration, antigen presentation and analytical conditions differ. The comparison requires method-specific evidence and the transplant programme's interpretation. A signal ratio alone does not establish a biological concentration ratio or a universal risk category.

Case 80 — Antibody and rejection are different observations

A post-transplant specimen shows a new donor-specific antibody. A student reports biopsy-proven rejection even though no tissue assessment or complete clinical evaluation is available.

Reasoned answer. The antibody is an important finding that may require prompt specialist evaluation, but it does not supply evidence from a biopsy that has not occurred. Rejection assessment uses the programme's clinical and pathological framework. The report should state the antibody finding and its significance without inventing a completed diagnosis.

21 | Tumour Immunology and Immune Monitoring

A malignant cell is a living host cell that has acquired properties allowing abnormal survival, growth and spread. It is not simply a foreign microorganism that can be removed without regard to the surrounding tissue. Immune recognition of a tumour therefore takes place in a difficult environment: many target molecules are shared with healthy cells, tumour populations evolve, and the local tissue can inhibit an otherwise capable immune response. Laboratory measurements help describe this environment, but a marker is not a complete explanation of tumour behaviour. [FA31]

21.1 How a tumour becomes immunologically visible

Changes in a malignant cell can generate altered proteins whose peptides are displayed by HLA molecules. Some of these peptides may be recognised by T cells as neoantigens. Other targets are normal proteins expressed at an unusual level or in a different developmental setting. The distinction matters. A target that is genuinely restricted to malignant cells offers a different therapeutic opportunity from a target also expressed by essential healthy tissue.

Recognition depends on more than the presence of a mutation. The altered sequence must be expressed, processed and presented in a form that an available T-cell receptor can recognise. A tumour can carry many sequence changes without generating a strong, effective antitumour response. Equally, a tumour with fewer mutations can contain a particularly relevant antigen. Mutation burden, antigen presentation and immune activity are related but nonidentical measurements.

Tumour antigens may be taken up by dendritic cells and presented to T cells. This cross-presentation can help initiate a response against cells that do not themselves provide all the signals required to activate naive lymphocytes. At the tumour site, effective killing also depends on trafficking, access, receptor engagement and

maintenance of effector function. A blood count of lymphocytes cannot demonstrate that this sequence has occurred inside a tumour.

21.2 Escape and the tumour microenvironment

A tumour population is heterogeneous. Cells with different antigen expression, growth characteristics and resistance mechanisms can coexist. An immune response or treatment may eliminate susceptible cells while allowing other populations to expand. This selection helps explain why a target detected at diagnosis may be reduced or absent at relapse. A historical result remains part of the record, but it may not describe the present disease.

Escape can involve altered antigen presentation, inhibitory signalling, exclusion of immune cells, suppressive cell populations or metabolic conditions that impair effector function. These mechanisms can operate together. The phrase "the immune system failed" hides this complexity and wrongly suggests a single measurable defect. The relevant question is which barriers are operating in this patient, tissue and treatment setting.

A biopsy is also a sample. A section from one region does not necessarily represent every metastatic site or every part of a large lesion. Necrosis, fixation, tissue age and the proportion of viable tumour affect interpretation. An apparently negative marker may describe the sampled material rather than prove uniform absence throughout the patient. Reports should retain specimen identity, site, method and adequacy information.

21.3 Checkpoints and why inhibition can be protective

Immune checkpoints are regulatory pathways that help constrain activation and tissue injury. Therapeutic blockade of pathways such as PD-1/PD-L1 or CTLA-4 can enhance antitumour activity in selected circumstances. Removing inhibition is not equivalent to directing a response only at cancer. Immune-related adverse effects can involve healthy organs, and their investigation requires clinical assessment rather than an assumption that every new symptom is tumour progression. [FA31]

A checkpoint marker is interpreted within a specific treatment and assay framework. PD-L1 assays can differ in antibody reagent, platform, scoring method, cell populations counted and eligibility threshold. A tumour-proportion score and a combined-positive score are not interchangeable labels for the same calculation. A laboratory should not transfer a threshold validated for one tumour type, drug or scoring system to another merely because the marker name is the same.

Predictive and prognostic roles also differ. A predictive biomarker concerns the likelihood of benefit or response under a particular intervention. A prognostic biomarker concerns outcome in a defined clinical context, whether or not a particular treatment is given. A marker can have both roles, neither role, or a role that is established only in a specified population. The report must not claim more than the relevant evidence supports.

21.4 Cellular therapies and changing analytical patterns

Chimeric antigen receptor T-cell therapy provides engineered recognition that is not identical to conventional peptide–HLA recognition. The receptor design, target and cell product determine its behaviour. Laboratory monitoring may include cell populations, disease-associated markers and safety investigations, but the required tests and timing belong to the authorised treatment programme.

Therapy can change the cells and proteins that a laboratory expects to observe. A target-positive population may decrease, while residual disease may have altered expression. B-cell-directed treatment can affect normal B cells as well as malignant populations. A low circulating B-cell count after treatment is therefore not interpreted in the same way as an unexplained low count before treatment. The temporal relationship to therapy is essential metadata.

Cytokine release and neurological toxicity are clinical syndromes that require urgent recognition in the appropriate setting. No single cytokine concentration should be presented as a universal diagnostic substitute for the clinical criteria used by the treating programme.

Laboratory staff contribute by producing timely, reliable results, communicating critical findings under local policy and recognising when a specimen belongs to a high-risk treatment pathway. They do not independently grade or treat the syndrome from an isolated assay.

21.5 Monitoring must distinguish biology from measurement

Serial measurements can be affected by changes in reagent lot, instrument, specimen type or analytical sensitivity. Suppose a fictional research assay reports a tumour-associated marker of 120 units before treatment and 70 units afterward. The apparent reduction is 50 units, or about 41.7% of the starting value. That arithmetic does not establish clinical response. The assay's precision, biological variability, sampling interval and relationship to validated response criteria must still be considered.

An immune-monitoring study should specify its endpoint before examining the results. A rise in activated T cells, a fall in tumour burden and improved survival answer different questions. Selecting whichever marker changed most after the study can create a misleading impression of success. The laboratory scientist should preserve the original measurement plan, document deviations and distinguish exploratory findings from validated decision tools.

Antigen escape and on-target effects in a changing tumour

A therapy directed at one antigen can select for cells with reduced or absent expression of that antigen. This is one mechanism of antigen escape. It does not prove that every relapse follows the same route, but it explains why reassessment of the target may be important. A diagnostic marker measured before treatment may no longer describe the residual population afterward. [FA31]

An on-target, off-tumour effect occurs when the intended target is also present on healthy tissue. The therapy may be recognising exactly what it was designed to recognise while still causing harm outside the malignancy. This differs from an unintended molecular

cross-reaction. The distinction is useful when interpreting both therapeutic benefit and adverse effects.

Tumour heterogeneity makes sampling important. A single biopsy can miss a target-negative population elsewhere. Liquid-biopsy, tissue and cellular measurements may provide complementary information, but none automatically represents the whole disease. The analytical sensitivity and biological compartment of each method should remain visible in the interpretation.

Immune-cell infiltration can also have different meanings. A tumour with many lymphocytes is not necessarily being effectively controlled. The cells may be excluded from critical regions, inhibited or directed at irrelevant targets. Conversely, a small but effective population may be important. Counting cells and assessing their functional state are different tasks.

Clinical response criteria therefore integrate more than an immune-marker panel. Imaging, pathology, symptoms and validated laboratory measures may all contribute. A textbook should teach the mechanism behind a biomarker while avoiding the claim that a single laboratory change proves success or failure of a complex therapy.

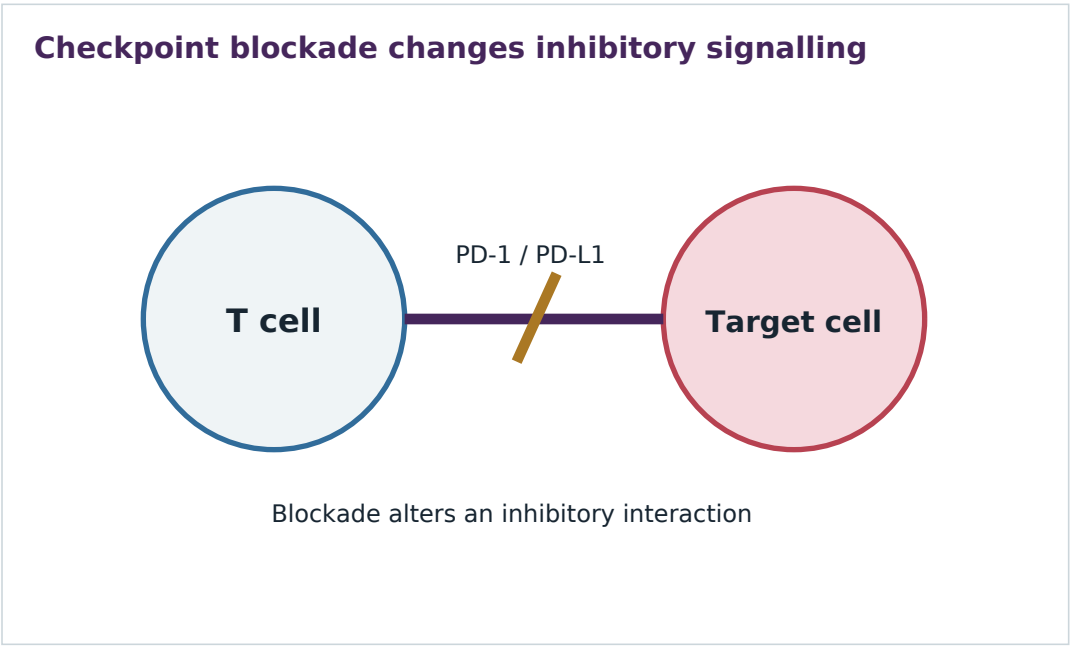


Figure FA21. PD-1–PD-L1 is shown as one inhibitory interaction between a T cell and a target cell. Blocking the interaction can alter immune activity; it does not guarantee tumour response or eliminate immune toxicity. Original CDIL educational schematic; not patient data.

Worked cases 81–84

Case 81 — A score transferred between systems

A learner sees a PD-L1 result from one scoring system and applies the eligibility threshold used for a different tumour and treatment. The numerical values look similar.

Reasoned answer. Similar numbers do not establish equivalent scoring. The counted cell populations, denominator, assay and clinical validation may differ. The correct action is to identify the exact score, method and intended treatment framework, then use the authorised interpretation for that combination. A laboratory report should not convert between systems without a validated basis. This is an example of a decision threshold belonging to a method and clinical question, not merely to an analyte name.

Case 82 — Negative blood, persistent tissue disease

A patient has few detectable abnormal cells in peripheral blood, but a tissue investigation demonstrates persistent disease. A student calls the findings contradictory.

Reasoned answer. Blood and tissue sample different compartments. A negative or low-level blood result does not prove that all tissue sites are free of disease. Differences in analytical sensitivity and specimen adequacy may add further explanation. The findings should be integrated rather than forcing one to invalidate the other. Monitoring plans must state which compartment is being assessed and what a negative result can reasonably exclude.

Case 83 — New symptoms during checkpoint treatment

A fictional patient develops diarrhoea after checkpoint therapy. The team considers infection, medication effects, tumour-related causes and immune-mediated inflammation. A trainee wants to label the symptom an immune adverse event before investigation.

Reasoned answer. The treatment creates a relevant possibility, not a diagnosis by timing alone. Competing causes may require different management and can coexist. Appropriate clinical assessment and targeted investigations are needed. The laboratory's responsibility is accurate testing and communication within the treatment pathway, not premature attribution. This case shows why knowledge of therapy should sharpen the differential rather than replace it.

Case 84 — A percentage without a response definition

A marker falls from 120 to 70 units. An analyst reports a 41.7% reduction and adds "treatment successful."

Reasoned answer. The percentage calculation is correct: 50 divided by 120 is approximately 0.417. The added clinical conclusion is not established by that calculation. A validated relationship between the marker, timing and response criteria is required. Analytical variation, specimen differences and other disease measures must be considered. The report can describe the measured change while reserving treatment-response classification for the appropriate evidence-based framework.

22 | Protein Electrophoresis, Immunofixation and Free Light Chains

Serum contains many proteins with different concentrations, structures and functions. Electrophoretic methods separate proteins according to their behaviour under defined analytical conditions, creating a pattern that can reveal broad changes or a restricted component. The pattern is not a photograph of an individual antibody clone. It is a measurement system whose interpretation depends on specimen quality, method, distribution and confirmatory investigations. [FA32]

22.1 Understanding the electrophoretic pattern

Serum protein electrophoresis commonly displays an albumin region and several globulin regions. The location and appearance of a component depend on the separation method. A sharp restricted component can occur in a region other than the gamma region, so inspection should not be limited to one familiar part of the trace. The laboratory should evaluate the entire pattern and relevant quality information.

A polyclonal increase generally reflects contributions from multiple immunoglobulin populations and often produces a broad distribution. A monoclonal component is associated with a more restricted population, but morphology alone does not establish its exact immunoglobulin type or clinical significance. A small clone can produce a clinically important protein, while a larger measured component may require a different interpretation. Quantity, identity and clinical effect must be considered separately.

The specimen matters. Plasma can contain fibrinogen that produces a component not expected in properly clotted serum. Incomplete clotting, haemolysis and other specimen problems can complicate interpretation. The report should not turn every discrete band into a

diagnosis of plasma-cell malignancy. When the pattern is unexpected, specimen type and preparation are part of the first investigation.

22.2 Immunofixation identifies a restricted component

Immunofixation combines separation with immunological recognition of selected heavy- and light-chain classes. A matching restricted component in appropriate lanes can support identification of an immunoglobulin type. The result should be described using the observed pattern and the validated interpretive rules for the method. Immunofixation is not simply a more sensitive quantitative version of electrophoresis; its principal role is characterisation.

The absence of a conventional heavy-chain band does not automatically establish that the patient has only free light chains. The sensitivity and panel coverage of the method, antigen excess, sample treatment and unusual proteins may need consideration. Similarly, two bands can reflect biclonality, oligoclonal responses, treatment-related patterns or other explanations. The clinical and treatment history is indispensable.

Therapeutic monoclonal antibodies can appear in electrophoretic or immunofixation investigations. Daratumumab is an important example because an administered IgG-kappa antibody may complicate assessment of an endogenous component. A laboratory should use the validated approach appropriate to the drug and method rather than interpreting the treatment-derived band as new or persistent disease by default. [FA23, FA29]

22.3 Free light chains require more than one number

Immunoglobulin-producing cells can release free kappa and lambda light chains. Assays quantify these proteins and commonly provide a ratio. A ratio is useful because it compares the two measurements, but it is not independent of them. A ratio can change because the numerator rises, the denominator falls, or both change. The actual concentrations must remain visible in the interpretation.

Kidney function affects the handling of free light chains. Reference intervals and interpretive approaches may therefore differ with renal function and analytical method. A single universal ratio range should

not be imposed on every platform or patient group. The laboratory must use the validated interval and clinical framework for its method. An abnormal ratio is evidence requiring interpretation, not a standalone diagnosis of malignancy.

A synthetic example illustrates the calculation. Kappa of 48 mg/L and lambda of 24 mg/L produce a kappa/lambda ratio of 2.0. Kappa of 8 mg/L and lambda of 4 mg/L also produce a ratio of 2.0. The identical ratios conceal very different concentrations. The clinical meaning cannot be recovered from the ratio alone, and neither example should be labelled normal or abnormal without the appropriate method-specific framework.

22.4 Quantification and interference

Densitometric estimation of a restricted component requires a defined integration approach. A component that overlaps another protein region may be difficult to quantify accurately. Changes in integration boundaries can create an apparent trend. Laboratories should maintain consistent methods, document limitations and avoid implying precision beyond what the separation supports.

Nephelometric or turbidimetric immunoglobulin measurements answer a related but different question: they estimate the concentration of a class or protein according to the assay design. A total IgG concentration is not necessarily identical to the quantity of a monoclonal IgG component. Polyclonal background and method-specific behaviour may contribute. Comparing unlike measurements as if they were interchangeable can produce false discrepancies.

Antigen excess is another concern in immunochemical measurement. Extremely high concentrations can lead to misleadingly low results in susceptible methods if the assay's safeguards do not adequately detect the condition. A result that conflicts strongly with the electrophoretic pattern should prompt a method-aware investigation, which may include appropriate dilution or an alternative method. Repeating the same undiluted sample without changing the analytical conditions may reproduce the error.

22.5 Following a patient over time

A meaningful serial interpretation compares the same measurand, method and reporting convention where possible. Changes in treatment can produce oligoclonal patterns or therapeutic-antibody interference. Changes in kidney function can influence light-chain concentrations. A laboratory should communicate these contextual limitations rather than reducing monitoring to a single rising or falling number.

The purpose of monitoring also matters. Screening for a monoclonal protein, characterising a newly detected component, and following a known disorder are different tasks. The most appropriate combination of serum, urine and other investigations depends on the clinical question and current specialist guidance. A negative result in one test does not automatically exclude every monoclonal-protein disorder.

Restricted proteins can injure without being abundant

A monoclonal protein's clinical importance depends partly on its biochemical properties. A relatively small quantity can have important effects if it deposits in tissue, interferes with physiological processes or has particular binding activity. Conversely, the presence of a larger component does not by itself determine the full clinical syndrome. Concentration and pathogenic potential are related only in context. [FA32]

The investigation may therefore combine electrophoresis, immunofixation, free-light-chain measurement and selected tissue or organ assessments. The laboratory should not promise that a negative result in one method excludes every disorder associated with a monoclonal protein. Sensitivity differs by protein type, concentration and specimen, and the clinical question determines the appropriate combination of tests.

A restricted band can migrate in a region that overlaps other proteins. This can complicate quantification and serial comparison. The laboratory should preserve a consistent integration approach and state when the component cannot be separated reliably from

background. A visually attractive number is not necessarily a valid measurement.

Urine investigations have their own collection and analytical considerations. A spot specimen, a timed collection and a serum measurement are not interchangeable. Incomplete timed collection can affect excretion estimates, while concentration methods can influence detection. The report must state the specimen and method rather than presenting all light-chain findings as if they were measured in the same way.

Finally, a clonal laboratory finding requires an appropriate clinical classification. It should not be converted automatically into the name of a malignancy without the necessary diagnostic evidence. The laboratory's strongest contribution is accurate characterisation, quantification where valid, and a clear explanation of what the pattern does and does not establish.

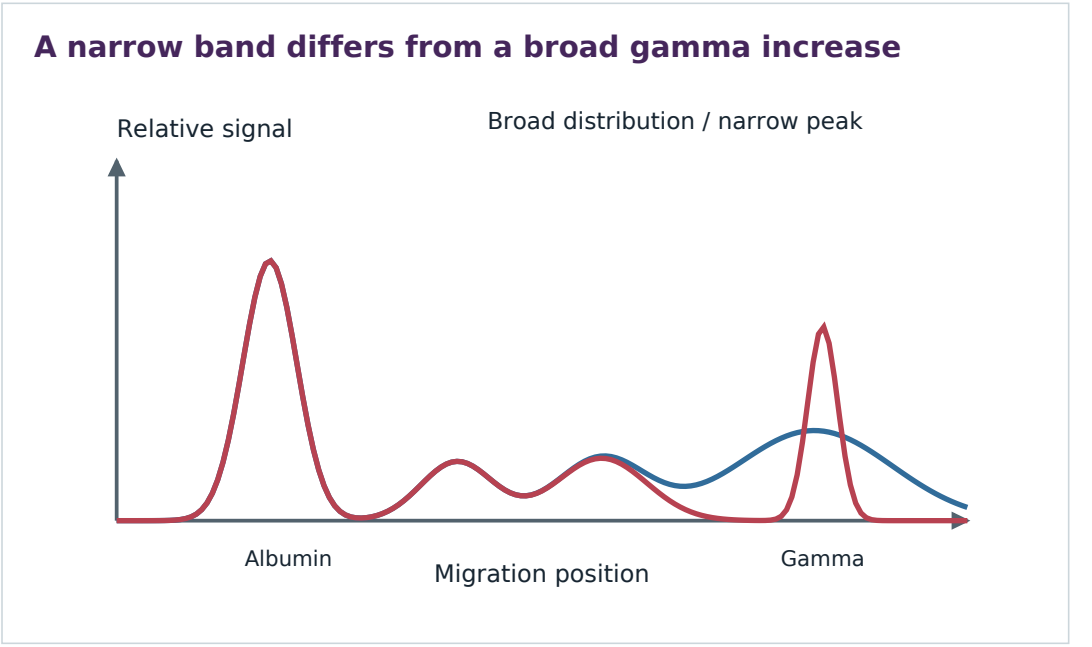


Figure FA22. Original synthetic electrophoretic profiles, not patient traces. A narrow peak prompts characterisation; a broad increase suggests a different distribution. Immunofixation and free light chains add complementary evidence. Original CDIL educational schematic; not patient data.

Worked cases 85–88

Case 85 — The plasma band

A restricted-looking component appears in a specimen submitted as plasma. The previous serum specimen did not show it. A learner immediately reports a new monoclonal protein.

Reasoned answer. Plasma contains fibrinogen, which can contribute a component that is absent from properly prepared serum. The specimen type and pattern must be reviewed before assigning a clonal interpretation. Appropriate repeat or confirmatory testing should follow the laboratory's validated process. The finding may still require investigation, but the difference in specimen matrix provides a concrete analytical explanation that must not be ignored.

Case 86 — The same ratio, different concentrations

Two fictional specimens both have a kappa/lambda ratio of 2.0. One has concentrations of 48 and 24 mg/L; the other has 8 and 4 mg/L. A student describes them as analytically identical.

Reasoned answer. The ratio is identical, but the measured concentrations differ sixfold. Interpretation requires both concentrations, the method-specific interval, kidney function and the clinical question. Ratios compress information. They are useful summaries, not replacements for the underlying data. Neither result should be assigned a disease label from the ratio alone.

Case 87 — A band after a new therapy

A patient receiving an IgG-kappa therapeutic antibody develops a small IgG-kappa component on immunofixation. The laboratory was not told about the treatment.

Reasoned answer. The drug may contribute to the observed pattern. The laboratory should obtain the treatment history and apply a validated interference-resolution approach where available. It must not assume that every matching band is drug-related, because endogenous disease can coexist. The correct interpretation distinguishes an analytical possibility from a demonstrated explanation and records how the interference was investigated.

Case 88 — The apparently low immunoglobulin

A very prominent restricted component is visible, but an immunochemical measurement gives an unexpectedly low result. Routine repetition produces the same number.

Reasoned answer. A repeat under identical conditions demonstrates reproducibility, not necessarily accuracy. Antigen excess or another method-specific interference may need investigation. Appropriate dilution, assay flags, calibration and an alternative method can help resolve the discrepancy under validated procedures. The laboratory should not choose the result that looks more reassuring without understanding the conflict.

23 | Complex Immunodeficiency Investigations

An immune investigation becomes complex when the history, cell counts, protein measurements and functional tests do not point to one simple explanation. Complexity is not a reason to order every available test at once. A disciplined approach begins with the clinical pattern, the relevant immune compartment and the quality of the existing evidence. The aim is to distinguish a biological defect from secondary effects, treatment consequences and analytical limitations. [FA16, FA17]

23.1 Start with the pattern, not the panel

The type, severity, frequency and anatomical distribution of infections provide clues. Recurrent infections with common organisms may have a different significance from invasive infection with an unusual organism. Noninfectious features such as autoimmunity, inflammation, lymphoproliferation, growth problems or syndromic findings can also be central to an inborn error of immunity. The outdated idea that every such disorder presents only as recurrent infection can delay recognition.

Age at presentation is informative but not absolute. Some disorders become evident in infancy; others are recognised in adolescence or adulthood. A later diagnosis does not prove an acquired cause. Conversely, a childhood presentation does not establish a genetic disorder. Secondary causes, environmental exposures, anatomical abnormalities and treatment effects remain part of the differential.

Family history can support investigation, but an apparently negative history does not exclude a genetic condition. Small families, variable expression, new variants and unrecognised illness can obscure inheritance patterns. The laboratory should record the clinical question and relevant history without converting a suggestive pedigree into a molecular diagnosis.

23.2 Separate quantity, distribution and function

A complete blood count gives an initial view of cell abundance. Flow cytometry can define populations more specifically. Immunoglobulin measurements describe selected soluble products. Functional assays evaluate performance under specified conditions. These layers complement one another; they are not interchangeable stages in which one normal result makes all later testing unnecessary.

A patient may have a normal total lymphocyte count but a reduced or abnormal subset. A normal B-cell count does not guarantee effective antibody production. Normal total IgG does not necessarily establish an adequate response to a particular antigen. Each test should be linked to a mechanistic question so that a normal result is not overinterpreted.

Age-specific interpretation is especially important. Lymphocyte populations and immunoglobulin concentrations change during development. Maternal antibody influences early measurements, while vaccination and exposure history affect specific antibodies. Adult reference intervals can misclassify a young child's result. The appropriate interval must belong to the age group, specimen and analytical method.

23.3 Specific antibody responses are contextual measurements

Assessment of antibody responses may involve selected antigens and appropriately timed specimens. The interpretation depends on prior vaccination, the type of antigen, baseline concentration, interval after exposure, age and assay. A fold increase can be misleading when the baseline is already high or close to the analytical detection limit. Absolute concentrations and validated response criteria may also be relevant.

An example illustrates the limitation. A concentration rising from 0.1 to 0.4 units is a fourfold increase; a rise from 10 to 20 units is twofold. The larger fold change does not automatically represent the better protective response. The antigens, units, assay limits and

decision criteria must be known. Protection is not established by a mathematical ratio detached from biological and clinical evidence.

Immunoglobulin replacement introduces another complication. Administered antibodies can contribute to measured concentrations and specific-antibody results. Testing after replacement may not describe the patient's own production. The laboratory should know the treatment timing and avoid interpretations that require an untreated baseline when that baseline is unavailable.

23.4 Functional tests depend on living systems

Lymphocyte proliferation, oxidative burst and cytotoxicity investigations depend on viable cells and controlled experimental conditions. Delayed transport, inappropriate temperature, insufficient cell numbers or medication effects can alter results. A poor response in a compromised specimen is not equivalent to a demonstrated inherited functional defect.

Controls help distinguish these possibilities. A contemporaneous healthy control may reveal transport-related deterioration, while internal stimulation controls assess the assay's capacity to produce an expected response. Controls must be appropriate to the method; no single control can answer every quality question. The report should state important limitations rather than presenting a technically questionable measurement as definitive.

Functional testing can also be affected by acute illness and therapy. A result obtained during severe systemic illness may differ from one obtained after recovery. The clinical team decides whether and when repeat testing is appropriate. The laboratory contributes by explaining what the assay measures and which preanalytical or treatment factors can compromise interpretation.

23.5 Molecular findings require phenotype-aware interpretation

A pathogenic or likely pathogenic variant can help establish a diagnosis when it fits the inheritance pattern and phenotype. A variant of uncertain significance is not a confirmed causal explanation. The word "variant" describes a sequence difference; its classification depends on evidence. Laboratories should not promote uncertain findings to diagnoses because the gene is associated with an attractive hypothesis.

A negative panel also has boundaries. Coverage, variant types detectable, gene content and current knowledge affect what is excluded. Some disorders involve mechanisms not captured by a particular assay. Reanalysis can become relevant as knowledge changes, but it should follow a controlled process rather than an assumption that every unresolved patient must have a hidden variant in the same panel.

The strongest interpretation integrates clinical, immunological and molecular evidence. Discordance should stimulate review of all three, including sample identity and analytical quality. A laboratory finding is most useful when it narrows the question honestly, even when it does not immediately produce a complete diagnosis.

A staged investigation reduces both missed disease and noise

A staged approach begins with a small set of investigations that match the phenotype and then expands according to the findings. This is not a rule that every patient must wait through a slow sequence. Severe presentations may justify several investigations at once. The principle is that each test has a defined purpose and that urgency, rather than curiosity alone, determines the pace. [FA16, FA17]

A basic count may identify a major quantitative abnormality. Subset analysis can locate it more precisely. Protein and functional measurements can distinguish a numerical deficiency from impaired performance. Molecular testing can then address a plausible causal

mechanism or, in selected circumstances, provide an early broad investigation. The sequence is flexible, but the reasoning should remain explicit.

Broad panels can generate incidental or uncertain findings. When many analytes are measured, at least one may fall outside its reference interval by chance or because the interval does not fit the patient. The response should be to examine the pattern, quality and clinical relevance, not to attach a separate diagnosis to every flagged result.

Reproducibility is important when a finding would lead to a major diagnosis. Appropriate repeat testing can distinguish persistent biology from transient illness or specimen problems. However, repeat testing must not delay urgent action when a severe, credible abnormality requires immediate specialist attention. The laboratory and clinical team should agree on what must be communicated now and what can be clarified subsequently.

The final report should preserve uncertainty at the correct level. It may establish a cellular phenotype without establishing a molecular cause, or identify a causal variant without fully predicting severity. Good reporting does not weaken the evidence; it prevents a valid finding from being stretched beyond what it supports.

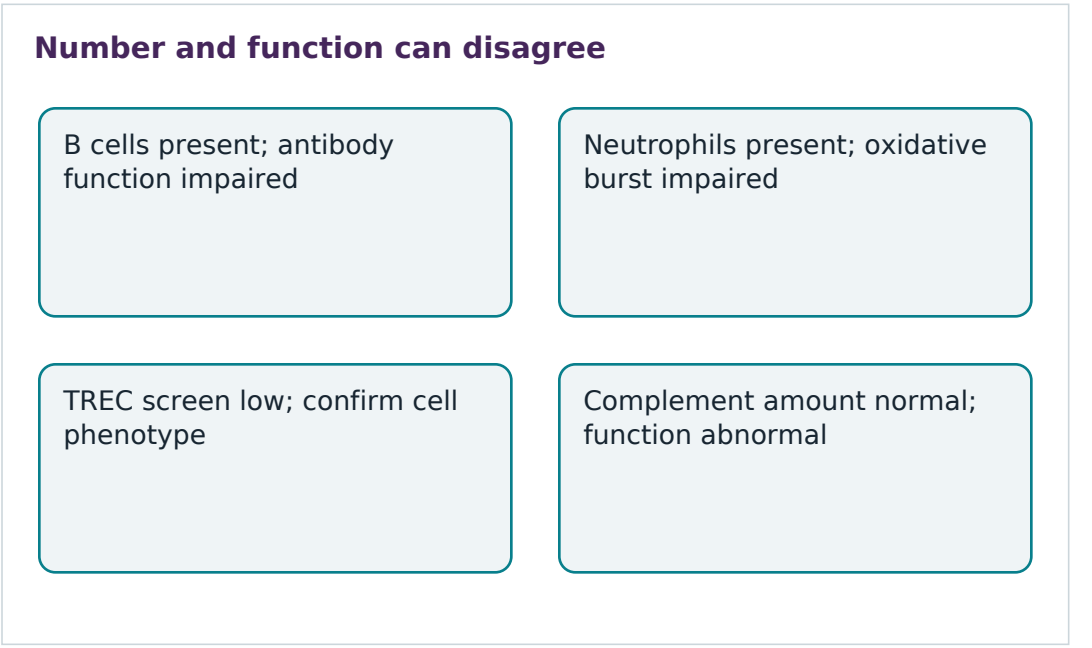


Figure FA23. An adequate count does not establish normal function. Each example identifies a different measurement level and a reason to use a mechanism-directed follow-up test. Original CDIL educational schematic; not patient data.

Worked cases 89–92

Case 89 — A normal total count

A child has a normal total lymphocyte count but a substantially reduced T-cell population on a valid age-referenced investigation. A trainee dismisses the subset result because the total was normal.

Reasoned answer. A total combines populations and can conceal an important subset abnormality. The result should be evaluated with absolute subset counts, age-appropriate intervals, specimen quality and the clinical presentation. The normal total does not invalidate the more specific measurement. It also does not establish the cause of the subset abnormality; further investigation may be required.

Case 90 — A response measured after replacement

Specific antibodies are detected after immunoglobulin replacement. The report draft states that the patient's own antibody production is normal.

Reasoned answer. Administered immunoglobulin can contain relevant antibodies. The result may therefore reflect passive transfer as well as endogenous production. Without an appropriate pretreatment baseline or a validated alternative approach, the proposed conclusion is not supported. Treatment history is part of analytical interpretation, not an optional administrative detail.

Case 91 — A weak proliferation result after delayed transport

A cell-function specimen arrives late, and the accompanying control also shows poor performance. The patient result is low.

Reasoned answer. The control raises concern about specimen or assay conditions. The result cannot be treated as a clean demonstration of biological dysfunction. The laboratory should follow its acceptance and reporting policy, explain the limitation and discuss appropriate recollection with the clinical team. Repeating a compromised preparation may not restore the information lost during transport.

Case 92 — A variant of uncertain significance

A gene panel identifies a variant of uncertain significance in a gene associated with immune dysfunction. The phenotype only partly matches the known disorder.

Reasoned answer. The classification explicitly indicates insufficient evidence for a definitive causal conclusion. Phenotype, inheritance, family studies, functional evidence and future reclassification may help, but the variant should not be reported as a confirmed diagnosis. The clinical team should continue to evaluate the patient rather than allowing an uncertain molecular result to end the investigation prematurely.

24 | The Immunoassay Interference Workbench

An unexpected result can be caused by disease, treatment, specimen problems or interference within the measurement system. The laboratory's challenge is to investigate without assuming that the most dramatic explanation is the correct one. Interference work is a controlled experiment: each additional test should distinguish between plausible mechanisms while preserving specimen identity and the original evidence. [FA19, FA20, FA23]

24.1 Define the discrepancy precisely

"The result looks wrong" is not yet an investigable statement. A useful discrepancy describes the result, method, clinical context, expected relationship and reason for concern. Examples include a result inconsistent with related measurements, an abrupt change without a plausible time course, or disagreement between two methods measuring a similar target. The discrepancy should be documented before the original specimen is exhausted.

A repeat on the same analyser is useful for detecting some random or handling errors, but it may reproduce a systematic interference. Reproducibility means that the system gives a similar answer under similar conditions. Accuracy concerns closeness to an appropriate reference or true value. The two properties should not be confused.

Communication with the clinical team is often the most efficient first investigation. Relevant supplements, therapeutic antibodies, recent procedures, pregnancy, transfusion, immunoglobulin administration and unusual protein states can change the list of plausible explanations. The laboratory should ask targeted questions rather than request an unlimited history that does not influence the next analytical step.

24.2 Dilution is an experiment with assumptions

Serial dilution can reveal nonparallel behaviour or recover a result affected by antigen excess. However, dilution changes the matrix as well as the analyte concentration. The diluent, permitted range and validated recovery criteria belong to the method. A neat mathematical multiplication is not enough to validate a diluted result.

Consider a synthetic specimen measured at 18 units undiluted. A validated 1:10 dilution measures 9 units, producing a corrected estimate of 90 units. A 1:20 dilution measures 4.6 units, producing 92 units. The large disagreement with the undiluted result and agreement between the two corrected diluted results suggest a concentration-dependent analytical problem. They do not, by themselves, identify its exact cause. The assay design and additional investigation determine whether antigen excess or another mechanism is likely.

A different pattern may be less interpretable. Corrected estimates of 90, 150 and 260 units across increasing dilutions show nonparallel behaviour but do not provide a stable concentration to report. Selecting the highest value because it seems most plausible would be arbitrary. The laboratory should follow its validated interference policy and communicate unresolved uncertainty.

24.3 Alternative methods can isolate a mechanism

An alternative platform may use different antibodies, labels, capture systems or signal architecture. Agreement or disagreement can therefore provide mechanistic information. Nevertheless, two methods may not measure exactly the same molecular forms or use identical calibration. A difference is not automatically proof that one is wrong.

The comparison should be planned. A biotin-independent method can be informative when a biotin-dependent architecture is suspected. A method with different antibody species or blocking chemistry may help investigate heterophile effects. An assay designed to distinguish a therapeutic antibody from an endogenous component can address a specific interference question. The value lies in the relevant

difference between methods, not merely in obtaining another number.

Clinical interpretation must also remain assay-specific. A decision threshold established for one platform may not transfer directly to another. When an alternative method is used, the report should identify it and use the appropriate interpretive framework. The aim is not to conceal disagreement but to explain how the laboratory reached the final conclusion.

24.4 Blocking, precipitation and specimen treatment

Blocking reagents can reduce some antibody-mediated interferences, but a change after treatment does not always establish a unique mechanism. Treatment can also affect the analyte or matrix. Recovery controls and validated acceptance criteria are therefore important. A negative blocking experiment does not exclude every heterophile or autoantibody effect.

Precipitation methods may help investigate macroforms or other complexes, depending on the assay and validated protocol. Recovery must be interpreted against method-specific expectations. A low recovery is not a universal disease diagnosis. The laboratory should distinguish the observed treatment effect from the biological explanation inferred from it.

Specimen treatment must not become an improvised bedside procedure. Reagent handling, safety, validation, competency and authorisation remain laboratory responsibilities. A textbook explains the reasoning behind an investigation; it does not replace the current manufacturer's instructions or a validated standard operating procedure.

24.5 Biotin illustrates architecture-dependent risk

Biotin can interfere with assays that depend on streptavidin–biotin interactions. The direction and magnitude depend on the design, circulating concentration, timing and other factors. In susceptible sandwich assays, results may be falsely low; in susceptible competitive assays, they may be falsely high. These are design-linked patterns, not a claim that every assay for the same analyte is affected. [FA20]

No universal supplement-withholding interval can safely be applied to every dose, patient and platform. Manufacturer guidance, dose history, kidney function, urgency and availability of an unaffected method may influence the response. In an urgent clinical situation, simply postponing all testing can be unsafe. The laboratory and treating team should choose a validated strategy that addresses both analytical reliability and clinical time sensitivity.

The final report should be concise but specific. It may state the original discrepancy, the method used to resolve it, the result considered reportable and any remaining limitation. It should avoid an unsupported declaration that "all interference was excluded." An investigation can reduce uncertainty without proving the absence of every possible analytical problem.

A practical hierarchy for an interference investigation

The first layer is identity and specimen quality. Confirm that the result belongs to the correct patient and collection event, and review visible or instrument-detected specimen problems. The second layer is analytical performance: calibration, controls, flags and recent method changes. The third layer is mechanism-specific testing, selected from the plausible interferences rather than applied indiscriminately. [FA19, FA20, FA22]

This hierarchy prevents an expensive investigation from being built on a simple error. A result from a misidentified specimen cannot be repaired by dilution studies. A failed analytical run should not be interpreted as a rare endogenous antibody effect before routine

quality problems are resolved. Basic checks remain scientifically important even in an advanced laboratory.

Once the basic layers are satisfactory, choose experiments that change one relevant feature. Dilution changes concentration and matrix. An alternative platform changes recognition or signal architecture. Blocking reagents test selected antibody-mediated effects. Specimen treatment may remove or alter particular complexes. Each experiment should have a stated hypothesis and an expected informative outcome.

The final interpretation should integrate converging evidence. Agreement between several appropriately chosen methods is more persuasive than repeated testing that shares the same vulnerability. Even then, the conclusion should state the specific interference addressed and the limits of the investigation. No finite set of tests demonstrates that every conceivable interference is absent.

Documentation makes the result reusable. Record the original method, treatment applied, controls, corrected values, decision and communication. A future specimen from the same patient may show the same problem, and a traceable record can prevent delay or contradictory reporting. The investigation is complete only when the reliable result and its limitations reach the people making the clinical decision.

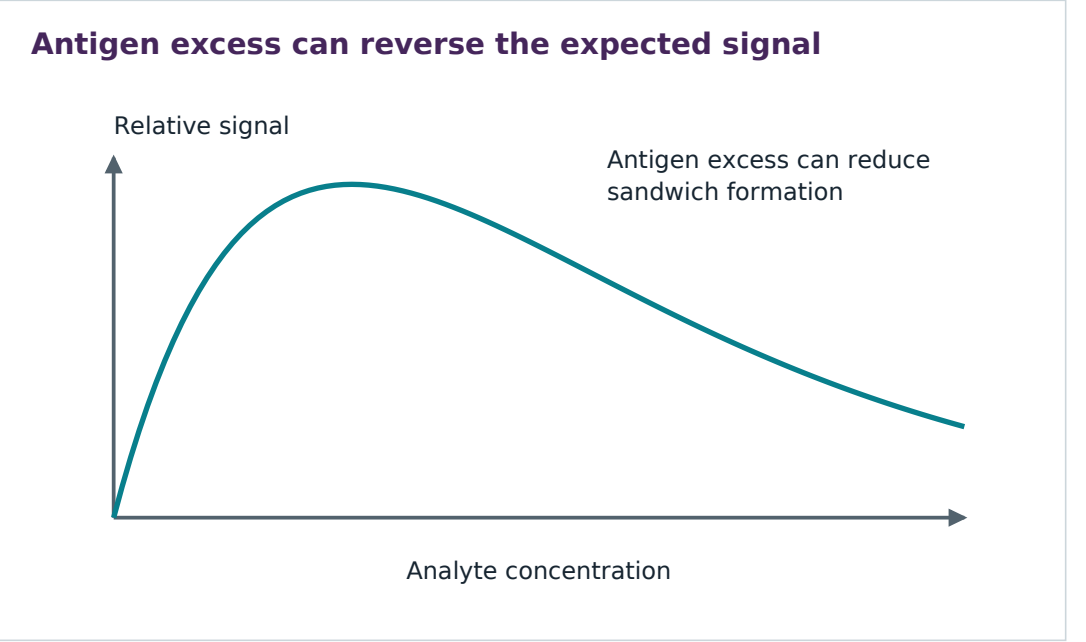


Figure FA24. Synthetic high-dose hook model. Signal rises, then falls at extreme analyte concentration in a susceptible assay. Appropriate dilution can uncover the inconsistency; not every immunoassay has this response. Original CDIL educational schematic; not patient data.

Worked cases 93–96

Case 93 — Dilution restores agreement

A specimen gives 18 units undiluted, 9 units at a validated 1:10 dilution and 4.6 units at 1:20. A student averages all three raw readings.

Reasoned answer. Raw readings from different dilutions are not directly comparable. Corrected diluted estimates are 90 and 92 units, whereas the undiluted result is discordant. This pattern supports investigation of a concentration-dependent problem. Averaging would hide the mechanism and produce a meaningless value. Reporting must follow the assay's validated dilution and interference procedure, not an improvised arithmetic compromise.

Case 94 — A second method with a different target

Two tests are described loosely as measuring the same hormone, but they detect different molecular forms. Their results differ.

Reasoned answer. The comparison may concern different measurands rather than interference. The laboratory should establish what each method detects, its calibration and intended use. An alternative method is informative only when its relationship to the original measurement is understood. The disagreement should not be resolved by choosing the method with the more familiar name.

Case 95 — A supplement in an urgent investigation

A patient taking a high-dose biotin product requires urgent testing. The local assay is potentially susceptible, but an alternative validated method is available.

Reasoned answer. The laboratory should communicate the exposure and use the appropriate validated pathway, which may include the alternative method. A blanket delay based on a generic withholding interval may not be appropriate. The clinical urgency and assay architecture must both be addressed. The report should identify the method and relevant limitation rather than imply that all biotin exposures produce the same effect.

Case 96 — A blocking experiment with no change

A blocking reagent produces no meaningful change in a discordant result. A draft report says that antibody interference is completely excluded.

Reasoned answer. A negative blocking experiment excludes only what that validated experiment can reasonably detect. Different interfering antibodies or mechanisms may not respond. The laboratory should integrate dilution, alternative-method, clinical and quality evidence, and report the investigation's actual limits. "No effect with this treatment" is a defensible observation; "all antibody interference excluded" is usually a much broader claim.

25 | Integrated Immunology Case Conference

An integrated case conference is not a contest to name a rare disease from one abnormal result. It is a structured exercise in combining mechanisms, measurements and clinical context. Good reasoning states what is known, what remains uncertain, which alternative explanations matter and what additional evidence would change the conclusion. The cases in this chapter bring together the principles developed throughout the book. They are fictional teaching constructions, not patient records.

25.1 Build an evidence map

Begin with the clinical question in ordinary language. Is the team investigating infection, antibody production, immune-mediated injury, treatment interference or another problem? Then identify the available observations and separate them by type: symptoms and history, anatomy, cell counts, protein concentrations, functional results, molecular findings and analytical quality. Mixing these categories too early can create false equivalence.

Next, connect each observation to the mechanism it can support. A low immunoglobulin concentration concerns abundance, not necessarily the full capacity to respond to every antigen. A positive autoantibody concerns a detected specificity, not a complete diagnosis. An abnormal functional result concerns performance under defined conditions. The evidence map should make these boundaries visible.

Finally, identify the weakest link in the proposed explanation. The missing link may be clinical context, a compromised specimen, an unverified treatment history or an untested analytical assumption. The best next step is often the one that resolves this specific weakness, rather than the broadest available panel.

25.2 Use probability without pretending certainty

Pretest probability influences the meaning of positive and negative results. It arises from the clinical setting and population, not from a laboratory value alone. A highly specific test can still generate a substantial proportion of false positives when used indiscriminately in a very low-prevalence population. Conversely, a negative result may leave important residual risk when the prior probability is high or sensitivity is limited.

Probability should guide communication, not produce false numerical precision. Published sensitivity and specificity may not apply exactly to a different population, disease stage or laboratory method. When a calculation is used, its assumptions should be stated. A result can be described as supporting, weakening or failing to resolve a hypothesis without assigning an unjustified percentage to an individual patient.

Discordance can be informative. A result that does not fit the clinical picture may reveal an unexpected disease mechanism, but it may also expose a preanalytical or analytical problem. The correct response is neither automatic rejection of the laboratory nor automatic rejection of the clinical assessment. The two teams should examine the mismatch together.

25.3 Protect the patient while uncertainty is resolved

Some investigations are time sensitive. A suspected severe immune defect, serious transfusion-related interference or rapidly evolving clinical syndrome may require prompt specialist communication even before every result is final. The laboratory should follow its critical-result and escalation policies. Waiting for a perfect explanatory narrative must not delay communication of an important, valid finding.

At the same time, urgency does not justify inventing certainty. A provisional report can state the observation, its immediate implication and the investigation still underway. The distinction between provisional and final interpretation should remain clear in the record. Subsequent corrections must be traceable and communicated to the appropriate recipients.

Patient identity, specimen identity and chronology remain foundational throughout this process. A beautifully reasoned explanation applied to the wrong specimen is unsafe. Every case conference should preserve the chain from the patient and collection event to the analytical result and clinical decision. This is not merely an administrative requirement; it is part of the scientific validity of the conclusion.

25.4 Case conference record and handover

A useful conference record is concise enough to be used but complete enough to show the reasoning. It states the question, decisive evidence, important alternatives, analytical limitations, agreed next steps and responsibility for follow-up. It should not reproduce every result without explaining its relevance. A list of numbers is not an interpretation.

The laboratory's contribution should be within its authorised scope. Staff can explain assay design, quality findings, interferences and the meaning of a pattern. Diagnostic and treatment decisions are integrated by appropriately authorised clinicians and specialists. Clear role boundaries improve collaboration because each participant knows which expertise is being provided.

The final learning question is transferable: what would make this conclusion wrong? Asking it encourages the learner to search for contradictory evidence, hidden assumptions and missing context. A strong interpretation remains open to correction while being sufficiently clear to support the next safe action.

Turning a case conclusion into a teachable explanation

A strong explanation begins with the decisive observation rather than with the name of a disease. For example, "the corrected diluted measurements agree while the undiluted result is discordant" states the analytical evidence. The next sentence explains the mechanism suggested by that pattern. The final sentence states the remaining uncertainty and the appropriate next step. This structure makes the reasoning visible and easier to challenge constructively.

Learners should distinguish an observation, an inference and a decision. "The antibody screen is reactive" is an observation. "An alloantibody may be present" is an inference. "Further identification is required before routine compatibility selection" is a decision within a defined pathway. Collapsing the three into one sentence can hide assumptions and make an uncertain interpretation sound definitive.

Counterfactual questions test understanding. What result would weaken the proposed explanation? What specimen problem could create the same pattern? What change in history would alter the next step? These questions move the learner beyond recognising a familiar example toward applying a mechanism in a new setting.

The same discipline improves communication with patients and colleagues. A clear explanation does not require excessive technical language. It requires a correct statement of what was found, why it matters and what happens next. Where uncertainty remains, naming it specifically is more useful than either absolute reassurance or a vague warning that "anything is possible."

The purpose of the case conference is therefore both scientific and professional. It develops the ability to connect laboratory evidence to safe action while respecting the limits of the measurement, the patient's context and the authorised responsibilities of the team.

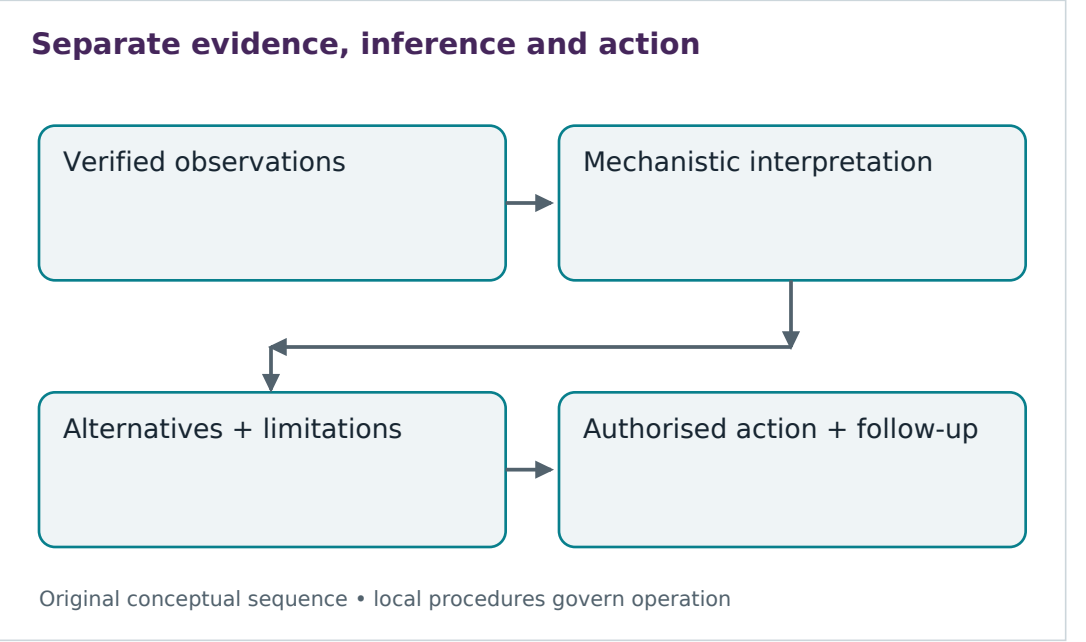


Figure FA25. A case conclusion is strongest when observed results, the proposed mechanism, uncertainty and the clinical action remain distinguishable. Original CDIL educational schematic; not patient data.

Worked cases 97–100

Case 97 — Antibody deficiency or passive treatment effect?

A fictional adult has recurrent respiratory infections. Total IgG is within the laboratory interval after recent immunoglobulin replacement. Several specific antibodies are detectable. The pretreatment record is incomplete, and a trainee proposes that antibody deficiency has been excluded.

Reasoned answer. The treatment can contribute both total and specific antibodies. The post-treatment results therefore cannot automatically establish normal endogenous production. The conference should recover pretreatment history and measurements where possible, evaluate the clinical pattern and involve the relevant specialist in planning further assessment. The normal current concentration may be useful for treatment monitoring under the appropriate framework, but it answers a different question from whether the patient produced those antibodies independently. The conclusion must distinguish measured circulating antibody from demonstrated immune competence.

Case 98 — Autoantibody, kidney findings and an uncertain assay

A patient with findings suggestive of systemic disease has a positive autoantibody result. The specimen also shows unusual protein behaviour, and the result differs substantially on another method. The team is deciding whether the discrepancy is clinically important.

Reasoned answer. Neither the positive result nor the disagreement should be dismissed. The conference should identify the antigen and assay design, compare the methods' intended measurands and review specimen quality and possible interference. Clinical evidence of organ involvement remains important and may justify continued assessment irrespective of one disputed antibody result. The laboratory should report what each method demonstrated and how the discrepancy was investigated. A diagnosis should not depend on an unexamined assumption that assays with similar names are analytically identical.

Case 99 — An unexpected infectious serology result

A low-risk patient has a reactive screening antibody test during a broad, untargeted panel. A confirmatory or linked test is not yet available. The learner tells the patient that active infection is proven.

Reasoned answer. The claim exceeds the evidence. Screening reactivity can reflect current infection, past exposure, cross-reactivity or other explanations, depending on the organism and assay. The low-prevalence context affects positive predictive value. The appropriate next step is the current organism-specific testing algorithm, with attention to timing and clinical risk. Communication should explain that further interpretation is required rather than assigning a definitive infection label. This protects the patient from both unnecessary alarm and false reassurance.

Case 100 — A convincing diagnosis built on the wrong chronology

A case conference links a new immune abnormality to a treatment started on Monday. Review of the specimen record shows that the sample was collected the previous Friday, although the result was released on Tuesday.

Reasoned answer. The treatment cannot explain a biological change in a specimen collected before it began. Collection time, not result-release time, anchors the patient's biological state represented by the sample. The conference must revise the causal explanation and reconsider the pretreatment differential. This case demonstrates why chronology and specimen traceability are part of scientific reasoning. A plausible mechanism becomes incorrect when attached to the wrong sequence of events.

Workshop 1 | Dilutions, Volumes and the Identity of the Reported Result

A dilution calculation is a statement about material balance. It links the amount of original specimen transferred into a mixture with the final volume of that mixture. The arithmetic is simple, but the clinical consequence of a misplaced factor can be large. This workshop follows one fictional specimen through serial preparation, measurement and reporting. The volumes and concentrations are invented for teaching; actual pipetting and dilution procedures must follow the validated assay. [FA19, FA21]

The denominator is the final volume

A learner combines 100 µL of serum with 900 µL of diluent. The final mixture contains 1,000 µL, of which 100 µL came from the original specimen. The specimen fraction is therefore 100/1,000 = 0.10, and the dilution factor is 10. A concentration measured in that mixture is multiplied by 10 to estimate the original concentration, provided the assay response is valid and the preparation is accurate.

If the learner instead combines 100 µL of serum with 1,000 µL of diluent, the final volume is 1,100 µL and the factor is 11. Calling both preparations "one to ten" hides the error. The unambiguous record states the specimen volume, diluent volume, final volume and factor. This also makes the calculation auditable when another person reviews the result.

Preparation	Specimen	Diluent	Final volume	Dilution factor
A	100 µL	900 µL	1,000 µL	10
B	100 µL	1,000 µL	1,100 µL	11
C	50 µL	450 µL	500 µL	10

Preparation	Specimen	Diluent	Final volume	Dilution factor
D	25 µL	475 µL	500 µL	20

Preparations A and C have the same nominal factor but do not necessarily have the same practical uncertainty. Pipettes have specified operating ranges, and small volumes can be more vulnerable to technique and calibration limitations. A laboratory should use a validated preparation rather than select a smaller volume solely to save specimen.

Serial factors multiply

The fictional assay requires a 1:100 preparation. The learner first makes preparation A, then transfers 100 µL of that diluted material into 900 µL of fresh diluent. The second tube contains one tenth of the first tube's concentration, and the first tube contained one tenth of the original concentration. The overall specimen fraction is 0.10 × 0.10 = 0.01, so the overall factor is 100.

If the second transfer were 200 µL into 800 µL, that step would be a factor of 5 and the overall factor would be 10 × 5 = 50. The factors multiply; they are not added. A worksheet that adds 10 + 5 and reports a factor of 15 has lost the physical meaning of the preparation.

Mixing is part of the process. The aliquot transferred to the next tube must represent the previous mixture. An incompletely mixed first tube can create a concentration error even when every written volume is correct. The laboratory therefore validates both the numerical plan and the physical procedure.

Recover the original concentration once

The instrument measures 2.4 mg/L in a correctly prepared 1:100 dilution. If the instrument output is the concentration in the measured mixture, the original concentration is 2.4 × 100 = 240 mg/L. However, some systems already apply an entered dilution factor. If the displayed result is already 240 mg/L after automatic correction, multiplying it again would create a false value of 24,000 mg/L.

The operator must identify what the number represents. The screen label, method configuration and result interface should make clear whether the factor has been applied. A safe report retains the dilution factor and the original analytical result in the audit trail, even when the patient-facing report displays only the final corrected value.

Unit conversion is a separate operation. Since 1 g/L equals 1,000 mg/L, 240 mg/L equals 0.240 g/L. The dilution factor does not change because the reporting unit changes. Keeping the two steps separate helps prevent a thousandfold error from being mistaken for a dilution correction.

Check the measured range before correcting

Suppose the fictional validated measuring interval is 1–50 mg/L in the analysed mixture. A diluted result of 2.4 mg/L lies within that interval. A diluted result of 0.2 mg/L does not become valid simply because multiplying by 100 produces a clinically plausible original concentration. The instrument's ability to measure the mixture remains the limiting condition.

Likewise, a result above 50 mg/L should not be extrapolated indefinitely from the calibration curve. Further dilution may be appropriate under the validated method. The final reportable interval depends on both the analytical interval and the allowed dilution procedure. It is not established by arithmetic alone.

A dilution study can also test consistency. If corrected values from suitable dilutions agree within established criteria, the result is more credible. Disagreement can indicate nonlinearity, interference,

preparation error or an invalid dilution matrix. Agreement is supportive, not proof that every possible interference is absent.

A complete worked record

A specimen is diluted 1:10, then 1:5. The instrument reports 6.2 mg/L in the final mixture without automatic correction. The overall factor is 50, so the original concentration is 310 mg/L, or 0.310 g/L. A second preparation at an overall factor of 100 gives 3.1 mg/L, also corresponding to 310 mg/L. Both measured values are within the fictional interval, and the corrected results agree exactly in this constructed example.

The report should not claim zero uncertainty. Real pipetting, calibration and measurement have variation even when two rounded results agree. The useful conclusion is that the two valid preparations support a consistent corrected result under the stated method.

Transfer questions with reasoned answers

Question 1. A 40 µL specimen is combined with 760 µL diluent. What is the factor? **Answer.** Final volume is 800 µL, so the factor is 800/40 = 20. Using 760/40 = 19 would compare diluent with specimen rather than total mixture with specimen.

Question 2. A 1:4 preparation is followed by a 1:8 preparation. What is the overall factor? **Answer.** 4 × 8 = 32. The final tube contains one thirty-second of the original concentration under ideal mixing and volume assumptions.

Question 3. A measured value of 0.4 is below the method's quantification limit. Does a factor of 100 make the corrected value 40 reliable? **Answer.** No. Multiplication cannot restore information that the assay could not quantify in the measured mixture. Use the validated reporting or repeat-testing pathway.

Question 4. Two valid dilutions give corrected results of 90 and 160. Should they be averaged? **Answer.** Not automatically. The discrepancy first requires investigation of preparation, method linearity and interference. An average can conceal a systematic problem rather than solve it.

Workshop 2 | From an Immunoassay Signal to a Concentration

An immunoassay does not directly display a biological truth. It measures a signal produced by a chain of binding, separation, labelling and detection events. Calibration connects that signal with assigned material concentrations under defined conditions. This workshop compares a simple linear model with a saturating model, then examines what controls can and cannot establish. All data and equations are fictional teaching constructions. [FA19–FA21]

A linear segment is a local relationship

Within a limited validated interval, suppose a model assay follows S = 0.050 + 0.025C, where S is signal and C is concentration in the measured mixture. The intercept represents the model's signal at zero concentration; the slope describes the change in signal per concentration unit. Solving for concentration gives C = (S − 0.050)/0.025.

For S = 0.800, C = 30.0 units. If the measured mixture was a validated 1:5 dilution and the instrument has not already corrected it, the original specimen concentration is 150 units. The subtraction of the intercept and division by the slope occur before the dilution correction.

Concentration in mixture	Model signal
0	0.050
10	0.300
20	0.550
30	0.800
40	1.050

A calibration equation should not be extended beyond its validated range merely because the algebra produces a number. Immunoassay responses often become nonlinear, and a straight line fitted to a central segment may perform poorly near the extremes. The method specifies the allowable model and interval.

A saturating response requires a different inverse

A second fictional assay follows S = 0.050 + 1.50C/(50 + C). The response approaches an upper limit as concentration increases. Subtracting 0.050 gives R = 1.50C/(50 + C). Rearrangement produces C = 50R/(1.50 − R), provided the result lies within the validated model's domain.

For a signal of 0.950, R is 0.900 and C is 50 × 0.900/(1.50 − 0.900) = 75.0 units. A validated 1:5 dilution would correspond to 375 units in the original specimen. It would be wrong to apply the first assay's linear equation to this second assay simply because both use a similar optical signal.

Concentration	Saturating-model signal
0	0.050
10	0.300
25	0.550
50	0.800
100	1.050
200	1.250

Notice that equal changes in concentration no longer produce equal changes in signal. Near saturation, a small signal error can correspond to a large concentration difference. This is one reason that quantitative performance must be characterised across the measuring interval rather than inferred from a single central control.

Calibration and control material have different roles

Calibrators establish the signal–concentration relationship. Independent control material evaluates whether the method performs as expected after calibration. A control should not be used merely to force the calibration to appear correct. If calibrator and control errors are linked through the same assigned material or preparation, apparent agreement can conceal a shared bias.

Suppose a control assigned 50 units gives 50.2, 49.8 and 50.1. These observations suggest close repeatability around the assigned value in this small constructed set. They do not prove equivalent performance at 2 units or 200 units, nor do they establish clinical sensitivity for every patient condition. Each claim requires suitable evidence.

Patient specimens can differ from control material in matrix and interfering substances. A passing control does not exclude heterophile antibodies, biotin effects or antigen excess in one patient. When the clinical pattern and assay result conflict, the investigation should examine the patient-specific mechanism rather than dismiss the concern because routine controls passed.

A plate map is part of the evidence

In a manual or semiautomated plate assay, the location of each calibrator, control and specimen must remain traceable. A correct signal interpreted against the wrong well identity becomes a wrong result. The plate map should identify duplicates, dilution factors and any repeated wells without ambiguous overwriting.

Edge effects, inconsistent timing, incomplete washing or uneven reagent delivery can create spatial patterns. A cluster of high results along one edge may suggest a process effect rather than a biological relationship among the patients. Reviewing the arrangement of wells can therefore reveal information lost in a simple sorted result list.

Duplicates should be compared using a predefined rule. A large difference should trigger the validated investigation, not selection of the more plausible value. Repeating only one well can be appropriate in some procedures, but the decision should be documented and consistent with the method's requirements.

Interpret a constructed failure

A plate has acceptable calibrators and controls, but all specimens in the final row show unexpectedly low signal. Their duplicate wells are adjacent and similarly low. The learner concludes that duplicate agreement proves accuracy.

The duplicates share the same position and may share the same process failure. Agreement between them is therefore not independent evidence against a row-specific problem. The investigation should review reagent delivery, washing, timing and plate handling, and repeat affected specimens under the validated procedure. The pattern is a clue to the mechanism.

Transfer questions with reasoned answers

Question 1. In the linear model, S = 0.550. What is C? **Answer.** (0.550 − 0.050)/0.025 = 20 units. Omitting the intercept gives 22 and creates a systematic error.

Question 2. In the saturating model, S = 1.050. What is C? **Answer.** R = 1.000; C = 50 × 1/(1.50 − 1) = 100 units. A linear interpolation from an unrelated assay is inappropriate.

Question 3. A control passes but one patient's result changes markedly after an appropriate blocking treatment. What does this suggest? **Answer.** A patient-specific interference is possible. Routine controls monitor the process but may not contain the interfering antibody or substance present in that specimen. Further method-specific confirmation is needed.

Question 4. Two duplicates agree after both were assigned the wrong specimen identifier. Are they valid patient results? **Answer.** No. Analytical agreement cannot repair an identity failure. The specimen-to-result relationship must be correct before the number can support patient care.

Workshop 3 | Diagnostic Probability Without Denominator Errors

A diagnostic test changes the probability of a condition; it does not usually replace the need to consider the patient's starting probability. Sensitivity and specificity describe performance under a defined reference-standard study. Predictive values describe what a positive or negative result means in a population with a particular prevalence. This workshop uses exact fictional counts to make those distinctions visible. [FA21]

Construct the reference-standard cohort

Consider 10,000 people, of whom 400 have the condition by an appropriate reference standard and 9,600 do not. A test with 95% sensitivity detects 380 of the 400 affected people and misses 20. With 98% specificity, it correctly classifies 9,408 of the 9,600 unaffected people and produces 192 false positives.

Test result	Condition present	Condition absent	Total
Positive	380	192	572
Negative	20	9,408	9,428
Total	400	9,600	10,000

Sensitivity is 380/400 = 95%. Specificity is 9,408/9,600 = 98%. Positive predictive value is 380/572, approximately 66.4%. Negative predictive value is 9,408/9,428, approximately 99.8%. The same table supports all four measures, but each uses a different denominator.

The 192 false positives outnumber the 20 false negatives, even though the test has high specificity. This is not a contradiction. There are many more unaffected people available to generate false-positive results. The population structure matters.

Change prevalence while preserving the model performance

Now consider another 10,000-person population with 1% prevalence. There are 100 affected people and 9,900 unaffected people. Under the same assumed sensitivity and specificity, the expected exact counts are 95 true positives, 5 false negatives, 198 false positives and 9,702 true negatives.

Positive predictive value becomes 95/(95 + 198), approximately 32.4%. The test's assumed sensitivity and specificity did not change, but a positive result is less likely to represent the condition because the starting probability is lower. This is why indiscriminate testing in a low-probability population can create a substantial confirmation burden.

In real practice, sensitivity and specificity may also vary with patient spectrum, disease stage, specimen quality and threshold. The workshop holds them constant to isolate the effect of prevalence. It should not be interpreted as a promise that a test has identical performance in every setting.

Likelihood ratios express the change in odds

For the model test, the positive likelihood ratio is sensitivity divided by one minus specificity: 0.95/0.02 = 47.5. The negative likelihood ratio is one minus sensitivity divided by specificity: 0.05/0.98, approximately 0.0510. These ratios can update pretest odds under the assumptions of the performance estimate.

At 4% pretest probability, pretest odds are 0.04/0.96 = 0.04167. Multiplying by 47.5 gives post-test odds of approximately 1.979. Converting odds back to probability gives 1.979/(1 + 1.979), approximately 66.4%, matching the table's positive predictive value.

Odds are not the same as probability. A common error is multiplying the pretest probability directly by the likelihood ratio and calling the result a probability. In this example 0.04 × 47.5 = 1.9, which is greater than one and cannot be a probability. The odds conversion prevents that mistake.

A comparator is not automatically a reference standard

Suppose a new assay is compared with an existing assay, but neither independently establishes the condition. Agreement can be measured, yet discordance cannot automatically be assigned as an error by the new test. Positive percentage agreement and negative percentage agreement may be the appropriate terms. Calling them sensitivity and specificity can overstate what the study established.

Resolving disagreements only by repeating the favoured assay can bias the conclusion. The study needs a predefined plan, appropriate independent evidence and transparent reporting of unresolved results. Excluding difficult or indeterminate specimens after seeing their results can make performance look better while reducing its relevance to actual patients.

The reference standard itself can have limitations. Clinical adjudication, culture, molecular testing and composite definitions each introduce different uncertainties. A high-quality report states how the condition was established and whether the assessors knew the index-test result.

Thresholds trade one error type against another

Lowering a positivity threshold can identify more affected people but may also classify more unaffected people as positive. Raising it can improve specificity while missing some affected people. The appropriate trade-off depends on the consequences of error, the availability of confirmation and the intended use.

A screening pathway may tolerate more false positives when confirmatory testing is available and missed disease has serious consequences. A result used for an irreversible intervention may require stronger confirmation. These are pathway decisions, not merely mathematical preferences for the largest percentage.

Transfer questions with reasoned answers

Question 1. Does 95% sensitivity mean that a person with a positive result has a 95% chance of disease? **Answer.** No. Sensitivity conditions on disease status. The probability of disease after a positive result is the positive predictive value, which depends on pretest probability and specificity as well as sensitivity.

Question 2. In the 4% cohort, what fraction of positive results are false positives? **Answer.** 192/572, approximately 33.6%. This is one minus the positive predictive value, not one minus specificity.

Question 3. A study removes all equivocal results before calculating performance. What question should be asked? **Answer.** How equivocal results occur in intended use and how their exclusion changes the estimate. They should be handled according to a predefined analysis plan rather than discarded to improve the apparent result.

Question 4. Can a very high negative predictive value coexist with missed cases? **Answer.** Yes. In the 4% model, 20 affected people test negative while the negative predictive value remains about 99.8%. A reassuring percentage does not mean zero false negatives.

Workshop 4 | Flow-Cytometry Gates, Absolute Counts and Compensation

Flow cytometry measures events that pass through a detection system. A reported cell population is constructed through specimen preparation, instrument settings, controls and a sequence of analysis gates. This workshop uses synthetic event counts and fluorescence values to show why percentages, absolute counts and corrected signals must remain distinct. It is not an instrument operating procedure. [FA18]

Follow the population through the gates

A fictional acquisition contains 100,000 events. After removing unstable acquisition periods and obvious debris, 94,000 remain. A singlet gate retains 90,000. A viability gate retains 85,500. A defined lymphocyte gate contains 30,000 of those viable singlets. Within that lymphocyte gate, 12,000 events meet the CD4 T-cell definition used in the exercise.

The CD4 fraction of gated lymphocytes is 12,000/30,000 = 40%. It is not 12%, which would use all acquired events as the denominator. Both numbers can be calculated, but they answer different questions. The report must state the biologically relevant denominator and the gating definition.

Stage	Events retained	What the number represents
All acquired events	100,000	Includes noncellular and unsuitable events
After acquisition/debris review	94,000	Selected stable event set
Singlets	90,000	Doublets excluded by the defined gate

Stage	Events retained	What the number represents
Viable singlets	85,500	Events passing viability criteria
Lymphocyte gate	30,000	Defined lymphocyte population
CD4 T-cell gate	12,000	Defined subset within the lymphocyte gate

A change in a gate can change the reported fraction without any change in the underlying specimen. This does not make flow cytometry arbitrary; it makes validation, controls and reproducible analysis essential. The reader should ask whether the same biological population was compared at each time point.

Percentages can rise while absolute counts fall

At the first visit, the absolute lymphocyte count is 2,000/µL and the CD4 fraction is 30%. The calculated CD4 count is 600/µL. At the second visit, the lymphocyte count is 1,000/µL and the fraction is 40%, giving 400/µL. The percentage increased, but the absolute count decreased.

This example explains why a percentage alone can mislead. A subset can occupy a larger share of a shrinking population. The method used to combine haematology and flow measurements should be validated, including specimen timing and the compatibility of the denominator definitions.

Single-platform bead methods use a different route to an absolute count. They compare the number of cell events with a known bead quantity or concentration under specified conditions. The calculation still requires correct volumes, recovery assumptions and a suitable acquisition.

Work an absolute-count bead example

Suppose a preparation contains 20,000 counting beads. The instrument acquires 5,000 bead events and 800 events in the target-cell gate. Under the simplified model, the target-cell count in the prepared material is 800/5,000 × 20,000 = 3,200. If that material represents 40 µL of original blood, the estimated concentration is 3,200/40 = 80 cells/µL.

The calculation assumes that the bead and cell observations are representative and that preparation and acquisition satisfy the method. Bead settling, incomplete mixing, incorrect volume or unsuitable gating can invalidate the result. A precise ratio calculated from a flawed acquisition remains flawed.

The label "20,000 beads" must also be interpreted correctly. A vial's assigned bead concentration is not the same as the total number added unless the volume is known. The operator should use the manufacturer's defined quantity and the laboratory's validated formula.

Compensation corrects spectral overlap, not biology

A fluorochrome can emit light detected in more than one channel. Compensation estimates and removes the contribution of one fluorochrome from another detector's signal under the established optical configuration. It does not determine whether the cell truly expresses the biological marker.

In a simple one-way teaching model, detector A records the true A signal plus 12% of the B signal. If true A is 200 and B is 1,000, the observed A signal is 320. Subtracting 0.12 × 1,000 restores 200. Applying a coefficient of 20% would give 120 and overcorrect the signal; applying 5% would give 270 and leave residual spillover.

Real multicolour compensation involves a matrix rather than one isolated subtraction. Appropriate single-colour controls, sufficient signal and matched acquisition settings are required. Fluorescence-minus-one controls help assess gating in the presence of spread from other colours, but they do not replace the controls used to calculate compensation.

Distinguish compensation from spreading error

Even after average spillover is corrected, measurement variation can broaden a negative population in another channel. This spreading affects the ability to separate dim positive events from background. A correctly compensated plot can therefore still have limited resolution for a particular marker combination.

The panel design should consider fluorochrome brightness, antigen density, co-expression and spillover relationships. Increasing the number of colours is not automatically an improvement if the clinically important dim population becomes difficult to distinguish. The question is whether the panel can answer the intended biological question reliably.

Transfer questions with reasoned answers

Question 1. What is the CD4 percentage in the stated lymphocyte denominator? **Answer.** 40%. The 12% figure uses all acquired events and does not describe the same population.

Question 2. A subset fraction rises from 20% to 25% while the parent count falls from 2,000 to 1,200/µL. What happens to the absolute count? **Answer.** It falls from 400 to 300/µL. The percentage increase does not imply an increase in cell number.

Question 3. Can compensation remove dead cells from the analysis? **Answer.** No. Compensation addresses spectral overlap. Viability assessment and appropriate gating address dead-cell inclusion; these are different controls.

Question 4. A bead ratio is stable but the original sample volume was entered incorrectly. Is the absolute count reliable? **Answer.** No. The ratio may be repeatable while the concentration calculation is systematically wrong. Volume identity and unit checking are essential parts of the measurement.

Workshop 5 | Electrophoresis, Immunofixation and Free Light Chains

Protein investigations combine distribution, identity and quantity. Electrophoresis separates proteins into a pattern, immunofixation characterises selected immunoglobulin components, and free-light-chain assays measure unbound kappa and lambda chains. No single result supplies every part of the diagnosis. This workshop uses invented traces and numerical values to practise that integration. [FA23, FA32]

Read the distribution before naming the disease

A broad gamma-region increase suggests a wide distribution of immunoglobulins, often described as polyclonal in the appropriate context. A narrow band or peak raises the possibility of a restricted protein population and requires characterisation. The visual distinction is useful, but electrophoresis alone does not determine whether a monoclonal protein represents malignancy, a precursor condition or another clinical situation.

Migration position also matters. A monoclonal protein may overlap other fractions rather than appear as a clean isolated gamma spike. An apparently modest or inconspicuous electrophoretic abnormality can therefore require additional testing when the clinical question remains important. A normal-looking serum trace does not exclude every light-chain disorder.

The reader should distinguish the trace from the report. A report may include a measured paraprotein concentration, an immunotype and a comment based on additional tests. These are not all directly visible in the same line on the electrophoretic graph.

Convert fractions into concentrations carefully

A fictional specimen has total protein 80 g/L. The integrated fractions are albumin 56%, alpha-1 3%, alpha-2 9%, beta 12% and gamma 20%. Multiplying each fraction by total protein gives 44.8, 2.4, 7.2, 9.6 and 16.0 g/L, respectively. The fractions sum to 100%, and the concentrations sum to 80 g/L.

Fraction	Percentage	Concentration from 80 g/L total
Albumin	56%	44.8 g/L
Alpha-1	3%	2.4 g/L
Alpha-2	9%	7.2 g/L
Beta	12%	9.6 g/L
Gamma	20%	16.0 g/L

If a discrete peak is assigned 8% of the total area under a validated measurement procedure, its estimated concentration is 6.4 g/L. It would be incorrect to multiply 8% by the gamma concentration of 16 g/L unless the 8% had specifically been defined as a fraction of the gamma region. The denominator must match the measurement.

Peak integration can be uncertain when a restricted protein overlaps normal proteins or a broad background. The laboratory's method and reporting practice should be followed. The number should not be presented with more precision than the separation and integration support.

Immunofixation asks which immunoglobulin components align

In a simplified immunofixation model, a restricted band at the same migration position in the IgG lane and the kappa lane supports an IgG-kappa component. A band in a light-chain lane without a corresponding heavy-chain band can require a different interpretation and further investigation. The pattern should be read with the method's controls and limitations.

The same light-chain type does not prove that two bands are the same clone, and a single image cannot resolve every oligoclonal or treatment-related pattern. Therapeutic monoclonal antibodies can appear in protein investigations and mimic or overlap an endogenous component. Medication history and appropriate specialised methods may be needed.

Immunofixation is primarily a characterisation method, not a direct replacement for quantitative measurement. A more intense-looking band on two differently exposed images should not automatically be interpreted as a proportional increase in concentration.

Ratios require both numerator and denominator

A specimen has free kappa 48 mg/L and free lambda 24 mg/L, giving a kappa/lambda ratio of 2.0. Another has kappa 8 mg/L and lambda 4 mg/L, also giving 2.0. The identical ratio does not mean that the two specimens have the same absolute light-chain burden.

Both chains can rise in settings involving reduced renal clearance or polyclonal immune activity. The ratio, absolute concentrations, renal function, assay-specific reference information and clinical context should be integrated. A reference interval from one method or population should not be imported uncritically into another.

A markedly abnormal ratio can support a clonal process in context, but the final diagnosis requires the broader clinical assessment. Conversely, a ratio near the reference interval does not exclude every plasma-cell disorder. Analytical antigen excess and nonlinearity can also affect measurement and should be investigated when results are discordant.

Work a longitudinal comparison

At visit A, a fictional measured component is 8.0 g/L; at visit B it is 6.0 g/L using the same validated method and comparable specimen conditions. The absolute change is −2.0 g/L, and the relative decrease from baseline is 2/8 = 25%. It is not a 2% decrease, and it is not a 33.3% decrease unless the later value is deliberately used as the denominator for a different question.

Whether the change is clinically meaningful depends on measurement variation, disease context and the monitoring framework. A percentage calculation alone cannot establish treatment response. Method changes between visits can further complicate comparison and should be identified in the record.

Transfer questions with reasoned answers

Question 1. Does a narrow electrophoretic peak prove multiple myeloma? **Answer.** No. It identifies a pattern requiring characterisation and clinical evaluation. The diagnosis depends on additional findings and the applicable criteria.

Question 2. Total protein is 70 g/L and a validated peak fraction is 6% of total. What concentration corresponds to the peak? **Answer.** 70 × 0.06 = 4.2 g/L. The denominator is total protein because the fraction was defined against the total area.

Question 3. Why should a free-light-chain ratio be reported with both concentrations? **Answer.** The same ratio can arise from very different absolute values. Concentrations and context are needed to understand the biological and analytical pattern.

Question 4. A new IgG-kappa band appears after therapeutic monoclonal-antibody treatment. What must be considered? **Answer.** The drug may contribute to the observed pattern. Review treatment history and use the appropriate laboratory investigation before assuming that the band represents a new endogenous clone.

Workshop 6 | An Interference Investigation That Answers a Defined Question

An unexpected immunoassay result can arise from the patient's biology, the specimen, the assay architecture or a processing error. A useful investigation asks a series of discriminating questions rather than repeating the same test until a plausible number appears. This fictional case follows a markedly discordant result through dilution, an alternative method and a blocking experiment. [FA19–FA21, FA23, FA29]

Preserve the original observation

The initial result is 18 units, but the patient's clinical pattern and a previous measurement suggest that the value may not represent the intended analyte accurately. The laboratory first confirms identity, specimen integrity, collection timing, units, method and any transcription or dilution factor. These checks are not glamorous, but they prevent an elaborate interference investigation from being built around a simple record error.

The original result and raw signal should be retained. Deleting it after a repeat result appears reassuring removes evidence about the failure. The report can be amended through a controlled process, but the audit trail should preserve the chronology and reason for change.

Medication and supplement history may identify a relevant mechanism. Biotin, therapeutic monoclonal antibodies and other substances affect selected assays, not all assays in the same direction. The investigation must connect the history to the actual architecture rather than rely on a generic list of "interfering drugs".

Use dilution as an experiment

The fictional method permits specified dilutions and has a measuring interval of 2–100 units in the analysed mixture. The following results are obtained from independently prepared aliquots. Automatic dilution correction is disabled for this exercise.

Preparation	Measured mixture result	Factor	Corrected result
Undiluted	18.0	1	18.0
1:5	18.0	5	90.0
1:10	9.2	10	92.0
1:20	4.55	20	91.0
1:40	2.30	40	92.0

The diluted results cluster near 90–92 units, whereas the undiluted result is much lower. This pattern suggests that dilution altered an interference or an antigen-excess effect. It does not by itself identify which mechanism is responsible. The corrected values must also meet the laboratory's predefined agreement criteria and dilution validation.

The 1:40 result is close to the lower measuring limit, so its uncertainty may be relatively important. A further 1:80 dilution might produce a value below the validated interval and would not automatically strengthen the investigation. More dilution is not always better.

An alternative method adds a different analytical view

A second method using a different reagent architecture gives 89 units. Agreement with the valid diluted results supports the possibility that the original undiluted result was misleading. However, the methods may not have identical calibration or analyte recognition, so exact numerical equivalence should not be assumed.

The value of an alternative method depends on how independent it is from the suspected mechanism. Two platforms using the same susceptible binding design may share an interference. A different manufacturer name alone does not establish analytical independence. The laboratory should understand the relevant capture, detection and separation principles.

The report should state which method produced the released result and why. A clinician comparing it with earlier results needs to know when the method or interpretation changed. Silent substitution can create an apparent biological trend that is actually analytical.

Blocking experiments provide supportive, not absolute, evidence

A heterophile-blocking treatment changes the initial result from 18 to 88 units, while a suitable control specimen remains within its expected behaviour. This supports an interference hypothesis involving a blockable interaction. It does not prove that every possible heterophile antibody has been removed or that the treated matrix is equivalent to untreated patient serum.

Blocking reagents can affect recovery and may not neutralise every interfering antibody. The experiment should therefore be interpreted alongside dilution, alternative-method findings and clinical context. A negative blocking experiment does not exclude all antibody-related interference; a positive effect does not uniquely identify the responsible molecule.

The final conclusion can be appropriately specific: the initial undiluted result was inconsistent with validated dilution and alternative-method findings, and a blocking experiment supports assay interference. The corrected report should use the established laboratory pathway and communicate any residual limitation.

Do not average incompatible analytical states

Averaging 18, 90, 92 and 89 would produce a number with no defensible measurement meaning. The observations were generated under different analytical conditions, one of which is suspected to be invalid. Averaging does not distinguish valid from invalid evidence.

Similarly, choosing the result closest to the clinical expectation is not enough. The clinical discrepancy triggered investigation, but the released result should be supported by the analytical findings. The goal is an evidence-based resolution, not confirmation of a preferred story.

Transfer questions with reasoned answers

Question 1. Why is agreement among the corrected diluted results useful? **Answer.** It supports consistent recovery under those validated preparations and identifies a contrast with the undiluted state. It does not uniquely identify the interference or prove universal assay accuracy.

Question 2. Why should an alternative method have a different relevant architecture? **Answer.** Shared architecture can produce shared susceptibility. Independence from the suspected mechanism makes concordant results more informative.

Question 3. Does an unchanged result after blocking exclude heterophile interference? **Answer.** No. The blocking treatment may not address the particular interaction. Interpretation requires the full investigation.

Question 4. What should accompany an amended result? **Answer.** A controlled record of the original observation, investigation, released method/result, reason for amendment and communication of clinically important consequences.

Workshop 7 | Complement Patterns and the Specimen-Handling Trap

Complement measurements include protein concentrations and functional assessments. A functional assay depends on multiple components remaining active through collection, transport and testing. A low result can therefore reflect an in vivo defect, consumption, treatment or an in vitro loss of activity. The workshop uses simplified fictional patterns to practise discrimination, not to assign a diagnosis from one table. [FA03, FA07, FA28]

Map the pathway before interpreting the result

A classical-pathway functional assay requires the relevant early classical components and the common terminal pathway. An alternative-pathway functional assay requires alternative-pathway components and the common terminal pathway. A defect affecting a shared component can reduce both, while a selective early-pathway defect may reduce one more than the other.

Protein concentration and function are not interchangeable. A protein can be present in a usual amount but function abnormally. Conversely, a low concentration may result from consumption rather than inherited absence. The patient's history and the specimen's handling conditions help determine which explanation is plausible.

Fictional pattern	Classical function	Alternative function	Initial interpretation to investigate
A	Markedly reduced	Preserved	Early classical-pathway problem is one possibility

Fictional pattern	Classical function	Alternative function	Initial interpretation to investigate
B	Preserved	Markedly reduced	Alternative-pathway problem is one possibility
C	Markedly reduced	Markedly reduced	Shared component, broad consumption, treatment or handling
D	Preserved	Preserved	Major defect detectable by these assays less likely; not all immune problems excluded

These patterns organise the next question. They do not identify one gene or one disease. Method-specific sensitivity, inhibitors and specimen conditions can alter the pattern, and the appropriate follow-up is selected by a qualified laboratory and clinical team.

A delayed warm specimen creates an alternative explanation

A sample arrives after prolonged transport under conditions outside the receiving laboratory's validated requirements. Both functional results are markedly reduced, but protein concentrations are not comparably abnormal. The learner immediately labels a terminal-pathway deficiency.

The handling history weakens that conclusion. Functional activity may have deteriorated in vitro. The correct response is to assess specimen suitability and obtain a properly handled repeat where indicated, without ignoring any urgent clinical concern. A technically reported number from an unsuitable specimen should not be promoted to a lifelong diagnosis.

The receiving laboratory's instructions are the operative requirements. Some assays need prompt separation, freezing or

controlled transport; others have different stability characteristics. A universal storage instruction copied across all complement tests would be unsafe. Record the actual method's requirements and the specimen's journey.

C3 and C4 describe a different part of the picture

C3 and C4 concentrations can support assessment of complement consumption or deficiency in context, but normal concentrations do not guarantee normal pathway function. Acute-phase production, partial defects and timing can affect the relationship. A single normal concentration should not automatically terminate a clinically justified functional investigation.

A low C4 can be useful in an angioedema investigation but is not sufficient to establish a particular hereditary syndrome. C1 inhibitor protein and function are central to the relevant pathway, and abnormal findings require confirmation in the appropriate clinical context. Normal-C1-inhibitor forms involve a different diagnostic problem.

The report should preserve the distinction between "protein low," "function low" and "clinical diagnosis established." Collapsing these into a single label hides the evidence required for the conclusion.

Work an angioedema pattern

A fictional patient has recurrent swelling without urticaria, low C4, low C1 inhibitor function and a C1 inhibitor protein concentration within the method's reference interval. This pattern is compatible with a dysfunctional-protein mechanism in the appropriate clinical setting, but repeat confirmation and specialist evaluation are required. The normal protein quantity does not contradict the low functional result because the tests measure different properties.

Another patient has low protein and low function. This suggests a different pattern but still requires evaluation of hereditary and acquired possibilities. The patient's age, family history, associated conditions and additional tests matter. The absence of a known family history does not alone exclude a hereditary mechanism.

Laboratory confirmation should not delay emergency treatment of a threatening airway episode. Diagnostic completeness and immediate clinical safety operate on different time scales. The laboratory supports both by communicating clearly and preserving the necessary evidence.

Transfer questions with reasoned answers

Question 1. Does low activity in both pathways uniquely identify a terminal-component deficiency? **Answer.** No. Shared-component defects are one possibility, but consumption, treatment and specimen deterioration can produce similar findings. The pattern needs a mechanism-directed investigation.

Question 2. Why can C1 inhibitor protein be normal while function is low? **Answer.** The amount of protein and its inhibitory activity are different properties. A dysfunctional protein can be present without adequate activity.

Question 3. What does a normal C3 concentration exclude? **Answer.** It excludes neither every complement defect nor every immune disorder. It is one measurement interpreted with function, timing and clinical context.

Question 4. Should a poorly transported specimen be repeated under suitable conditions? **Answer.** Where the handling failure can affect the assay, a suitable repeat is important. The service should document the limitation and coordinate any urgent clinical response rather than issuing an unqualified inherited-deficiency diagnosis.

Workshop 8 | Infectious Serology as a Timeline and a Linked Pathway

Serology detects a host response or a microbial antigen under a specified method. The result depends on exposure timing, immune status, vaccination, passive antibody and the test's specificity. This workshop follows hepatitis B, hepatitis C, syphilis and EBV examples to show why a marker name is not a complete diagnosis. The patient patterns are fictional and do not replace current clinical algorithms. [FA12–FA14, FA24–FA27]

Hepatitis B: interpret the combination

The HBV triple panel includes HBsAg, anti-HBs and total anti-HBc. These markers answer different questions. Surface antigen detection supports current infection in the appropriate context. Anti-HBs can reflect immunity after vaccination or recovery. Total anti-HBc indicates exposure to core antigen and is not produced by ordinary surface-antigen vaccination alone.

Fictional pattern	HBsAg	Anti-HBs	Total anti-HBc	Interpretation to assess
A	Negative	Positive	Negative	Compatible with vaccine-associated immunity
B	Negative	Positive	Positive	Compatible with resolved infection
C	Positive	Negative	Positive	Current infection; acute/chronic distinction needs further evidence

Fictional pattern	HBsAg	Anti-HBs	Total anti-HBc	Interpretation to assess
D	Negative	Negative	Positive	Isolated core-antibody pattern; several explanations remain
E	Negative	Negative	Negative	Susceptibility or an early/other context requiring appropriate assessment

Pattern C should not automatically be called chronic solely from this one panel. Timing, prior results, IgM anti-HBc and other evidence may be relevant. Pattern D can arise in more than one setting, including past infection with waning anti-HBs, a false-positive result, occult infection or a window-period context. The next step follows current guidance and patient risk.

An immunocompromised patient may require a different level of caution. A negative antibody result can be less reassuring when the expected immune response is impaired. The laboratory should not assume that all patients follow the same seroconversion timetable.

Hepatitis C: antibody and RNA are different endpoints

A reactive HCV antibody result indicates that the assay detected antibody, but it does not establish current viraemia. Reflex HCV RNA testing identifies current infection in the recommended pathway. A reactive antibody with RNA not detected can represent resolved infection or a biological false-positive antibody result, depending on context.

Recent exposure can require RNA-based or repeat testing even when antibody is initially negative. The window before a detectable antibody response is a biological limitation, not necessarily an assay

malfunction. The patient's exposure history and timing should therefore accompany interpretation.

A report that simply says "HCV positive" without identifying antibody or RNA can create avoidable misunderstanding. The analyte and the linked interpretation should be explicit. Clinical linkage and treatment decisions follow the appropriate confirmed pathway.

Syphilis: two test families provide complementary evidence

Treponemal and nontreponemal tests have different roles. In a traditional sequence, a reactive nontreponemal screen is followed by treponemal testing. In a reverse sequence, an initial treponemal immunoassay is followed by a quantitative nontreponemal test and, when indicated, a different treponemal assay to resolve discordance. The specific algorithm follows current guidance.

A nontreponemal titre is a dilution endpoint. A change from 1:8 to 1:32 is fourfold, spanning two twofold dilution steps. A change from 1:8 to 1:16 is twofold. Meaningful comparison requires the appropriate method and clinical context; results from different nontreponemal methods or laboratories may not be directly interchangeable.

Treponemal reactivity can persist after treatment. It should not be used alone as a quantitative marker of treatment response. Conversely, a low nontreponemal titre does not independently establish that infection is old, adequately treated or clinically unimportant.

EBV: marker timing is more useful than a single IgM slogan

VCA IgM, VCA IgG and EBNA antibodies develop and persist differently. A pattern with VCA IgM and no EBNA antibody can support recent primary infection in the appropriate context, whereas VCA IgG with EBNA antibody commonly reflects past infection. Atypical patterns and immunocompromised patients require additional interpretation.

Heterophile tests have limitations, including reduced sensitivity in some groups and nonspecific results. They are not interchangeable with EBV-specific serology. The clinician's question, symptom timing and the assay used should remain visible in the report.

For congenital CMV, maternal or infant antibody alone does not establish congenital infection. Early virological testing is required within the appropriate time window to distinguish congenital from later acquisition. This is another example of why "IgG positive" cannot answer every infectious-disease question.

Transfer questions with reasoned answers

Question 1. HBsAg is positive and total anti-HBc is positive in one specimen. Is chronic infection proven? **Answer.** No. Current infection is supported, but the acute/chronic distinction requires timing and additional evidence under the current pathway.

Question 2. HCV antibody is reactive and RNA is not detected. Does the antibody prove current viraemia? **Answer.** No. RNA testing addresses current viraemia; the combined pattern requires the appropriate interpretation and follow-up for recent exposure or other relevant circumstances.

Question 3. A syphilis titre changes from 1:16 to 1:64. What is the fold change? **Answer.** Fourfold. The denominator increased by a factor of four, spanning two twofold steps. Clinical interpretation still requires comparable methods and context.

Question 4. Can infant CMV IgG distinguish congenital infection from transferred maternal antibody? **Answer.** No. The appropriate early virological investigation is required. Antibody detection alone does not establish the timing or source of infection.

Workshop 9 | Antibody Function, Vaccination and Inborn Errors of Immunity

Immune competence is not a single concentration. A patient can have measurable immunoglobulin and still respond inadequately to selected antigens, while another patient can have a low measurement because of treatment, loss, age or a secondary condition rather than a primary genetic defect. This workshop integrates quantities, cell counts and antigen-specific responses without inventing a universal diagnostic threshold. [FA16–FA18]

Begin with the clinical pattern and secondary causes

The fictional patient has recurrent respiratory infections. The initial review examines infection type, severity, frequency, microbiological evidence, age of onset, family history, vaccination, medicines and conditions that can cause secondary immune impairment. The word recurrent is not enough: repeated uncomplicated viral illnesses and invasive bacterial infections raise different questions.

Laboratory quantities should be compared with appropriate age- and method-specific reference information. A child's immunoglobulin pattern should not be judged against an adult interval. Protein loss, haematological disease, immunosuppressive treatment and passive immunoglobulin administration can also alter the findings.

The investigation should be staged to answer the most likely mechanism while remaining alert to severe patterns requiring urgent specialist care. A broad genetic panel is not a substitute for confirming specimen identity, basic phenotype and the clinical question.

A fold rise does not equal a protective concentration

Five fictional antigen-specific measurements are obtained before and after an authorised diagnostic vaccination challenge. The values are arbitrary assay units, not clinical protective thresholds.

Antigen	Before	After	Fold rise
A	0.10	0.40	4.0
B	1.00	2.00	2.0
C	8.00	12.00	1.5
D	0.20	0.20	1.0
E	0.50	3.00	6.0

Antigen A has a larger fold rise than C, yet its final measured value is much lower. Antigen C begins at a high value and may have less room for a large fold increase. The example shows why fold rise and final concentration should not be treated as interchangeable evidence.

Actual interpretation depends on the antigen, vaccine, age, timing, method, baseline status and validated clinical criteria. A threshold used for one vaccine study or population should not be copied as a universal definition of individual protection. The specialist evaluates the complete response pattern rather than selecting the most favourable percentage.

Passive antibody can create a misleading appearance of response

A patient receives immunoglobulin replacement between the two specimens. The later sample contains antigen-specific antibodies, but they may have been passively transferred rather than produced by the patient's own B cells. The timing of treatment therefore changes what the measured increase can establish.

Similarly, transfusion or other antibody-containing products can complicate selected serological investigations. The laboratory should know the treatment history and avoid interpreting a post-treatment antibody result as unqualified evidence of endogenous immune competence.

The solution is not simply to stop medically necessary treatment for a textbook exercise. Diagnostic planning must be coordinated with the treating specialist so that testing answers the question without compromising care.

Cell numbers and functional pathways complement each other

Flow cytometry can identify T-, B- and NK-cell populations and selected maturation patterns. A normal B-cell count does not prove normal antibody production or antigen-specific memory. A low count suggests a different line of investigation but does not by itself identify one gene.

A neutrophil oxidative-burst assay examines a functional pathway rather than merely counting neutrophils. TREC newborn screening examines a marker related to T-cell development, not a complete measure of every aspect of immune function. These distinctions help the learner choose a test that matches the suspected defect.

The IUIS classification organises a large and changing set of inborn errors. A classification category is useful for reasoning, but a gene name should not be assigned from a superficial similarity to one textbook case. Molecular findings require appropriate variant interpretation and clinical correlation.

Work a discordant case

The fictional patient has normal total IgG, a usual circulating B-cell count and an inadequate pattern of selected antigen-specific responses under the specialist's validated assessment. The learner says that normal IgG excludes an antibody problem.

That conclusion confuses quantity with function. Total IgG combines antibodies of many specificities and does not establish an adequate response to every relevant antigen. The findings should be interpreted with infection history, age, timing, secondary causes and the complete immune phenotype. The laboratory report should state the measured evidence rather than overreach to a final diagnosis from one component.

Transfer questions with reasoned answers

Question 1. Which antigen in the table has the greatest fold rise? **Answer.** E, with 3.00/0.50 = 6. The result does not alone establish protection because the units and clinical threshold are not defined for that purpose.

Question 2. Why can a high baseline reduce the usefulness of a fold-rise criterion? **Answer.** A patient already has substantial measured antibody, so a smaller proportional increase may occur despite a meaningful final concentration. Interpretation must include both baseline and final values.

Question 3. Does a normal neutrophil count establish normal oxidative killing? **Answer.** No. Number and function are different properties. A mechanism-directed functional assay may be required.

Question 4. What is the key limitation after immunoglobulin replacement? **Answer.** Measured antibodies may be passive. The result cannot automatically be attributed to the patient's own response without considering treatment timing and the diagnostic plan.

Workshop 10 | Verification, Quality Control and the Difference Between a Shift and Scatter

Quality assessment should distinguish random variation, systematic displacement and clinically important classification errors. This workshop uses a small fictional quantitative dataset, a lot comparison and a diagnostic agreement study. The calculations illustrate reasoning; they are not a universal validation protocol or acceptance limit. [FA19, FA21, FA22]

Calculate repeatability from the actual observations

A control is measured five times: 9.8, 10.1, 10.0, 9.9 and 10.2 units. The mean is 10.0. The deviations from the mean are −0.2, +0.1, 0, −0.1 and +0.2. Squaring and summing gives 0.10. For the sample variance, divide by n − 1 = 4, giving 0.025. The sample standard deviation is the square root of 0.025, approximately 0.158.

The coefficient of variation is standard deviation divided by mean, multiplied by 100: 0.158/10 × 100, approximately 1.58%. The sample is small and describes only the conditions represented. It does not establish long-term precision across operators, days, instruments or concentration levels.

Observation	Result	Deviation from mean	Squared deviation
1	9.8	−0.2	0.04
2	10.1	+0.1	0.01
3	10.0	0.0	0.00
4	9.9	−0.1	0.01

Observation	Result	Deviation from mean	Squared deviation
5	10.2	+0.2	0.04

A method can produce a small coefficient of variation while being systematically biased. If the appropriate target is 12 rather than 10, the close cluster around 10 is repeatable but displaced. Precision is not a synonym for accuracy.

A reagent-lot comparison needs paired evidence

Five fictional specimens are tested with old and new reagent lots. The old-lot values are 10, 20, 40, 80 and 160; the new-lot values are 11, 22, 44, 88 and 176. Each new result is 10% higher than its paired old result. The agreement in rank is perfect, but the systematic proportional difference is clinically important to assess.

A correlation coefficient can be very high in this situation because both methods increase together. Correlation does not establish absence of bias. The laboratory should examine paired differences, proportional effects, concentration dependence and the consequences near decision limits.

The old lot is not automatically the truth. The investigation should consider calibrator assignment, control behaviour, traceability and any appropriate independent evidence. The aim is to determine whether the new lot meets the intended requirements, not merely to force it to reproduce a potentially biased predecessor.

A decision boundary can magnify a small shift

Suppose a fictional qualitative interpretation uses a threshold of 1.00 signal units under a validated method. A control near 0.98 and another near 1.02 challenge the boundary more directly than a strong negative at 0.10 and a strong positive at 8.0. A small shift may leave the strong controls unchanged while altering classifications near the threshold.

This does not mean that every routine control must sit exactly on a decision boundary. The control strategy should be designed for the

failure modes and stability of the method. The example explains why a strong positive alone cannot demonstrate sensitivity to weak reactivity.

The laboratory should also define how equivocal results are handled. Reclassifying them after seeing the desired outcome undermines the study. A transparent plan preserves their frequency and clinical consequences.

Agreement without a reference standard

A new assay and an existing assay are compared in 100 specimens. Both are positive in 28, both negative in 65, the new assay alone positive in 4, and the existing assay alone positive in 3. The observed overall agreement is 93/100 = 93%. Using the existing assay as comparator, positive percentage agreement is 28/31, approximately 90.3%, and negative percentage agreement is 65/69, approximately 94.2%.

These figures do not automatically become sensitivity and specificity because the comparator has not been established as an independent reference standard. The seven discordant specimens need a predefined investigation. Deleting them would produce a misleading 100% agreement among the remaining specimens.

A final verification report should state specimen selection, method, comparator, results, discrepancies, uncertainty, acceptance criteria and the authorised conclusion. The evidence should support the actual intended use rather than a broader claim invented after the study.

Transfer questions with reasoned answers

Question 1. Why divide the sum of squared deviations by four rather than five in the stated calculation? **Answer.** The exercise uses the sample variance estimator, with n − 1 in the denominator because the mean was estimated from the same five observations. A population variance calculation would answer a different mathematical question.

Question 2. Does perfect correlation between lots exclude a 10% proportional bias? **Answer.** No. Perfectly proportional results can correlate perfectly while differing systematically. Paired differences and clinical impact must be assessed.

Question 3. Why retain equivocal and discordant results in the study record? **Answer.** They are part of the method's intended-use performance. Removing them selectively can bias the estimate and conceal cases that require additional work in practice.

Question 4. What is the overall agreement in the 100-specimen example? **Answer.** 93%. The positive and negative agreement measures use different denominators and should not be confused with that overall percentage.

Mastery Assessment | 125 Questions with Reasoned Answers

Use these questions after studying the corresponding chapter. Answer before reading the explanation. The scenarios are fictional and test mechanism, interpretation and limitations rather than memorisation of an isolated label. A correct answer should identify the evidence, explain the mechanism and avoid claiming more than the observation supports. Chapter citations and the reference section support the underlying teaching.

Chapter 1 — Organisation and compartments

1. Why can a normal circulating lymphocyte count coexist with an important tissue-level immune problem?

Reasoned answer. Blood is one compartment. The count does not directly measure migration into tissues, organisation within lymphoid organs, receptor signalling or antigen-specific function. A normal number is useful evidence about quantity, but it does not establish that cells reach the relevant site or perform the required task. The follow-up should match the clinical pattern rather than dismiss it from one count.

2. A learner says that the spleen and lymph nodes filter the same fluid. What is the correction?

Reasoned answer. Lymph nodes receive lymph draining tissues, whereas the spleen surveys blood and has specialised roles involving blood-borne material and red cells. Both support immune organisation, but their inputs and architecture differ. The distinction helps explain why their dysfunction can produce different clinical consequences and why one organ cannot be described as a simple duplicate of the other.

3. What does the separation of B-cell follicles and T-cell zones accomplish?

Reasoned answer. It supports organised encounters among cells, antigen and signals required for activation and differentiation. Cells move between relevant regions during a response; the compartments are not sealed boxes. Their spatial organisation increases the likelihood that appropriate partners interact while preserving specialised microenvironments. A circulating-cell count does not describe that organisation.

4. Why is an epithelial barrier part of immunity rather than merely a covering?

Reasoned answer. It limits entry through physical structure and participates through secretions, local antimicrobial activity and signalling. Damage can change both access for microbes and the inflammatory response. The barrier therefore contributes to prevention and communication before a systemic antibody response is measured. An intact serum immunoglobulin concentration cannot compensate for every form of barrier failure.

5. Does detecting a microbial molecule in a tissue prove that adaptive immunity has already generated memory?

Reasoned answer. No. Detection can initiate innate recognition before a mature adaptive response develops. Memory requires the relevant activation and differentiation processes over time. The presence of antigen, an inflammatory response and durable antigen-specific memory are related but distinct observations. Testing should identify which of these the clinical question actually requires.

Chapter 2 — Innate recognition and killing

6. A neutrophil ingests bacteria but cannot generate an appropriate oxidative burst. Which distinction explains the finding?

Reasoned answer. Engulfment and intracellular killing are separate functions. Formation of a phagosome does not prove normal oxidase activity, phagolysosomal processing or other killing mechanisms. A cell count or an image of ingestion can therefore be normal while a

functional defect remains. A mechanism-directed assay is more informative than repeating the count alone.

7. Why can sterile tissue injury produce inflammation?

Reasoned answer. Innate systems can respond to damage-associated signals as well as microbial patterns. Inflammation is therefore not synonymous with infection. The response may help contain injury and begin repair, but it can also contribute to tissue damage. Microbiology, clinical context and other evidence are needed before an inflammatory marker is treated as proof of a pathogen.

8. Does a high inflammatory marker identify the responsible organism?

Reasoned answer. Usually not. Many inflammatory measurements reflect host response rather than organism identity, and similar changes occur in infectious and noninfectious conditions. The marker may support assessment of severity or trend within a defined context, but pathogen identification requires appropriate direct or indirect tests. The report should not convert a nonspecific response into a species diagnosis.

9. Why do pattern-recognition receptors not need a unique receptor rearrangement for every new exposure?

Reasoned answer. They recognise conserved classes of molecular patterns through germline-encoded recognition systems. This differs from the highly diverse rearranged receptors of adaptive lymphocytes. The distinction helps explain rapid innate responses and the later selection of antigen-specific adaptive clones. It does not mean that innate responses lack regulation, diversity or context-dependent behaviour.

10. What is the consequence of failing to terminate an inflammatory response?

Reasoned answer. Continued recruitment, mediator release and effector activity can damage host tissue even after the initiating threat has changed or resolved. Resolution is an active regulated process, not simply exhaustion of the system. The clinical

significance of an immune response depends on its location, duration and control as well as its ability to attack a target.

Chapter 3 — Adaptive activation and memory

11. Does antigen exposure instruct a lymphocyte to invent a matching receptor sequence?

Reasoned answer. The core clonal-selection model begins with a diverse pre-existing receptor repertoire. Antigen selects cells with suitable recognition, and those cells expand and differentiate under appropriate signals. Later processes can modify antibody affinity within activated B-cell lineages. The distinction prevents confusion between initial repertoire generation and selection during a response.

12. Why is receptor binding alone not always sufficient for full T-cell activation?

Reasoned answer. Co-stimulatory and cytokine signals help determine whether recognition leads to activation, differentiation, nonresponsiveness or another outcome. The immune system integrates context rather than treating every binding event as a command to attack. This regulation is important for tolerance and for understanding why antigen presentation can have different consequences in different settings.

13. A memory response is faster than the first response. Does this mean no new cell proliferation is needed?

Reasoned answer. No. Memory provides an altered starting population and response capacity, but expansion and differentiation can still occur. The timing, magnitude and quality of the response depend on the antigen, memory compartment and host condition. A faster response should not be simplified into an instantaneous or universally sterilising form of protection.

14. What is the difference between a plasma cell and a memory B cell?

Reasoned answer. A plasma cell is specialised for antibody secretion, while a memory B cell preserves antigen-experienced

response potential and can participate in later activation. Their persistence and locations differ, and both contribute to immune protection. Measuring circulating antibody does not directly count either population or describe all future response capacity.

15. Why can an immunosuppressive treatment change vaccine response without changing the vaccine antigen?

Reasoned answer. The host must process antigen, activate appropriate lymphocytes and generate effectors and memory. Treatment can alter one or more of these steps. The same antigen can therefore produce different responses in different host states. Interpretation should include treatment timing, mechanism and the validated clinical vaccination or testing pathway.

Chapter 4 — HLA and presentation

16. Which common presentation route links intracellular peptides with CD8 T cells?

Reasoned answer. Class I presentation commonly displays intracellularly derived peptides to CD8 T cells. The pathway is a foundational model rather than an exception-free rule; cross-presentation and specialised contexts exist. The key is to connect peptide source, processing, presenting molecule and responding cell rather than memorise CD8 as an isolated label.

17. Why is a peptide-binding HLA molecule not itself an antibody?

Reasoned answer. HLA molecules present peptides for cellular recognition and have a different structure, genetic organisation and function from immunoglobulins. Antibodies bind targets through immunoglobulin variable regions. Both participate in specificity, but they operate at different points in the immune response. Treating them as interchangeable recognition molecules obscures how T cells are activated.

18. Does HLA compatibility depend only on ABO group?

Reasoned answer. No. ABO and HLA are distinct systems with different molecules and clinical roles. Transplant assessment can

require donor and recipient HLA information, antibody specificity, crossmatch evidence and other clinical factors. Sharing an ABO group does not establish HLA compatibility, and HLA findings do not replace the applicable ABO requirements.

19. Why can inflammation alter antigen presentation in a tissue?

Reasoned answer. Inflammatory signals can change expression of presenting molecules, co-stimulatory pathways and antigen-processing components. The same tissue may therefore present a different immune context during inflammation. This does not mean every inflamed cell becomes an equivalent professional antigen-presenting cell; cell type and pathway remain important.

20. What does a virtual crossmatch compare?

Reasoned answer. It integrates the recipient's known antibody specificities with the donor's antigen information under a defined programme. It is not a physical mixture of donor cells and recipient serum. Its value depends on accurate typing, current antibody information and the limitations of the assays and interpretation rules used.

Chapter 5 — Antibody structure

21. Which region determines antigen recognition in an IgG molecule?

Reasoned answer. The variable regions of the antigen-binding portions form the binding sites. The Fc region contributes to interactions with receptors and complement-related effector mechanisms, depending on class and subclass. Recognition and effector function are linked within the molecule but are not the same structural task.

22. Can two antibodies recognise the same antigen but have different effector behaviour?

Reasoned answer. Yes. Differences in class, subclass, Fc structure, glycosylation and other properties can alter biological interactions even when target recognition overlaps. Antigen specificity alone does

not fully describe the antibody's effects. Clinical interpretation may require both what the antibody recognises and how it behaves.

23. Why is avidity not identical to affinity?

Reasoned answer. Affinity describes an individual binding interaction, whereas avidity reflects the combined stability of multiple interactions. Multivalent binding can produce a strong overall attachment even when each individual interaction is modest. This distinction is important in agglutination, maturation of antibody responses and interpretation of selected avidity assays.

24. What changes during class-switch recombination?

Reasoned answer. The heavy-chain constant-region expression changes, altering the antibody class and associated effector properties while retaining the rearranged variable-region specificity in the basic model. It is not simply creation of a new unrelated antigen target. Somatic hypermutation and selection can separately influence affinity within the responding lineage.

25. Does a positive binding assay prove that an antibody is neutralising?

Reasoned answer. No. Binding and neutralisation are different functional observations. An antibody can bind a microbial component without preventing the relevant infection or biological activity. A claim of neutralising function requires an appropriate functional assay or validated correlate rather than inference from binding alone.

Chapter 6 — Immunoglobulin classes and compartments

26. Why is serum IgA not a direct measurement of secretory mucosal IgA function?

Reasoned answer. The measurements concern different compartments and molecular contexts. Secretory IgA participates at mucosal surfaces, while serum concentration describes circulating material. A normal serum value does not directly establish secretion, localisation or antigen-specific activity at every mucosal site. The clinical question determines the relevant measurement.

27. Does IgM positivity always identify a new infection?

Reasoned answer. No. IgM can persist, cross-react or be affected by nonspecific and analytical factors. Timing and disease-specific marker combinations matter. A result labelled IgM should be interpreted through the validated infectious-disease pathway rather than used as a universal clock for recent acquisition.

28. Why can a newborn have antigen-specific IgG without producing it?

Reasoned answer. Maternal IgG can cross the placenta. The infant's measured antibody may therefore be passive rather than evidence of the infant's own response or infection. The appropriate diagnostic method must distinguish the biological source and timing relevant to the clinical question.

29. A patient has high total IgE. Is one specific allergy identified?

Reasoned answer. No. Total IgE is not an antigen-specific diagnosis. Even a positive specific-IgE result indicates sensitisation that must be connected with symptoms and exposure. Clinical allergy requires the appropriate assessment; the magnitude of a broad immunoglobulin measurement cannot identify one responsible trigger by itself.

30. Why might a normal total IgG conceal an inadequate response to selected antigens?

Reasoned answer. Total IgG combines many specificities. Its quantity does not show whether the patient has effective antibodies against the antigens relevant to the infection pattern. Antigen-specific functional assessment may be needed within a specialist pathway, with age, vaccination and treatment history considered.

Chapter 7 — Complement mechanisms

31. What is shared by classical, lectin and alternative complement pathways?

Reasoned answer. They converge on activation of central complement components and can feed common effector pathways. Their initiating and early components differ. This architecture explains why some defects affect one functional pathway more than another while shared-component problems can reduce multiple pathway assays.

32. Why is C3b described as an opsonin?

Reasoned answer. Its deposition can facilitate recognition and uptake by cells bearing appropriate complement receptors. Opsonisation makes a target more accessible to phagocytic mechanisms; it is not identical to direct membrane lysis. Complement has several effector routes, and their consequences depend on the target and regulatory environment.

33. Does complement activation always injure the host?

Reasoned answer. No. Controlled activation contributes to defence and clearance. Host injury can arise when activation is excessive, misdirected or inadequately regulated. The clinical problem may involve deficiency, consumption or dysregulation, so interpreting every low or high marker as the same mechanism would be incorrect.

34. What does a normal concentration fail to establish?

Reasoned answer. It does not prove normal function of the protein or the complete pathway. A dysfunctional protein can be present in a usual quantity, and an assay of one component does not test every required interaction. Functional and quantitative tests provide complementary evidence.

35. Why should complement functional specimens follow method-specific handling instructions?

Reasoned answer. Activity can deteriorate or change in vitro. Unsuitable delay, temperature or processing can create a low result that resembles an in vivo problem. The laboratory must assess the specimen's suitability before converting the measurement into a diagnostic conclusion.

Chapter 8 — Cytokines and chemokines

36. Can the same cytokine have different effects on different cells?

Reasoned answer. Yes. Receptor expression, signalling context, cell state and interacting mediators influence the response. A cytokine name does not specify one universal outcome. This helps explain why systemic concentration alone may not describe its local biological effect in every tissue.

37. Why is a chemokine gradient more informative than a single concentration for migration?

Reasoned answer. Directed migration depends on spatial information sensed through appropriate receptors. A uniform concentration and a gradient can produce different behaviour even if their average levels are similar. The cell also needs a functioning motility and signalling system to respond.

38. What is a risk of blocking a cytokine pathway?

Reasoned answer. The targeted pathway may contribute to harmful inflammation and also to normal defence. Blocking it can reduce one disease mechanism while changing susceptibility to infection or other effects. The benefit–risk assessment depends on the agent, patient and current clinical guidance, not solely on the cytokine's inflammatory reputation.

39. Does a low circulating cytokine exclude strong local signalling?

Reasoned answer. No. A mediator may act locally, be rapidly consumed or have a timing pattern not captured by the sample. The measured compartment and collection time limit the inference. A negative systemic measurement cannot automatically describe every tissue microenvironment.

40. Why can several cytokines produce overlapping effects?

Reasoned answer. Signalling pathways and cellular programmes can overlap, and mediators can influence one another. This redundancy and interaction make biological responses more complex

than one-mediator/one-effect diagrams. The simplified pathway is useful for learning but should not be mistaken for a complete network.

Chapter 9 — Hypersensitivity

41. How does type I hypersensitivity differ mechanistically from type IV?

Reasoned answer. The classic type I model involves IgE-associated mast-cell activation, whereas type IV describes T-cell-mediated mechanisms. Their timing and tissue effects can differ, but real conditions may combine pathways. The classification organises mechanisms rather than providing an automatic diagnosis from symptom timing alone.

42. Why can immune complexes cause injury away from the site of antigen entry?

Reasoned answer. Complexes can circulate, deposit or form in tissues and activate inflammatory mechanisms, including complement and recruited cells. Their distribution depends on multiple biological factors. Detecting an antibody in serum does not by itself show where complexes are located or whether they are causing the patient's injury.

43. Does sensitisation guarantee clinical allergy on every exposure?

Reasoned answer. No. A positive specific-IgE result indicates a recognised immune relationship, but symptoms depend on exposure, dose, route, cofactors and clinical context. Diagnosis requires the appropriate history and assessment. The test should not be interpreted as a universal prediction of severity.

44. Why can an antibody directed at a receptor alter function without destroying the cell?

Reasoned answer. Binding can stimulate, block or otherwise change receptor activity. Antibody-mediated disease is not limited to cell lysis. Understanding the target's function helps explain the clinical

pattern and why a test for tissue destruction may not capture the principal mechanism.

45. A delayed skin reaction is observed. Is its mechanism proven solely by the delay?

Reasoned answer. No. Timing is informative but not definitive. The exposure, lesion characteristics, test method and clinical context must be considered. Mechanistic categories should be supported by evidence rather than assigned from one temporal feature.

Chapter 10 — Autoimmunity and probability

46. Why can an autoantibody be present without a corresponding autoimmune disease?

Reasoned answer. Some autoantibodies occur in people without the clinical syndrome, and assays differ in specificity and thresholds. The significance depends on the target, titre, method and pretest probability. A positive result should prompt appropriate interpretation, not automatic conversion into a disease label.

47. What does central tolerance attempt to prevent?

Reasoned answer. It limits the survival or development of strongly self-reactive lymphocytes during maturation through relevant selection mechanisms. Peripheral tolerance provides additional controls after cells leave those developmental sites. Neither layer is an absolute guarantee that self-reactivity never occurs.

48. Does one positive antiphospholipid antibody establish APS?

Reasoned answer. No. Clinical features, persistence and the appropriate laboratory evidence matter, and research classification criteria are not identical to individual diagnostic judgement. Transient positivity and analytical limitations must be considered. The result should be interpreted through the current specialist framework.

49. Why can indiscriminate autoantibody screening generate misleading diagnoses?

Reasoned answer. In a low-probability population, false-positive or clinically nonspecific results can constitute a large share of positives. Testing without a defined question can therefore create unnecessary follow-up and labelling. Selection should be guided by the clinical pattern and the intended use of the assay.

50. What is the appropriate response to a result that conflicts with the clinical phenotype?

Reasoned answer. Reassess the clinical question, specimen, method, timing and possible interference. Consider appropriate confirmation or an alternative analytical route. Neither the laboratory result nor the clinical expectation should be accepted uncritically; the discrepancy is evidence that the explanation needs further work.

Chapter 11 — Immunodeficiency

51. Why should secondary causes be assessed before assuming a genetic immune defect?

Reasoned answer. Medicines, protein loss, infection, malignancy and other conditions can alter immune measurements and function. Identifying the cause changes interpretation and management. A primary inborn error remains possible, but the investigation should not skip common or clinically important acquired explanations.

52. Does a low immunoglobulin result in a child have the same interpretation as the same value in an adult?

Reasoned answer. Not necessarily. Age-related development and method-specific reference information matter. Applying an adult interval to a child can create a false abnormality or miss a relevant pattern. The report should use the appropriate population context.

53. What does a TREC newborn screen measure indirectly?

Reasoned answer. It uses a marker associated with T-cell development to identify infants needing prompt follow-up. It is a screening result, not a complete immune phenotype or a final genetic diagnosis. Abnormal results require timely confirmatory assessment under the newborn-screening pathway.

54. Why can a normal B-cell count coexist with impaired antibody function?

Reasoned answer. Cell presence does not prove normal activation, class switching, differentiation or antigen-specific response. Quantitative and functional tests answer different questions. The clinical infection pattern and specialist assessment guide which additional measurements are needed.

55. Does a variant of uncertain significance prove the cause of an immune disorder?

Reasoned answer. No. The relationship between the sequence change and disease remains uncertain. Interpretation requires appropriate genetic evidence, phenotype, inheritance and sometimes functional studies. A gene-panel result should not be promoted beyond its stated classification.

Chapter 12 — Flow cytometry

56. Why exclude doublets before analysing a cell population?

Reasoned answer. Two attached or coincident cells can generate a combined signal that resembles a different size or marker-expression pattern. Singlet gating reduces that source of misclassification. It is one quality step and does not replace viability, compensation or biological gating controls.

57. A CD4 fraction is 25% and the absolute lymphocyte count is 1,200/µL. What is the calculated count?

Reasoned answer. 0.25 × 1,200 = 300/µL, assuming compatible validated denominator definitions and specimen conditions. The fraction and absolute count should remain linked to the method used. Arithmetic alone cannot correct an unsuitable gate or mismatched sample timing.

58. What does a fluorescence-minus-one control help assess?

Reasoned answer. It helps evaluate gating in the presence of signals and spreading from the other fluorochromes in a panel. It is not a replacement for the single-colour controls used to calculate

compensation. Different controls address different analytical questions.

59. Can a percentage increase demonstrate an absolute-cell increase?

Reasoned answer. Not without the parent-population count. A subset can become a larger fraction while the total population shrinks. Reported percentages should therefore be interpreted with the relevant absolute measurements when the clinical question concerns cell number.

60. Why label synthetic flow plots explicitly?

Reasoned answer. A teaching plot illustrates a concept without representing a validated patient acquisition. Students must not treat its gate boundaries, event distribution or thresholds as a diagnostic reference. The distinction preserves the value of the model while preventing inappropriate clinical transfer.

Chapter 13 — Immunoassays

61. Why can excess biotin affect two assay formats in opposite directions?

Reasoned answer. The direction depends on how biotin–streptavidin binding participates in capture, separation and signal generation. A disrupted sandwich arrangement and a disrupted competitive arrangement can produce different apparent results. The actual assay architecture and current manufacturer information are necessary; one universal direction is unsafe.

62. What is the high-dose hook effect?

Reasoned answer. In a susceptible assay, extreme analyte abundance can interfere with formation of the signal-producing binding arrangement, producing an unexpectedly low signal. Appropriate validated dilution may reveal the discrepancy. It is not a universal property of every assay or an explanation to assume without evidence.

63. Does passing a control exclude patient-specific heterophile interference?

Reasoned answer. No. The control may not contain the interfering antibody present in the patient's specimen. It monitors selected aspects of process performance, not every possible matrix effect. A clinically discordant result may require a patient-specific investigation despite acceptable controls.

64. Why is an assay's measuring interval different from its dilution-extended reportable interval?

Reasoned answer. The measuring interval applies to the material actually analysed. A validated dilution can extend the concentration range reported for the original specimen. The extension depends on acceptable dilution performance; multiplication alone does not establish a valid interval.

65. Can a signal be compared directly between two unrelated platforms?

Reasoned answer. Usually not. Signal scale, calibration, reagent recognition and detection architecture can differ. Even reported concentrations may require method-specific interpretation. A raw optical or luminescent value has meaning within its own calibrated method, not as a universal biological unit.

Chapter 14 — Diagnostic performance

66. A study has 90 true positives and 10 false negatives. What is sensitivity?

Reasoned answer. 90/(90 + 10) = 90%. The denominator includes all reference-positive cases. False positives belong to a different denominator and should not be inserted into this calculation. The estimate also depends on the reference standard and study population.

67. A test has 36 true positives and 98 false positives. What is PPV?

Reasoned answer. 36/(36 + 98), approximately 26.9%. The denominator is all positive test results. The result demonstrates how a low-prevalence setting can yield many false positives despite apparently good sensitivity and specificity.

68. Why should agreement with another assay not automatically be called accuracy?

Reasoned answer. The comparator may itself be imperfect or may measure a somewhat different construct. Without an appropriate reference standard, agreement does not establish truth. Use terminology that matches the study design and investigate discrepancies through a predefined method.

69. What does a confidence interval add to a point estimate?

Reasoned answer. It communicates statistical uncertainty under the model and sampling assumptions. A percentage from a small study can be imprecise even when it looks impressive. The interval does not correct selection bias or an unsuitable reference standard, but it prevents false certainty from the point estimate alone.

70. Does lowering a threshold always improve a diagnostic pathway?

Reasoned answer. No. It may improve sensitivity while increasing false positives. The appropriate balance depends on the consequences of errors, confirmation options and intended use. A threshold should be selected and validated for the pathway rather than judged by one isolated metric.

Chapter 15 — Agglutination and titres

71. What is the reciprocal titre when the last reactive dilution is 1:64?

Reasoned answer. The reciprocal titre is 64 under the stated method. The endpoint depends on the dilution sequence, reaction criterion and assay conditions. It is semiquantitative and should not be treated as an exact concentration without an appropriate calibration relationship.

72. A titre changes from 1:8 to 1:32. How many twofold steps is this?

Reasoned answer. Two steps: 1:8 to 1:16, then 1:16 to 1:32. The overall change is fourfold. Clinical significance requires comparable testing conditions and the disease-specific interpretation framework.

73. Why can antibody excess reduce visible lattice formation?

Reasoned answer. Excess antibody can occupy available binding opportunities without forming the extensive cross-linked network needed for a visible reaction. The relationship between concentration and visible agglutination is therefore not always monotonic. Appropriate controls and dilution can help investigate a suspected zone effect.

74. Is rouleaux identical to immune agglutination?

Reasoned answer. No. Rouleaux reflects a different physical arrangement of cells, often influenced by the protein environment. The distinction matters because the corrective investigation and clinical inference differ. Appearance alone should be interpreted through the appropriate method and controls.

75. Why should serial titres be compared using consistent methods?

Reasoned answer. Reagents, endpoints, incubation and reading criteria can alter the reported titre. A numerical change across different methods may reflect analytical differences rather than biological change. The report should identify method changes and preserve the context of longitudinal comparisons.

Chapter 16 — Infectious serology

76. What does HCV antibody reactivity fail to prove?

Reasoned answer. It does not by itself establish current viraemia. Reflex RNA testing and the current clinical pathway distinguish current infection from other antibody-reactive states. Recent exposure and immune status can also affect interpretation of negative or discordant results.

77. Which HBV marker is not expected solely from ordinary surface-antigen vaccination?

Reasoned answer. Total anti-HBc reflects exposure to core antigen and is not produced by the ordinary surface-antigen vaccine alone. The combined triple-panel pattern is more informative than any

isolated marker. Unexpected patterns require the appropriate follow-up rather than a vaccine-history assumption.

78. Why can a treponemal test remain reactive after treatment?

Reasoned answer. The antibody response can persist beyond active infection. Treponemal reactivity is therefore not a simple quantitative measure of treatment response. Nontreponemal titres, treatment history and clinical context have distinct roles in the follow-up pathway.

79. What limits an early negative antibody test after exposure?

Reasoned answer. The host may not yet have generated a detectable response. The relevant window depends on the infection and assay. Direct detection or repeat testing may be required under current guidance; the initial negative does not universally exclude recent acquisition.

80. Why is congenital-CMV diagnosis a timing problem as well as a detection problem?

Reasoned answer. Virological detection must occur within the appropriate early window to distinguish congenital infection from later acquisition. Antibody alone cannot establish that timing because maternal antibody can be present. The specimen, method and age at collection are essential parts of the evidence.

Chapter 17 — Autoimmune serology

81. Does a homogeneous ANA pattern identify one disease uniquely?

Reasoned answer. No. Patterns organise observations and suggest appropriate follow-up, but they overlap across clinical conditions and can occur without the presumed disease. Titre, antigen-specific testing, method and phenotype must be integrated. A pattern is not a standalone diagnosis.

82. Why measure total IgA when interpreting selected coeliac serology?

Reasoned answer. IgA deficiency can make an IgA-based assay misleadingly negative. The appropriate alternative testing pathway depends on the patient's IgA status and current guidance. A negative result is only reassuring when the assay is suitable for the biological context.

83. How can a gluten-restricted diet affect coeliac testing?

Reasoned answer. Reduced exposure can change the immune response and reduce the sensitivity of selected investigations. The testing plan should be coordinated clinically rather than interpreted without dietary context. The textbook does not prescribe an unsupervised gluten challenge.

84. Why distinguish classification criteria from diagnosis?

Reasoned answer. Classification criteria are designed to create defined groups, often for research, with particular trade-offs in sensitivity and specificity. Individual diagnosis considers a broader clinical context. Meeting or failing a research threshold should not be treated as a universal substitute for specialist judgement.

85. What should be done when two autoimmune assays disagree?

Reasoned answer. Review their targets, methods, thresholds, specimen timing and clinical question. They may not be measuring identical epitopes or analytical constructs. Appropriate confirmation can resolve the discrepancy, but choosing the preferred result without investigating the difference is not sound interpretation.

Chapter 18 — Complement testing and angioedema

86. Low C1 inhibitor function with normal protein quantity suggests what general distinction?

Reasoned answer. A protein can be present but function inadequately. The pattern may support a dysfunctional-protein mechanism in the appropriate setting, but confirmation and specialist assessment are needed. Quantity and activity should not be collapsed into one measurement.

87. Does normal C4 exclude every form of hereditary angioedema?

Reasoned answer. No. The condition includes different mechanisms, and C4 is not a universal standalone exclusion test. The clinical pattern, C1 inhibitor studies and appropriate specialist investigation determine the next step. A single normal screening measurement should not override a compelling phenotype.

88. Why can a transport delay mimic complement deficiency?

Reasoned answer. Functional activity can deteriorate outside validated handling conditions. The resulting low activity may reflect the specimen rather than the patient. Suitability assessment and a correctly handled repeat can be essential before assigning a persistent biological defect.

89. Can both low classical and alternative functional results identify one exact gene?

Reasoned answer. No. The shared pattern has several possible explanations, including common-pathway defects, consumption, treatment and handling effects. Follow-up should test the proposed mechanism with appropriate quantitative, functional and clinical evidence.

90. Should confirmation of an angioedema mechanism delay emergency airway care?

Reasoned answer. No. Immediate clinical safety and definitive diagnosis have different time scales. The treating team follows the emergency pathway while the laboratory supports a timely, well-documented investigation. A request for diagnostic certainty must not postpone necessary urgent care.

Chapter 19 — Biologics and interference

91. Why can daratumumab affect an antibody screen?

Reasoned answer. The therapeutic antibody can interact with CD38 on reagent red cells and create indirect antiglobulin reactivity. The result reflects an interaction within the test system and is not

automatically a new red-cell alloantibody. Treatment history and a validated interference strategy are required.

92. What important limitation follows DTT treatment of red cells?

Reasoned answer. DTT alters selected antigens, including clinically important Kell determinants under commonly used procedures. A negative treated-cell result cannot exclude antibodies against targets that were destroyed. Component selection must account for those blind spots under the validated pathway.

93. Can therapeutic monoclonal antibodies affect protein electrophoresis or immunofixation?

Reasoned answer. Yes, selected therapeutic antibodies can contribute a detectable restricted pattern or overlap an endogenous component. The effect depends on the drug and method. Medication history and appropriate laboratory techniques help avoid misclassifying treatment-related material as a new patient clone.

94. Why should baseline testing precede selected biologic treatment where feasible?

Reasoned answer. Baseline information can preserve an interpretable reference before the therapy changes the assay or immune state. It may support later compatibility or disease-monitoring investigations. The exact testing plan follows the treatment and laboratory pathway; baseline testing does not eliminate all later uncertainty.

95. Does a drug-related analytical interference prove drug-induced haemolysis?

Reasoned answer. No. Interference in a test and destruction of red cells in the patient are different mechanisms. Clinical haemolysis requires its own evidence and, where appropriate, specialised drug-dependent antibody investigation. The terms should not be used interchangeably.

Chapter 20 — Transplant immunology

96. Does an HLA antibody result alone prove graft rejection?

Reasoned answer. No. Antibody specificity and donor relationship contribute to risk assessment, but graft function, tissue findings and the clinical pathway may be needed to establish rejection. The assay is one part of a broader investigation, not a complete diagnosis in isolation.

97. Why is MFI not a universal antibody concentration?

Reasoned answer. Mean fluorescence intensity is a method-dependent signal influenced by antigen density, binding conditions, interference and instrument factors. It can support semiquantitative interpretation within a validated context but should not be converted into a universal biological concentration or threshold across platforms.

98. What can make a virtual crossmatch unreliable?

Reasoned answer. Incomplete donor typing, outdated recipient antibody information, unrecognised assay limitations or an unsuitable interpretation rule can undermine it. The process relies on the quality and relevance of its inputs. A computer-generated conclusion cannot exceed the evidence entered into the system.

99. Why does a transplant programme distinguish pre-existing from newly detected donor-specific antibody?

Reasoned answer. Timing and immunological history affect the interpretation and follow-up question. The same specificity detected before transplantation and emerging later may have different implications in the clinical pathway. Neither timing category independently determines outcome without the rest of the assessment.

100. Can one numerical crossmatch result replace communication with the transplant team?

Reasoned answer. No. The team needs the method, specificity, uncertainty, donor information and clinical context. A number without its limits can produce inappropriate reassurance or alarm. Clear interpretation connects the laboratory evidence to the authorised transplant decision.

Chapter 21 — Tumour immunity

101. What does checkpoint blockade change?

Reasoned answer. It interferes with selected inhibitory immune interactions, potentially altering T-cell activity against tumour and other targets. It does not directly guarantee tumour destruction in every patient. The mechanism also helps explain immune-related adverse effects and the need for clinical monitoring.

102. Why can loss of a target antigen undermine a targeted cellular therapy?

Reasoned answer. Recognition depends on the target being present in a suitable form. A tumour population that reduces or loses the target may evade the therapy even when the effector cells remain active. Target expression and tumour heterogeneity therefore matter alongside the treatment's initial potency.

103. Does a falling tumour-associated laboratory marker prove that every tumour cell has been eliminated?

Reasoned answer. No. Marker production, clearance, treatment effects and assay behaviour can change independently of complete tumour eradication. The trend can support monitoring within a validated framework, but clinical and other disease assessments remain necessary.

104. Why can immune activation produce toxicity outside the tumour?

Reasoned answer. Immune pathways are not confined to malignant tissue. Altered regulation can affect normal organs, and effector responses may recognise or injure nonmalignant targets. The benefit–risk balance requires monitoring and specialist management, not the assumption that more immune activity is always better.

105. A marker falls from 120 to 70 units. What is the relative reduction from baseline?

Reasoned answer. The decrease is 50 units; 50/120 × 100 is approximately 41.7%. The arithmetic describes the marker change,

not a direct percentage of tumour cells destroyed. Clinical interpretation remains separate from the calculation.

Chapter 22 — Proteins and light chains

106. Why does a broad gamma increase differ from a narrow restricted peak?

Reasoned answer. The patterns describe different distributions of protein migration. A broad increase commonly reflects a wider immunoglobulin population, while a narrow peak prompts characterisation of a restricted component. Neither shape alone provides a complete clinical diagnosis.

107. What does matching migration in IgG and kappa immunofixation lanes support?

Reasoned answer. It supports an IgG-kappa component in the appropriate controlled interpretation. The result characterises the protein pattern but does not by itself determine whether the underlying condition is malignant or how it should be treated.

108. Kappa is 48 mg/L and lambda is 24 mg/L. What is the ratio?

Reasoned answer. The kappa/lambda ratio is 2.0. Both concentrations, renal function, assay-specific reference information and clinical context remain necessary. Another specimen can have the same ratio with very different absolute values.

109. Why can renal function affect free-light-chain interpretation?

Reasoned answer. Clearance contributes to circulating concentrations, so changes in renal function can alter the pattern independently of one simple clonal-production model. The method and appropriate reference context should be considered. A ratio or concentration should not be interpreted without relevant clinical information.

110. Does a normal serum electrophoretic appearance exclude every light-chain disorder?

Reasoned answer. No. Some clinically important light-chain patterns may not produce an obvious intact-immunoglobulin peak. Free-light-chain assays, immunofixation and other investigations can add information when indicated. The test pathway should match the suspected condition.

Chapter 23 — Complex immune investigations

111. Why is phenotype–genotype agreement important?

Reasoned answer. It helps determine whether a detected variant plausibly explains the patient's observed immune problem. Discordance can reveal an alternative diagnosis, incomplete penetrance, an analytical limitation or an uncertain variant interpretation. Genetic information should be integrated, not treated as automatically decisive.

112. Can a normal broad screen end every immunodeficiency investigation?

Reasoned answer. No. Screening tests cover selected quantities and functions. A strong clinical pattern may justify more specific testing even when broad measurements are normal. The next step should be mechanism-directed rather than indiscriminate repetition or an unqualified dismissal.

113. Why record infection organisms rather than only the number of infections?

Reasoned answer. The type of organism, site and severity can point toward different immune functions. Ten self-limited upper-respiratory illnesses and recurrent invasive infections do not convey the same risk. Accurate clinical characterisation improves the choice and interpretation of laboratory tests.

114. What can make a post-treatment immune phenotype difficult to interpret?

Reasoned answer. Treatment may alter cell populations, antibody quantities, function or assay behaviour. The result then describes the treated state rather than an untreated baseline. Timing and

mechanism should be recorded so the laboratory does not attribute every change to the underlying disease.

115. Why should an uncertain result be stated explicitly?

Reasoned answer. It identifies the limit of the current evidence and the question that further testing must answer. Concealing uncertainty can lead to an unsupported diagnosis or unsafe reassurance. Specific uncertainty is useful when linked to an appropriate action and follow-up plan.

Chapter 24 — Interference workbench

116. Corrected dilution results are 90, 92 and 91 while the undiluted result is 18. What is the key finding?

Reasoned answer. The undiluted state is inconsistent with valid diluted recovery. The pattern supports investigation of interference or antigen excess, but it does not uniquely identify the mechanism. Method validity, preparation and an independent analytical route should be considered before release.

117. Why is averaging the discordant undiluted and diluted values inappropriate?

Reasoned answer. The values may come from analytically different states, one of which is invalid. An average hides the discrepancy without establishing which measurement reflects the intended analyte. The investigation should resolve the mechanism rather than create a compromise number.

118. What does a result change after blocking demonstrate?

Reasoned answer. It supports an interaction affected by the blocking treatment, provided controls and recovery are suitable. It does not identify every interfering molecule or prove that all interference has been removed. The conclusion should remain linked to the experiment's limits.

119. Can an alternative method share the same interference?

Reasoned answer. Yes. Different instruments can use similar vulnerable binding architectures. The laboratory should select an

alternative that is informative about the suspected mechanism, not merely a different brand. Agreement is most useful when the relevant sources of error are sufficiently independent.

120. Why preserve raw data during an interference investigation?

Reasoned answer. Signals, dilution factors, timing and original observations allow the reasoning to be checked and the report to be amended transparently. Deleting inconvenient values can erase the evidence needed to understand the event or assess other affected results.

Chapter 25 — Integrated interpretation

121. What are the three levels that should not be collapsed in a case conclusion?

Reasoned answer. Observed evidence, mechanistic interpretation and the proposed clinical or laboratory action should remain distinguishable. The action may be justified even while some mechanism remains uncertain. Keeping the levels separate makes the conclusion auditable and easier to revise when new evidence appears.

122. Why is a correct disease label insufficient in a laboratory case assessment?

Reasoned answer. The learner must explain how the observations support the label, what alternatives were considered and which limitations remain. A memorised answer may fail when the next patient has a different specimen, treatment or timing. Reasoning supports transfer beyond the example.

123. What should change when a result is urgent but incomplete?

Reasoned answer. Communication and escalation should match the clinical risk. The laboratory should state what is known, what remains unresolved and the expected next information, while the authorised clinical team makes necessary decisions. Urgency does not justify inventing certainty or withholding a significant concern.

124. Why should a final report identify the method when it affects interpretation?

Reasoned answer. Assay targets, thresholds, calibration and interference susceptibility can differ. The method explains what the result can support and whether it is comparable with earlier measurements. A bare number can conceal a clinically important analytical change.

125. What is the strongest evidence that a learner understands a case?

Reasoned answer. The learner can explain the mechanism, select a discriminating follow-up, calculate with correct units and denominators, recognise the model's limits and adapt the reasoning to a changed scenario. Repeating the original conclusion without these links demonstrates recall, not yet reliable professional interpretation.

Applied glossary | Meaning, use and limits

These definitions support retrieval and interpretation. Each entry includes a source anchor, and the subject index points to teaching locations in this PDF. A concise definition does not replace the full mechanism and case discussion in the relevant chapter.

Absolute cell count

Number of cells in a defined blood volume, such as cells per microlitre. A percentage becomes an absolute count only when linked to the correct parent population and its absolute concentration. Changes in the denominator can make percentages and counts move in different directions. [FA18]

Accuracy

Closeness of a measurement to an appropriate reference value or accepted truth. It is not interchangeable with precision, which concerns repeatability. A method can produce tightly clustered but systematically biased results; assessment must consider the intended clinical use and reference approach. [FA19]

Adaptive immunity

Antigen-specific defence involving lymphocyte receptor diversity, selection, expansion and differentiation. Its response develops through interactions among cells, antigen and regulatory signals. Memory can alter later responses, but detection of one antibody does not describe every adaptive function or guarantee protection. [FA04]

Affinity

Strength of an individual binding interaction between an antibody binding site and its target epitope. It differs from avidity, which describes the combined stability of multiple interactions. Assay design, target presentation and antibody heterogeneity affect how binding is observed experimentally. [FA06]

Affinity maturation

Selection within an activated B-cell response that favours variants with improved antigen binding after somatic mutation and competition. It is not the same as generating the initial receptor repertoire or switching immunoglobulin class. The process depends on organised cellular help and selection. [FA04]

Agglutination

Visible aggregation of particles or cells through an appropriate binding lattice. The observed grade depends on the reaction system and method, not simply antibody concentration. Antigen or antibody excess can disrupt lattice formation and produce weaker apparent reactions despite abundant reactant. [FA06]

Allele

One of the alternative sequence forms at a genetic locus. Allelic differences can affect immune molecules or their expression, but a detected variant is not automatically a disease-causing finding. Interpretation requires the gene, inheritance, phenotype, assay coverage and evidence for functional consequence. [FA16]

Allergen

An antigen capable of provoking an allergic response in a sensitised context. Demonstrating sensitisation by a laboratory test is not identical to demonstrating clinically relevant allergy. Exposure history, symptoms and the authorised diagnostic pathway determine how the laboratory finding should be used. [FA09]

Alloantibody

Antibody directed against an antigen that differs among members of the same species and is absent from the person's relevant native antigen profile. Transfusion and transplantation provide important exposure settings. Its significance depends on specificity, method, history and clinical context. [FA20]

Analytical interference

An effect that alters the measured signal or reported result without representing the intended measurand in the expected way. Heterophile antibodies, biotin and therapeutic antibodies are examples in appropriate assays. Direction and magnitude are method-dependent and require evidence, not assumption. [FA20]

Analytical sensitivity

Ability of an analytical method to detect or measure small amounts of its target under defined conditions. It should not be confused with clinical sensitivity, which is the proportion of affected people testing positive in a specified population and study design. [FA21]

Anaphylaxis

A serious systemic hypersensitivity reaction requiring prompt clinical recognition and emergency management. Laboratory markers may support selected investigations but must not delay treatment. A normal or absent laboratory result does not alone exclude the clinical event or replace assessment of its timing and presentation. [FA09]

Antibody

Immunoglobulin molecule with antigen-binding regions and class-dependent effector properties. Binding specificity and effector function are related but distinct. An antibody can be measured by concentration, binding activity or another assay-defined property; those measurements are not interchangeable descriptions of protective function. [FA06]

Antibody avidity

Combined stability of multiple antibody-target interactions in a defined system. Avidity testing may help selected timing questions, but its interpretation is assay- and infection-specific. A high or low result should not be generalised into a universal clock for every infection. [FA26]

Antigen

A molecular structure recognised by an immune receptor or antibody. Recognition does not automatically imply harmfulness, immunogenicity or disease. Antigen detection measures the target or an assay-defined part of it; antibody detection measures a host response or transferred antibody instead. [FA01]

Antigen presentation

Processing and display of antigen-derived material in a form recognised by relevant lymphocytes, commonly peptide presentation by HLA molecules to T cells. The source, processing route, presenting cell and co-stimulatory context influence the response. Presentation alone does not always produce activation. [FA05]

Antigen-presenting cell

Cell that displays antigen in a context relevant to lymphocyte recognition. Professional antigen-presenting cells have specialised roles in initiating and regulating responses. Expression of an HLA molecule does not make every cell equivalent to a dendritic cell in its capacity to activate naive T cells. [FA05]

Apoptosis

A regulated cell-death process with characteristic signalling and structural changes. It contributes to development, immune regulation and removal of selected cells. It is not simply a synonym for any cell death, nor does every non-apoptotic death represent an entirely unregulated accident. [FA04]

Area under the curve

A summary measure for a receiver-operating-characteristic curve across decision thresholds. It describes discrimination within the studied data, not calibration, clinical benefit or performance at one chosen threshold. Population selection and verification methods affect its interpretation. [FA21]

Autoantibody

Antibody recognising a self-associated target. Some autoantibodies are clinically useful markers or contribute to injury; others occur without the disease a learner expects. Interpretation requires specificity, titre or concentration where relevant, method, pretest probability and the clinical pattern. [FA10]

Autoimmunity

Immune recognition and response directed against self-associated structures. It is not equivalent to autoimmune disease, which requires clinically meaningful pathology. The presence of an autoantibody supports a defined question but does not automatically establish a diagnosis or explain every symptom. [FA09]

Autoinflammation

Inflammatory disease mechanisms strongly involving innate immune dysregulation rather than a simple model of antigen-specific autoantibody injury. Boundaries with other immune disorders can overlap. The clinical pattern and molecular or functional evidence guide investigation more reliably than a broad label alone. [FA16]

Avidity

Overall binding stability arising from multiple interactions between binding partners. It depends on affinity, valency and geometry. A pentameric antibody can have high avidity even when individual binding-site affinities are modest, illustrating why single-site strength and whole-complex behaviour are different concepts. [FA06]

B cell

Lymphocyte lineage capable of antigen recognition through immunoglobulin receptors and differentiation into antibody-secreting and memory populations. B-cell number, phenotype and function are separate questions. A normal count does not establish normal class switching, antibody specificity or response to immunisation. [FA04]

Background signal

Assay response not attributable solely to the intended target-specific interaction. It may arise from nonspecific binding, optical effects or other components of the system. Appropriate blanks and controls help characterise it, but subtracting a background value does not correct every interference. [FA19]

Basophil

Circulating granulocyte involved in selected inflammatory and hypersensitivity responses. Its number and activation are different measurements. A functional basophil assay requires a defined clinical question, controlled stimulation and appropriate interpretation; it is not interchangeable with a total IgE concentration. [FA09]

Bayesian reasoning

Updating the probability of a hypothesis using prior probability and the likelihood of observed evidence. In diagnostic testing, pretest probability and test performance jointly shape post-test probability. A positive result cannot have the same meaning in every population regardless of prevalence and selection. [FA21]

Bias

Systematic difference between a measurement and an appropriate comparator or reference. It can coexist with excellent repeatability. In research, bias also describes systematic distortion from study design or conduct. The relevant meaning should be identified rather than treating all disagreement as random error. [FA19]

Biological variation

Variation in a measurand within or between people arising from biological processes. It differs from analytical imprecision. Interpretation of serial results should consider timing, physiology, treatment and measurement characteristics rather than assume that every numerical change represents a new disease process. [FA19]

Biotin interference

Method-dependent disturbance of assays that use biotin-related binding architecture. The result may be falsely high or low depending on the assay design. Supplement or treatment history, timing and the actual platform matter; a single universal direction or withholding interval is unsafe. [FA20]

Bystander injury

Damage to host tissue occurring during an immune response even when that tissue is not the intended primary target. Mediators, recruited cells and altered local conditions can contribute. The concept explains why an effective defence can still produce clinically important collateral effects. [FA08]

Calibration

Establishment of the relationship between an assay signal and assigned values of calibrators. It supports calculation within a validated range and model. Calibration does not independently prove specimen suitability, absence of interference or correctness of every patient result. [FA19]

Chemokine

Signalling molecule with an important role in directing cell movement and positioning. Gradients and receptor expression help organise recruitment and tissue localisation. A circulating concentration alone does not fully describe the spatial signal experienced by cells within an inflamed tissue. [FA08]

Class switching

Change in the immunoglobulin heavy-chain constant region used by an activated B-cell lineage while preserving the rearranged antigen-binding specificity. It changes effector properties and distribution. It is distinct from somatic mutation and affinity selection, although these processes can occur in related responses. [FA04]

Clinical sensitivity

Proportion of people with the defined condition who test positive in an appropriately designed study. It depends on disease definition, spectrum, stage and method. It is not the same as positive predictive value or the smallest analytical quantity the assay can detect. [FA21]

Clinical specificity

Proportion of people without the defined condition who test negative in the study context. It is not a guarantee that every positive result represents disease. Pretest probability and the consequences of false positives remain essential when using the result clinically. [FA21]

Clonal expansion

Increase in cells derived from an activated clone. It amplifies a selected response but does not imply that all expanded cells have identical later function or location. Differentiation, survival and regulation determine how the expanded population contributes to immunity or pathology. [FA04]

Clonal selection

Preferential activation and expansion of cells whose pre-existing receptors recognise an encountered target in an appropriate context. It differs from the idea that an antigen directly instructs a lymphocyte to invent a matching initial receptor. Later mutation and selection can further alter B-cell responses. [FA04]

Co-inhibition

Regulatory signalling that limits or changes immune activation. Checkpoint pathways illustrate how inhibitory signals contribute to tolerance and response control. Blocking such a pathway can enhance antitumour activity but also disturb normal regulation; the effect is not equivalent to indiscriminate immune strengthening. [FA31]

Co-stimulation

Additional signalling that helps determine the outcome of antigen-receptor recognition, particularly in lymphocyte activation. It contributes to the distinction between an activating context and tolerance or nonresponsiveness. Receptor binding alone therefore cannot be treated as a universal command for full immune activation. [FA04]

Coefficient of variation

Standard deviation divided by the mean, commonly multiplied by 100 to express a percentage. It describes relative imprecision for suitable positive-valued data. Near-zero means and inappropriate data scales can make it misleading, and it does not measure systematic bias. [FA19]

Compensation

Correction for predictable fluorescence spillover between detection channels in a conventional flow-cytometry system. It requires appropriate controls and does not correct poor gating, nonspecific binding or every spectral problem. A compensated value can legitimately be negative after background-related correction. [FA18]

Competitive immunoassay

Assay in which the measured target competes within a binding system. Signal often decreases as target concentration increases in common designs, but the exact architecture governs interpretation. Interference direction cannot be inferred safely without understanding the platform and assay format. [FA19]

Complement

A regulated network of plasma and surface-associated proteins that supports opsonisation, inflammation and, in appropriate circumstances, membrane injury. Activation, consumption, deficiency and specimen handling can all affect testing. A single component concentration does not describe the function of the entire network. [FA03]

Complement consumption

Reduction in available components associated with activation and use within the complement system. Low values can support this hypothesis but also arise through inherited deficiency, synthesis changes or specimen problems. Functional and component tests must be interpreted together with the clinical pattern. [FA03]

Complement regulator

A molecule that restrains or directs complement activation and protects appropriate host surfaces. Failure of regulation can cause injury even when complement proteins are present. Testing therefore may need to address function and control, not merely whether each measured component is detectable. [FA28]

Confidence interval

A range generated by a statistical procedure that expresses uncertainty in an estimate under stated assumptions. It is not the range containing a fixed proportion of individual patient results. Sample size, study design and model assumptions determine what the interval can support. [FA21]

Conjugate

A labelled binding reagent used to convert a recognition event into a measurable signal. The label may be enzymatic, fluorescent or another type. Its specificity, stability, concentration and interaction with the assay system affect performance; a bright signal is not automatically a specific one. [FA19]

Cross-presentation

Presentation of externally acquired material through a pathway that can support class I-associated recognition by CD8 T cells. It qualifies the simple intracellular/class I and extracellular/class II teaching model. Exceptions should refine that model rather than make the underlying processing distinction meaningless. [FA05]

Cross-reactivity

Binding or response to more than one target because of shared or sufficiently similar recognised features. It can be biologically meaningful or analytically misleading. A positive assay may therefore require confirmation or contextual interpretation rather than assumed unique recognition of one disease-specific target. [FA06]

Cytokine

A signalling molecule that influences cell behaviour through relevant receptors and pathways. Its effect depends on concentration, location, timing, responding cells and other signals. The same cytokine can have different effects in different contexts; a one-word pro-inflammatory or anti-inflammatory label is often incomplete. [FA08]

Cytotoxic T cell

T-cell effector capable of killing appropriately recognised target cells through controlled mechanisms. Recognition and killing require more than the mere presence of CD8 on a cell. Counts, phenotype, antigen specificity and functional activity are distinct measurements in an immune investigation. [FA04]

Dendritic cell

Specialised immune cell important in antigen capture, processing, presentation and coordination of lymphocyte responses. It is different from a follicular dendritic cell, which has a distinct origin and role in displaying antigen-containing material within follicles. Similar names should not obscure different biological functions. [FA05]

Detection limit

A method-defined boundary for distinguishing a signal from background with specified performance. It does not necessarily establish the lowest concentration that can be quantified with acceptable precision and bias. Results near the boundary require careful wording and appropriate interpretation of uncertainty. [FA21]

Dilution factor

Ratio of total mixture volume to the volume of original sample used, when the dilution is prepared as specified. Sequential factors multiply. The original concentration is recovered only when the method permits that correction and the result remains within a validated measurement range. [FA19]

Direct antiglobulin test

Test examining detectable immunoglobulin or complement-related coating of sampled red cells. A positive result is not itself a diagnosis of haemolysis. The method, pretransfusion findings, clinical state and other evidence determine how the observation contributes to an immune or transfusion investigation. [FA23]

Doublet

Two cells or particles acquired together as an event in a flow-cytometry system. Doublets can distort apparent co-expression and population counts. Appropriate discrimination and controls help reduce the effect, but gating should not be used to remove inconvenient biology without a justified strategy. [FA18]

Effector function

Action through which an immune response influences a target, such as killing, neutralisation, recruitment or phagocytosis. Recognition and effector function are related but distinct. A binding assay may demonstrate recognition without proving that the measured antibody or cell performs the clinically relevant action. [FA06]

Epitope

The part of a target recognised by an antibody or immune receptor in the relevant context. Epitopes can depend on sequence, conformation or molecular arrangement. Altered target presentation in an assay can change binding, which is one reason different methods may produce different results. [FA06]

Ex vivo artefact

A change occurring after collection rather than reflecting the patient's in-vivo state. Handling-related complement activation, cell deterioration and other specimen effects can distort interpretation. Timing, temperature, transport and preparation must therefore be considered before assigning every abnormality to disease. [FA19]

Fc receptor

Receptor recognising the constant-region portion of appropriate immunoglobulins. Such interactions connect antibody binding with cellular effector responses or transport. Receptor type, antibody class or subclass and cellular context influence the outcome; Fc binding is not a single uniform biological event. [FA06]

FcRn

A receptor involved in IgG handling and transport, including protection from degradation and placental transfer in relevant contexts. Its role helps explain distribution and persistence of IgG. It should not be confused with every activating Fc receptor on an effector cell. [FA06]

Flow cytometry

Measurement of individual particles or cells passing through a detection system, often using scatter and fluorescence. Interpretation requires appropriate preparation, controls, gating and denominators. A coloured dot plot is not self-validating evidence of a cell identity or diagnosis. [FA18]

Fluorochrome

A molecule that emits light after suitable excitation and is used as a label in relevant assays. Excitation and emission properties, brightness, spillover and reagent performance affect panel design. A marker's apparent absence can reflect technical limitations as well as true biological non-expression. [FA18]

Follicular dendritic cell

A specialised stromal cell that helps organise follicles and display antigen-containing complexes for B-cell selection. It is not simply another conventional dendritic cell presenting processed peptide to naive T cells. The distinction links tissue architecture with the mechanisms of affinity selection. [FA04]

Free light chain

Immunoglobulin light chain circulating without an associated heavy chain. Concentrations and ratios support selected protein and plasma-cell investigations, but renal function, inflammation, method and the whole protein pattern affect interpretation. An abnormal ratio is not an automatic standalone diagnosis of malignancy. [FA32]

Functional assay

A test designed to observe a biological activity rather than only the amount or presence of a component. Its result depends on the complete tested pathway and specimen conditions. Normal concentration can coexist with abnormal function, while a functional abnormality may require localisation with additional tests. [FA16]

Gate

A defined region or rule used to select events for analysis, particularly in flow cytometry. The gate determines the population and denominator. It should be justified by controls and biology, not moved solely to obtain an expected percentage or remove an unexplained population. [FA18]

Germinal centre

Organised follicular site in which activated B-cell populations undergo processes including mutation, selection and differentiation with cellular help. It supports affinity maturation and response development. Its function cannot be inferred fully from a circulating B-cell count or total serum immunoglobulin concentration. [FA04]

Haemolysis

Destruction of red cells with release or clearance of their contents. It can be immune, nonimmune or specimen-related depending on context. A positive immune test does not by itself prove the mechanism, and a visibly haemolysed sample may not represent in-vivo haemolysis. [FA23]

Hapten

A small molecular structure that can be recognised immunologically but often requires association with a larger carrier to provoke an appropriate immune response. Recognition and immunogenicity are therefore not identical. The concept helps explain selected drug-associated and experimental immune responses. [FA09]

Heterophile antibody

An antibody capable of unintended interactions in an immunoassay, including binding assay reagents. It can produce method-dependent false results. Blocking studies or alternative methods may help, but no single interference test excludes every possible effect or establishes the true concentration automatically. [FA20]

Histocompatibility

The relationship between donor and recipient immune recognition relevant to transplantation. It involves more than ABO group alone. HLA typing, antibody specificity, crossmatch information, transplant type and clinical factors contribute to assessment under a specialised programme. [FA30]

HLA

Human leukocyte antigen system, including molecules involved in peptide presentation and major transplantation relationships. HLA antigens are distinct from ABO red-cell determinants. Typing and antibody tests answer different questions and require interpretation of allele, antigen and assay resolution. [FA05]

Hook effect

A misleading assay response caused by very high target concentration in susceptible assay architectures, often producing an inappropriately low reported result. A validated dilution or alternative method can help investigate it. The phenomenon is method-dependent and should not be assumed whenever results are discordant. [FA19]

Humoral immunity

Immune mechanisms involving soluble factors, including antibodies and complement. It is broader than total immunoglobulin concentration alone. Quantity, specificity, affinity, effector capability and pathway integrity all contribute, so a normal single concentration cannot certify every aspect of humoral defence. [FA01]

Hyper-IgM pattern

A pattern in which immunoglobulin class distribution raises a question about switching or related immune function. It is not one diagnosis and does not require the same numerical findings in every patient. Clinical features, age, treatment and targeted functional or molecular testing guide interpretation. [FA16]

Hypersensitivity

Immune-mediated injury arising from a response that is inappropriate in its target, magnitude, location or regulation. Classical categories organise mechanisms but do not capture every clinical overlap. Demonstrating sensitisation or an antibody is not equivalent to proving the mechanism of a patient's symptoms. [FA09]

Immune complex

A complex formed through antigen-antibody binding. Its size, clearance, location and complement interactions influence biological effects. Detection of binding in an assay does not automatically demonstrate pathogenic tissue deposition; clinical and laboratory context are needed to connect the complex with disease. [FA03]

Immune memory

Altered response capacity retained after an immune encounter through relevant cell populations and sustained products. It may change the speed and quality of later responses. A measurable antibody titre is one possible correlate, not a complete measure of all memory or guaranteed sterilising protection. [FA04]

Immunodeficiency

Impaired immune capability that may be inherited or acquired and may affect selected pathways rather than every immune function. Infection pattern, autoimmunity, inflammation and other features can guide investigation. A normal screening count or concentration does not exclude all functional defects. [FA16]

Immunofixation

A protein investigation that uses immunologic detection to characterise restricted immunoglobulin-related bands after separation. It complements electrophoresis and quantitative measurements. A detected pattern must be interpreted with clinical findings, renal function and other evidence rather than treated as an isolated disease label. [FA32]

Immunogen

Material capable of inducing an immune response in a suitable host and context. It is related to but not identical with an antigen, which is defined by recognition. Dose, route, adjuvant effects and host state can influence whether recognition develops into a measurable response. [FA04]

Immunoglobulin class

Category defined by the heavy-chain constant region, such as IgG, IgA, IgM, IgE or IgD. Classes differ in distribution and effector properties. Class alone does not establish antigen specificity, and a positive IgM result is not a universal marker of a newly acquired infection. [FA06]

Immunophenotype

Pattern of measurable cellular features, often surface or intracellular markers. It supports identification and characterisation of populations but is not synonymous with genotype or full function. Marker intensity, preparation, controls and gating affect the observed pattern. [FA18]

Immunoprecipitation

Formation or capture of complexes through antigen-antibody interaction in a defined system. Classical lattice formation depends on reactant proportions and other conditions. Lack of visible precipitate may reflect an unfavourable ratio or method limitation rather than complete absence of antigen or antibody. [FA06]

Imprecision

Random variability among repeated measurements under specified conditions. It may be described by standard deviation or coefficient of variation. Precision under repeatability conditions can differ from between-day or between-laboratory performance, and good precision does not rule out systematic bias. [FA19]

Inborn error of immunity

An inherited or genetically mediated immune disorder within a broad and evolving classification. Manifestations can include infection susceptibility, autoimmunity, inflammation, allergy and malignancy-related risk. The name does not imply that every disorder presents in infancy or is detectable with one screening test. [FA16]

Indirect immunofluorescence

A method using a labelled secondary reagent to reveal binding of an unlabelled primary antibody in a substrate-based system. Pattern, titre, substrate and reading conditions affect interpretation. A visual pattern can guide further testing but does not uniquely diagnose every associated disease. [FA10]

Innate immunity

Defence involving barriers, germline-encoded recognition, phagocytes and other rapid response systems. It interacts extensively with adaptive immunity. Rapid recognition does not mean unregulated or nonspecific in every sense; the response depends on pattern, tissue context and signalling networks. [FA08]

Interference investigation

A structured attempt to explain a result that may not reflect the intended measurand. It may compare dilution behaviour, alternative platforms, blocking or treatment studies and clinical evidence. No single manoeuvre is definitive in every assay, and the investigation must preserve its detection limits. [FA20]

Isotype

Immunoglobulin category determined by constant-region characteristics, including class and subclass. It influences effector function and distribution. It is different from antigen-binding specificity, so antibodies of different isotypes can recognise related targets while acting differently in the host. [FA06]

Likelihood ratio

A measure of how much a test result changes odds under a defined performance model. For a binary test, positive and negative likelihood ratios use sensitivity and specificity in different formulas. They should be applied to suitable pretest odds, not directly added to a probability. [FA21]

Limit of quantification

Lowest level at which a method can quantify a target with specified acceptable performance. It is distinct from merely detecting a signal above background. A detectable result below quantification capability should not be reported as an equally precise numerical concentration without appropriate qualification. [FA21]

Lymph node

Organised lymphoid structure receiving lymph from tissues and supporting immune-cell interactions. It differs from the spleen, which surveys blood. Its follicles, T-cell regions and flow pathways help explain how antigen location and cellular migration influence an immune response. [FA01]

Lymphocyte repertoire

The collection of antigen-receptor specificities within a lymphocyte population. Diversity, selection and expansion shape it over time. A total lymphocyte count does not describe repertoire breadth or the presence of an adequate response to a particular pathogen or antigen. [FA04]

Macrophage

A phagocytic cell with roles in tissue maintenance, microbial handling, inflammatory signalling and antigen-related interactions. Functions vary across tissues and activation states. Counting circulating monocytes does not directly measure every tissue macrophage population or its functional capacity. [FA08]

Major histocompatibility complex

Genetic region and associated molecular system central to antigen presentation and transplantation biology. In humans the HLA terminology is used. The simple class I/class II framework is foundational, but specialised presentation routes and expression patterns require appropriate qualification. [FA05]

Mean fluorescence intensity

A summary of fluorescence intensity in a defined population, with the exact statistic and scaling requiring specification. It is affected by instrument settings, reagents, controls and gating. It is not a universal concentration unit or a direct quantitative measure of biological injury. [FA18]

Memory B cell

Antigen-experienced B cell retaining capacity for a later response. It differs from a plasma cell specialised for sustained antibody secretion. Both contribute to humoral memory, but a serum concentration does not directly count either population or predict every future response. [FA04]

Monoclonal pattern

A restricted immunoglobulin-related pattern suggesting production by a limited clone in the relevant analytical context. It requires characterisation and clinical interpretation. A narrow band can have different causes, and therapeutic antibodies or other analytical effects may complicate protein studies. [FA32]

Mucosal immunity

Defence at surfaces such as the respiratory and gastrointestinal tracts, involving barriers, secretions, local cells and organised lymphoid structures. Serum measurements capture only part of this system. A normal circulating immunoglobulin value does not fully describe local protection or mucosal function. [FA01]

Natural killer cell

Lymphocyte contributing to innate effector responses through a balance of activating and inhibitory signals. Its function is not identical to that of an antigen-specific cytotoxic T cell. Number, phenotype and cytotoxic capability are distinct questions in an immune investigation. [FA08]

Negative predictive value

Proportion of negative test results that occur in people without the defined condition in the studied setting. It depends on prevalence and selection as well as test performance. A negative result can be highly reassuring in one context and insufficient in another. [FA21]

Neutralisation

Reduction of a target's biological activity through an appropriate immune interaction, such as blocking viral entry or toxin action. A binding antibody assay does not necessarily measure neutralisation. The assay used must match the functional question before a result is described as protective. [FA06]

Opsonisation

Coating of a target with factors that facilitate recognition and handling by relevant effector cells. Antibody and complement can contribute through different receptors. Binding, ingestion and killing remain separate steps, so successful coating does not prove that the entire defence pathway functions normally. [FA03]

Oxidative burst

Rapid production of reactive oxygen-related species by activated phagocytes through appropriate enzymatic pathways. Functional testing addresses a mechanism that a cell count cannot establish. Normal phagocyte numbers or ingestion do not exclude a defect in intracellular killing. [FA08]

Paraprotein

A restricted immunoglobulin-related product identified in an appropriate protein investigation. The term should not be used as a diagnosis by itself. Quantity, type, clinical state and additional studies determine significance, and analytical interference can complicate both detection and measurement. [FA32]

Pattern-recognition receptor

A receptor recognising classes of microbial or damage-related features through germline-encoded systems. It helps initiate and regulate innate responses. Recognition of damage can occur without infection, explaining why inflammation is not synonymous with the presence of a particular microorganism. [FA08]

Phagocytosis

Cellular uptake of particles or microbes into an internal compartment. It is one stage of a broader response that includes recognition, processing, killing and disposal. An image showing ingestion does not establish that the ingested organism was destroyed or that all related immune functions are intact. [FA08]

Plasma cell

A differentiated B-lineage cell specialised for antibody secretion. Plasma-cell products, survival niches and tissue distribution contribute to humoral responses. Measuring an antibody in blood does not directly reveal how many plasma cells produced it or whether the response is protective. [FA04]

Polyclonal pattern

A broad immunoglobulin-related pattern produced by multiple responding clones. It can occur in several inflammatory or immune contexts and is not a single diagnosis. Interpretation considers the electrophoretic shape, quantitative findings, clinical setting and any superimposed restricted component. [FA32]

Positive predictive value

Proportion of positive results that represent the defined condition in the studied population. It changes with pretest probability and selection. Even a test with high specificity can produce many false-positive results when used indiscriminately in a very low-prevalence population. [FA21]

Post-test probability

Probability assigned after incorporating the observed test evidence into an appropriate pretest assessment. It is not determined by the positive or negative label alone. Test performance, disease spectrum, timing and dependence among repeated tests affect the update. [FA21]

Precision

Closeness of repeated measurements to one another under stated conditions. Repeatability, intermediate precision and reproducibility describe different scopes of repetition. Precision does not measure bias, and a precise assay can consistently produce an incorrect result if calibration or interference is problematic. [FA19]

Pretest probability

Probability of a condition before the particular test result is considered. It reflects the clinical setting, symptoms, history and selection, not simply a national prevalence figure. A defensible estimate helps interpret positive and negative results without assuming they have identical meaning everywhere. [FA21]

Prozone

Reduced visible lattice formation associated with antibody excess in susceptible classical reactions. It is one explanation for an unexpectedly weak or negative result. A validated dilution strategy can help investigate it, but dilution can also change other interferences and must be interpreted within the method. [FA06]

Reference interval

A range derived from a defined reference population and measurement process. It is not automatically a disease decision threshold or a guarantee of health. Age, physiology, method and population affect applicability, and values inside the interval can coexist with clinically important disease. [FA19]

Regulatory T cell

A T-cell population with functions that help regulate immune responses and tolerance. Phenotypic markers support identification but do not by themselves prove every suppressive function. Immune regulation is a network, so one cell count cannot summarise the entire tolerance system. [FA04]

Repeatability

Precision assessed under closely similar conditions over a short interval, typically using the same method and environment. It differs from performance across days, lots, operators or laboratories. A small repeatability study should not be presented as complete validation of clinical performance. [FA19]

Reproducibility

Agreement of measurements across specified changed conditions, such as laboratories or methods, depending on the study definition. It is broader than repeating one specimen in one run. The sources of variation and intended use must be stated before comparing performance claims. [FA19]

Sandwich immunoassay

Assay architecture in which the target is recognised by capture and detection reagents in a defined arrangement. Signal commonly increases with target within the validated range. Excess target or interference can disrupt that relationship, so the calibration model is not valid without limits. [FA19]

Sensitisation

Development or demonstration of immune recognition relevant to a target. In allergy, laboratory sensitisation is not identical to symptomatic clinical allergy. In transfusion, cell sensitisation can mean antibody coating without visible agglutination. The intended meaning should be specified from the context. [FA09]

Seroconversion

Change from a previously nonreactive to a reactive antibody result in a defined testing context. It can support evidence of a developing response, but timing, assay comparability, passive antibody and analytical variation require consideration. One unpaired positive result does not document seroconversion. [FA12]

Serology

Investigation of antigen-antibody-related findings in serum or other appropriate specimens. It can assess host response, antigen presence or other immune phenomena depending on the method. The word does not imply that every positive result proves active infection or that every assay measures the same biological event. [FA15]

Somatic hypermutation

Introduction of mutations into immunoglobulin variable-region sequences in activated B-cell responses, followed by selection in an appropriate context. It differs from class switching and from inherited germline variation. Mutation alone does not guarantee improved affinity; selection determines which variants persist. [FA04]

Specificity

A context-dependent term that may describe molecular recognition or clinical test performance. Molecular specificity concerns target binding; clinical specificity concerns negative results among people without the defined condition. Confusing the meanings can make an analytically selective assay seem more diagnostically definitive than it is. [FA21]

Spillover

Detection of fluorescence from one label in an additional channel in a flow-cytometry system. Compensation or spectral analysis addresses relevant signal mixing under controlled conditions. Spillover is not the same as biological co-expression, and its correction does not repair every source of background or nonspecific binding. [FA18]

Standard deviation

A measure of dispersion around a mean under an appropriate numerical model. Sample and population formulas differ in their denominator. It describes variability in the measured data, not the uncertainty of every clinical conclusion or the magnitude of systematic bias. [FA19]

T-cell receptor

Antigen-recognition receptor expressed by T cells, with recognition commonly dependent on an appropriate presenting context. It differs structurally and functionally from a secreted antibody. T-cell number does not directly measure receptor diversity, antigen specificity or the functional quality of the response. [FA04]

Therapeutic antibody interference

Disturbance of an assay caused by an administered antibody interacting with the system or appearing as a measured protein. Anti-CD38 effects in transfusion testing illustrate one mechanism. The therapy, timing, platform and baseline information guide investigation; the interference is not automatically a new disease antibody. [FA23]

Titre

A method-defined endpoint, often the highest dilution still producing a specified reaction. It is not an absolute antibody concentration and depends on the assay and reading criteria. Comparisons require suitable method consistency, and fold changes must be interpreted within the disease-specific clinical question. [FA15]

Tolerance

Mechanisms limiting harmful immune responses against appropriate self or innocuous targets. Central and peripheral processes contribute, and failures can occur at different levels. Tolerance is not simply absence of immune cells; it involves active regulation, selection and context-dependent response control. [FA09]

Total analytical error

A framework combining relevant contributions of bias and imprecision for a specified measurement purpose. Its calculation and acceptance depend on the model and intended use. It should not be invoked as a universal single number that proves every clinical result is correct. [FA19]

TREC

T-cell receptor excision circle, a marker used in relevant newborn screening approaches to assess aspects of newly generated T-cell output. A screening result is not a complete immune diagnosis. Abnormal findings require the authorised follow-up pathway, and normal screening does not exclude every immune disorder. [FA17]

Valency

Number of binding interactions a molecule or target can support in a given context. It contributes to lattice formation and avidity but is not identical to single-site affinity. Geometry and accessibility influence whether the potential binding sites can actually interact simultaneously. [FA06]

Virtual crossmatch

An interpretation comparing recipient antibody information with donor antigen data under a defined transplant programme. It is not a physical mixture of cells and serum. Reliability depends on typing, current antibody evidence, assay limitations and the programme's validated criteria. [FA30]

Window period

Interval during which a developing infection or response may not yet be detectable by a particular test. Different targets and methods have different timing. A negative result must be interpreted against exposure timing and the appropriate testing pathway rather than as an unconditional exclusion. [FA24]

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[FA28] Maurer M and colleagues; WAO/EAACI. International guideline for hereditary angioedema: 2021 revision and update. Allergy. 2022;77:1961–1990. doi:10.1111/all.15214.

https://onlinelibrary.wiley.com/doi/10.1111/all.15214

C1 inhibitor function, protein and C4; confirmation and limitations.

[FA29] Chapuy CI and colleagues. Resolving the daratumumab interference with blood compatibility testing. Transfusion. 2015. doi:10.1111/trf.13069.

https://pubmed.ncbi.nlm.nih.gov/25764134/

Primary interference study; DTT changes antigen detection.

[FA30] van den Broek DAJ and colleagues; ESOT. Clinical utility of post-transplant monitoring of donor-specific antibodies in stable renal transplant recipients: consensus report. Transpl Int. 2023;36:11321.

https://www.frontierspartnerships.org/journals/transplant-international/articles/10.3389/ti.2023.11321/full

HLA antibody interpretation, MFI limitations and clinical integration.

[FA31] National Cancer Institute. Immune Checkpoint Inhibitors; CAR T Cells.

https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors

Checkpoint mechanisms. Companion: https://www.cancer.gov/about-cancer/treatment/research/car-t-cells . Source artwork not reproduced.

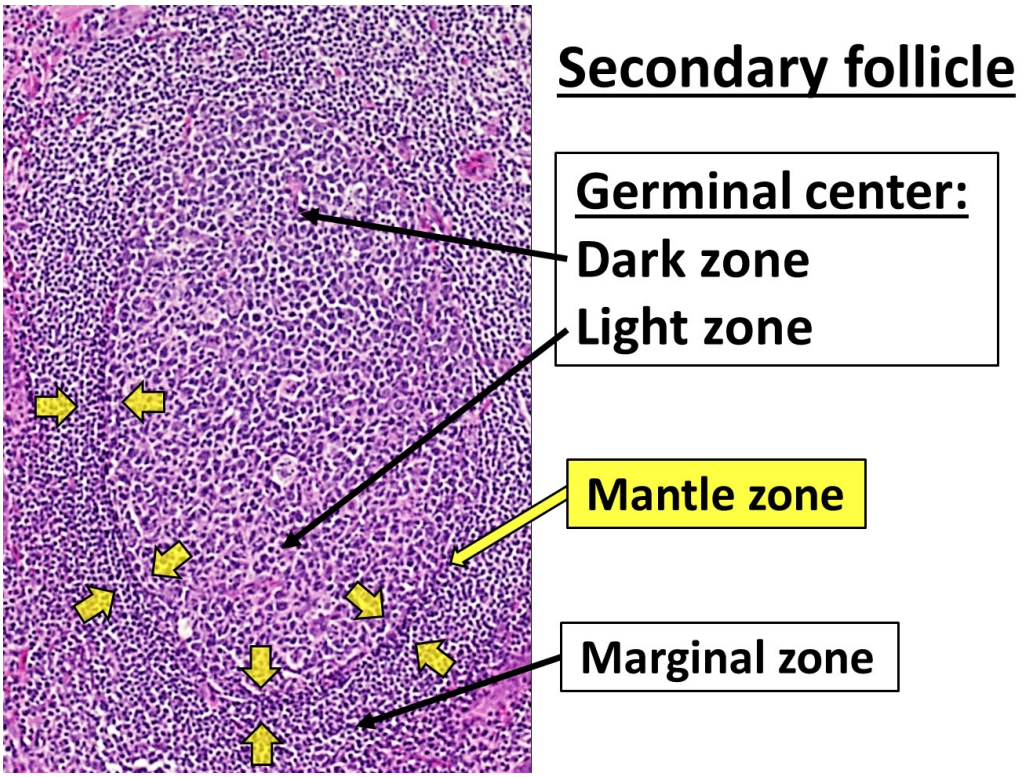
[FA32] Newcastle Hospitals Laboratories; Oxford University Hospitals. Serum electrophoresis, immunofixation and free immunoglobulin light chains.

https://laboratories.newcastle-hospitals.nhs.uk/test-directory/serum-electrophoresis/

Companion: https://laboratories.newcastle-hospitals.nhs.uk/test-directory/immunofixation/ and https://www.ouh.nhs.uk/immunology/diagnostic-tests/tests-catalogue/free-immunoglobulin-light-chains/ . Local reference intervals are not universal.

A real follicle: organisation in mucosal immunity

Photomicrograph • H&E-stained; secondary follicle in colonic mucosa-associated lymphoid tissue



This is a tissue observation, not a blood-cell count. The labelled dark and light zones belong to the germinal centre; surrounding mantle and marginal regions help organise the follicle. The section comes from colonic mucosal lymphoid tissue, not a lymph node. A two-dimensional section cannot display every three-dimensional relationship. [FA02, FA04]

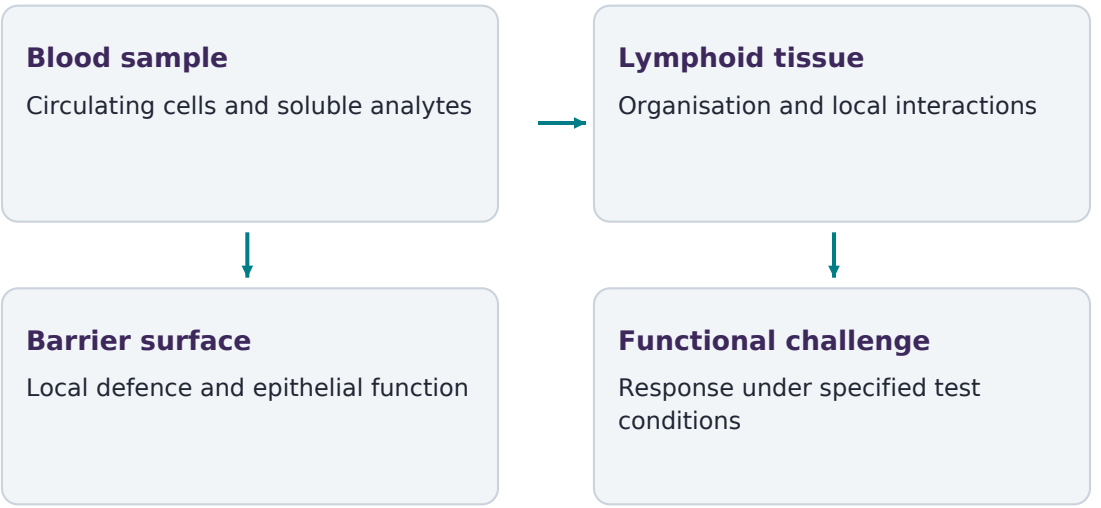
Explain it: Why can a normal circulating lymphocyte count coexist with impaired local immune function?

Reasoned answer: A count measures cells in the sampled blood compartment. It does not establish trafficking, receptor function, tissue organisation or the quality of the local response.

Photograph: Mikael Häggström, own work, 11 November 2022, CC0. Source: Wikimedia Commons, “Dark, light, mantle and marginal zones of a secondary follicle.png”. Reproduced from the supplied credited image; resized, not recoloured. No diagnostic claim.

Which compartment did the test observe?

An original location-and-measurement map • Read with Chapters 1, 6 and 12



The same patient can have measurements from several compartments that appear discordant because they answer different questions. Normal total immunoglobulin concentration does not, by itself, prove an adequate specific antibody response. A normal circulating cell count does not prove normal receptor signalling. The investigation should identify the function at issue before selecting a measurement. [FA02, FA06, FA16]

Explain it: A referral says “normal immune tests” after only a total white-cell count. What is the missing reasoning?

Reasoned answer: The report has generalised one measurement to the entire immune system. Identify the clinical pattern and which compartment or function was actually tested; further investigation depends on that question, not on a universal list of tests.

Original educational schematic. Sources: FA02, FA06, FA16. Boxes are categories of observations, not an order set. The arrows show possible comparisons, not anatomical flow.

A positive result: count the right denominator

Original fictional diagnostic dataset • Read with Chapter 14 and Workshop 3

	Condition present	Condition absent
Test positive	90	90
Test negative	10	810
Total	100	900

Sensitivity: 90/100

90% in this model

Positive predictive value

90/180 = 50%

Of the 180 positive results, 90 occur in people with the reference-defined condition. Sensitivity instead uses the 100 people with the condition as its denominator. The two percentages answer different questions. These invented values assume an appropriate reference standard and specified population; they are not an estimate for a real assay. [FA21]

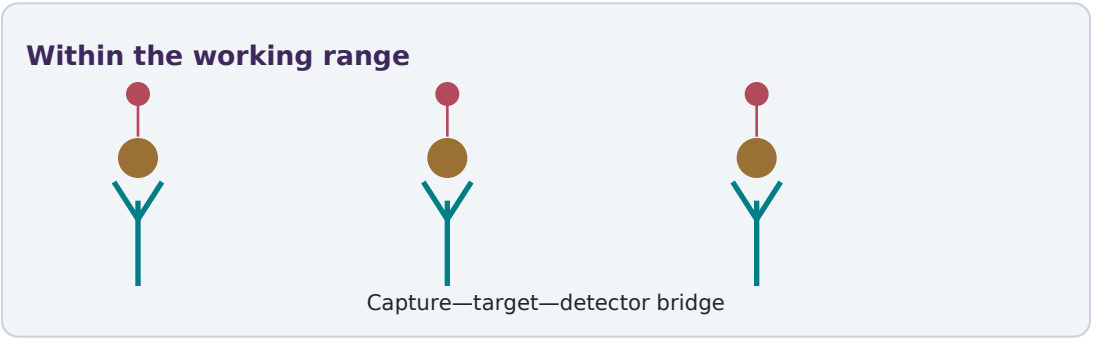
Explain it: Why is “90% sensitivity” not equivalent to “a positive result means a 90% chance of disease”?

Reasoned answer: Sensitivity conditions on having the condition. The predictive question conditions on a positive result and therefore includes the false-positive group. Population frequency and specificity affect that denominator.

Original synthetic data. Arithmetic: sensitivity 90%; specificity 90%; PPV 50%; NPV 810/820 ≈ 98.8%. Source framework: FA21. No assay performance is claimed.

Sandwich immunoassay: a bridge must form

Original molecular schematic • Read with Chapters 13 and 24



Some sandwich assays can give misleadingly low results at extreme antigen concentrations because the expected labelled bridge is not formed efficiently. The effect depends on assay design. An appropriate dilution or alternative validated method may help investigate a clinically discordant result; the instrument number alone does not establish the mechanism. [XFA01]

Explain it: Why might a dilution yield a higher dilution-corrected concentration than the undiluted specimen?

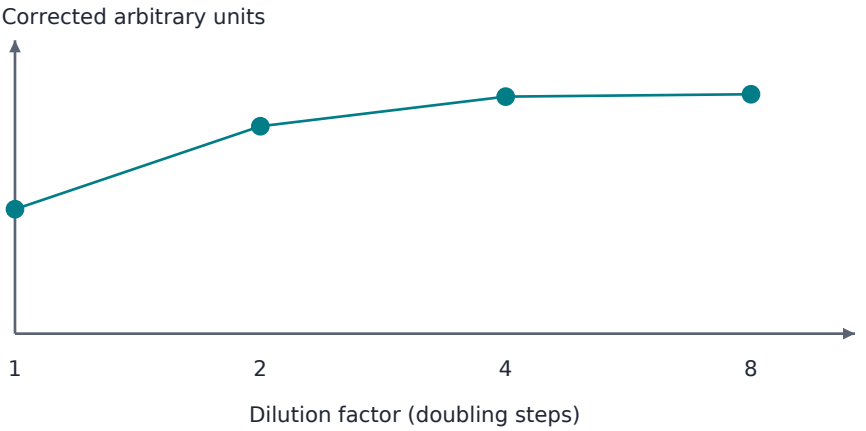
Reasoned answer: Dilution may reduce an interference or high-dose effect. The result supports further investigation only when the dilution is validated and interpreted within the assay range; it is not a licence to multiply every result without checking the method.

Original model, not to scale. XFA01: Raverot V et al. (2022), Prolactin immunoassay: does the high-dose hook effect still exist? DOI: 10.1007/s11102-022-01246-8; PMID 35793045. Study supports assay-dependent susceptibility, not a universal effect. No source artwork reproduced.

Dilution behaviour: parallel is not identical

Original fictional measurement series • Read with Workshop 6

Dilution factor	Measured	Corrected
1	42	42
2	35	70
4	20	80
8	10.1	80.8



The corrected value approaches a plateau rather than remaining constant from the first dilution. This pattern prompts an interference or range investigation; it does not identify the interfering substance. The model uses arbitrary units and assumes the specified dilutions are permitted for the assay. Real interpretation also considers analytical imprecision and specimen matrix. [FA19, FA20]

Explain it: Is 80.8 proof that the true patient value is exactly 80.8?

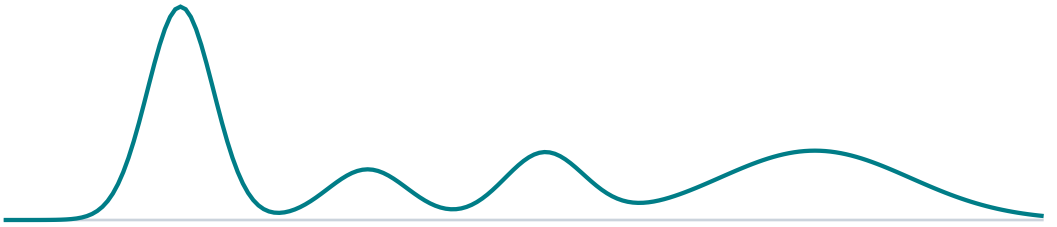
Reasoned answer: No. The convergence is one observation. Calibration, uncertainty, dilution validity and alternative explanations still matter. Reporting excess decimal places would imply precision not established by this experiment.

Original fictional data and vector plot. Corrected result = measured result × dilution factor. Reference framework: FA19–FA21. No clinical decision threshold.

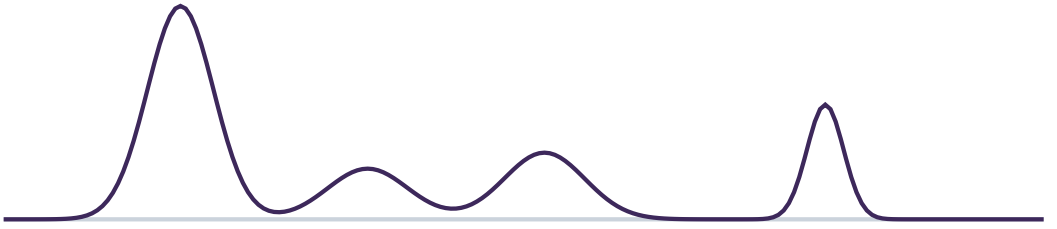
Broad and narrow protein patterns

Original synthetic electrophoresis profiles • Read with Chapter 22

Broad increase



Narrow restricted feature



Shape is an observation, not an isotype assignment. A restricted feature may require immunofixation or other follow-up, and a small clone may not produce a conspicuous spike. Therapeutic antibodies, specimen factors and method differences can complicate interpretation. These profiles are synthetic and do not represent measured patient concentrations. [FA23, FA32]

Explain it: Can a narrow feature alone establish malignant disease or its immunoglobulin class?

Reasoned answer: No. Follow-up testing characterises the protein, while clinical assessment establishes its significance. Neither a narrow shape nor an absent obvious spike answers every diagnostic question.

Original mathematical curves for teaching; no patient trace or external artwork. Sources: FA23 and FA32. Peak areas, distances and heights are not quantitatively calibrated.

Complement: interpret pathways and specimen integrity

Original functional comparison • Read with Chapters 7 and 18

Model pattern	Question to investigate
CH50 low; AH50 preserved	Classical-pathway component issue?
AH50 low; CH50 preserved	Alternative-pathway component issue?
Both low	Shared components, consumption or handling?
Both preserved	Other immune mechanisms still possible

A screening pattern helps choose the next investigation; it is not a diagnosis of a named deficiency. Pathway assays depend on multiple components and suitable specimen handling. Functional loss caused by collection or transport can imitate a biological abnormality. Concentration and function are different measurements. [FA03, FA07, FA28]

Explain it: Both functional screens are low after an uncertain transport delay. Should the report name a terminal complement deficiency?

Reasoned answer: No. Review specimen requirements and quality, obtain appropriate confirmation and correlate with the clinical question. A repeat suitable specimen may distinguish handling-related loss from persistent dysfunction; targeted component testing may then be indicated.

Original comparison, not a universal diagnostic algorithm. CH50/AH50 naming and reference intervals vary by service. Sources: FA03, FA07 and FA28; follow the testing laboratory’s specimen instructions.

Visual evidence, sources and study routes

The image, mechanism and measurement have different roles

Authentic microscopy

Page 294 contains a real H&E; image of a secondary follicle in colonic mucosal lymphoid tissue. It is not a patient-specific diagnostic case. Creator: Mikael Häggström; CC0 public-domain dedication. The existing credited image was resized without recolouring. Source: https://commons.wikimedia.org/wiki/File:Dark,_light,_mantle_and_marginal_zones_of_a_secondary_follicle.png

Original models

Pages 295–300 contain newly drawn conceptual diagrams and invented numerical examples. They are not manufacturer validations, clinical traces or diagnostic microscopy. Read the limitations with each figure, then return to the linked narrative chapter.

Evidence routes

FA02–FA06 support immune organisation and mechanisms. FA19–FA21 support method and statistical distinctions. FA23 and FA32 support therapeutic interference and protein investigation. FA07 and FA28 support complement/C1-inhibitor context. Full citations appear on pages 290–293.

Active retrieval

Explain the tissue compartment, identify the denominator, trace the required assay bridge, and calculate the dilution correction before reading the answers. The subject index follows this section and links selected teaching locations throughout the book.

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Numbers link to the relevant teaching. A glossary location gives a concise definition; use the chapter and worked cases for the complete explanation.

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