Diagnostic methods and assays in microbiology

From a clinical specimen to a reliable laboratory result

Direct detection • Host response • Culture and identification

Methods, indications, limitations and quality controls

Three complementary diagnostic approaches

A method is selected according to the clinical question and the specimen.

Direct methods

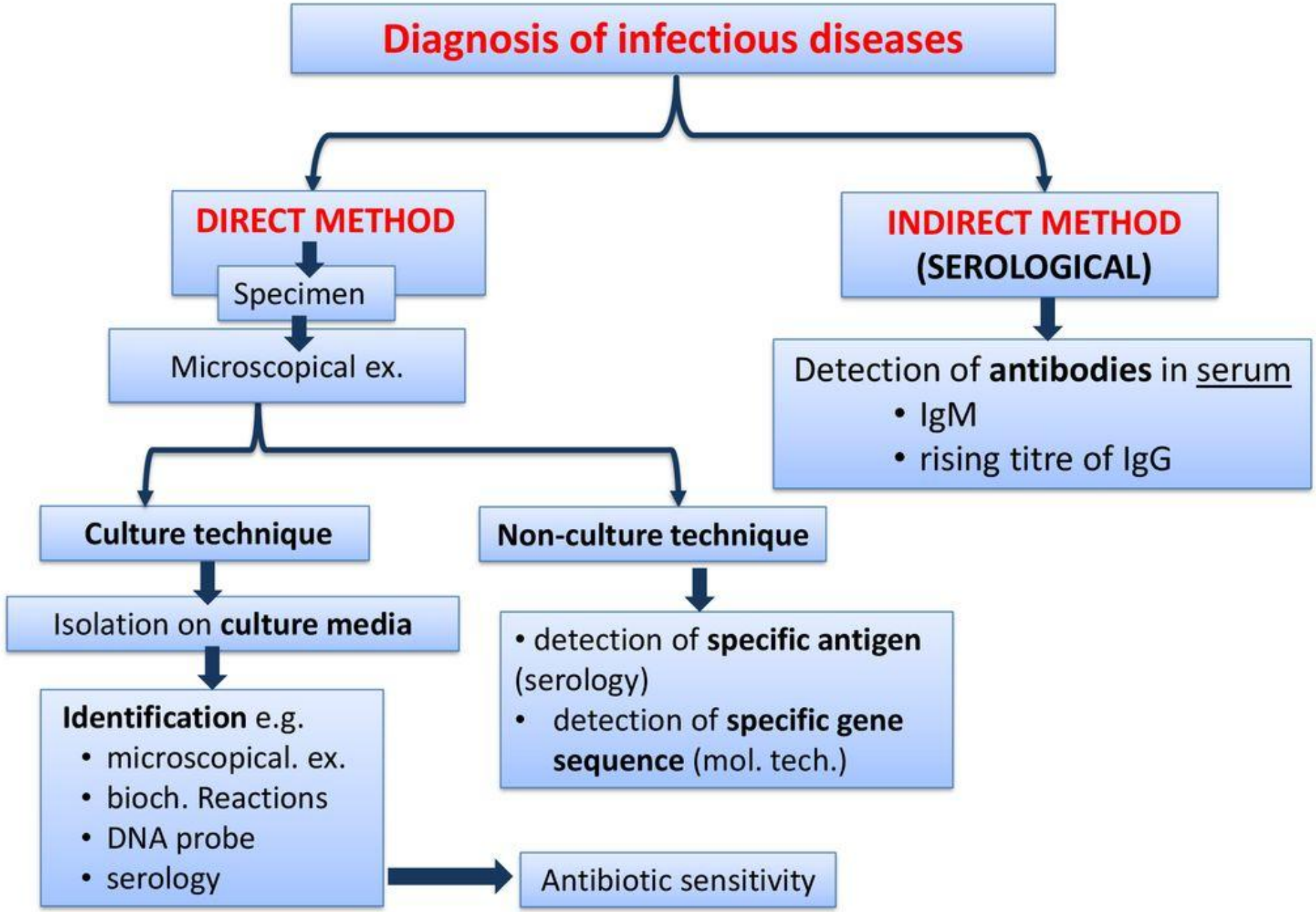
Detect the microorganism or its components: microscopy, culture, antigen, nucleic acid.

Indirect methods

Detect the host response: mainly pathogen-specific antibodies.

Rapid tests

Describe turnaround time and workflow, not a separate biological category.


Diagnosis of infectious diseases
DIRECT METHOD
Specimen
Microscopical ex.
INDIRECT METHOD (SEROLOGICAL)
Detection of antibodies in serum
• IgM
• rising titre of IgG
Culture technique
Isolation on culture media
Identification e.g.
• microscopical. ex.
• bioch. Reactions
• DNA probe
• serology
Antibiotic sensitivity
Non-culture technique
• detection of specific antigen (serology)
• detection of specific gene sequence (mol. tech.)

Begin with the clinical question

The result is only as informative as the specimen and its context.

Patient → specimen → appropriate assay → interpretation

Consider site of infection, stage of disease, urgency, prior treatment and pre-test probability.

What can the laboratory demonstrate?

Different methods measure different biological targets.

Microscopy

Morphology and staining features; fast but often not species-specific.

Culture

Viable, cultivable organisms; isolates support further identification and susceptibility testing.

Molecular/antigen

Microbial DNA/RNA or antigen; fast detection, but not always proof of viability.

Direct testing in clinical microbiology

Ask what a positive and a negative result actually mean.

Strengths

Often detects the agent early; can provide rapid, clinically actionable information.

Limitations

Depends on sample quality, target abundance, inhibitors, specimen timing and assay design.

Rapid tests and point-of-care testing

Rapid is a property of the workflow, not proof of higher accuracy.

Lateral-flow antigen

Antigen recognition by labeled antibodies; straightforward and rapid, with context-dependent sensitivity.

Molecular POCT

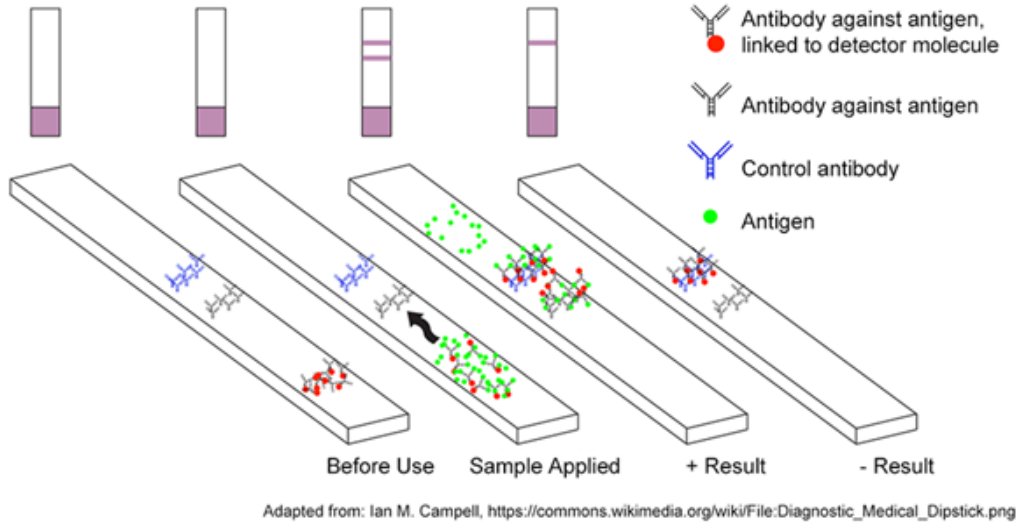
Integrated nucleic-acid amplification near the patient; quality assurance remains essential.

MALDI-TOF MS

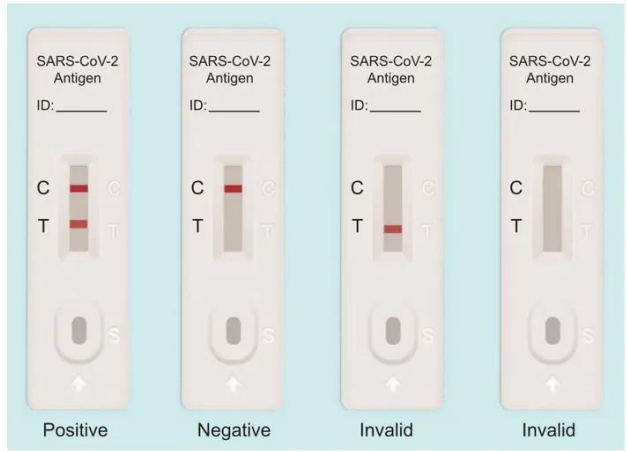
Rapid identification from characteristic mass spectra, usually after obtaining an isolate.

Rapid antigen tests

Immunochromatographic test principle



Adapted from: Ian M. Campell, https://commons.wikimedia.org/wiki/File:Diagnostic_Medical_Dipstick.png

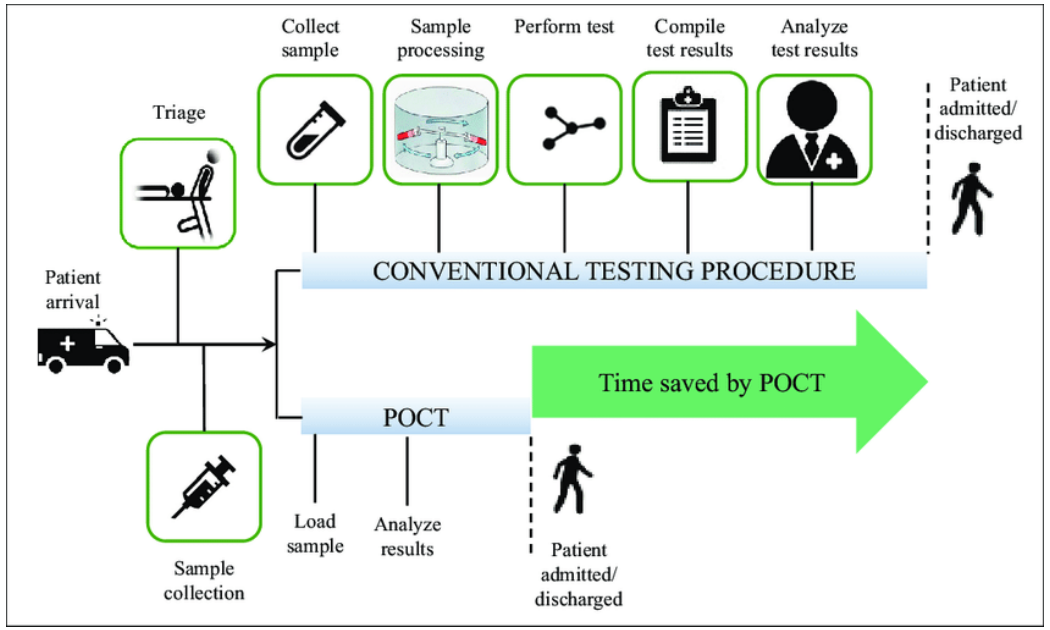


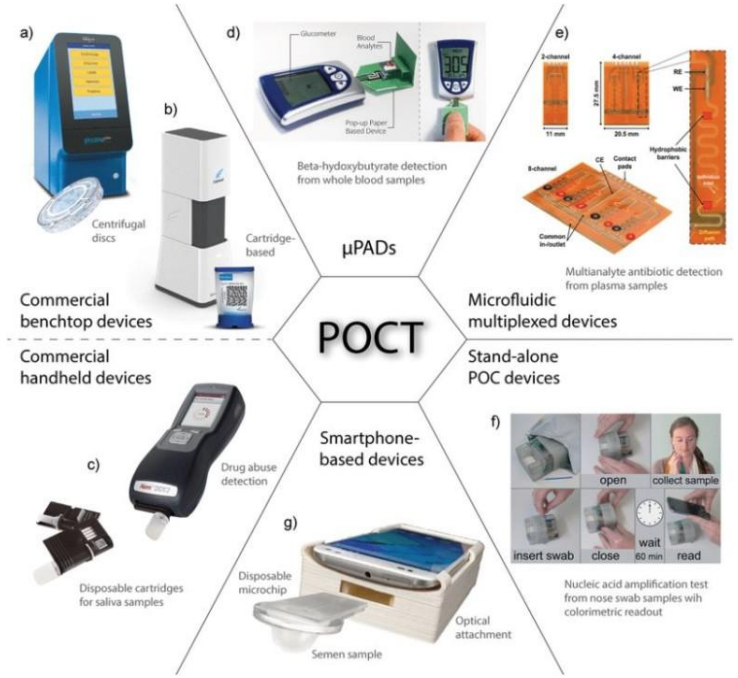
NOTE: This figure is only used as a reference.

Interpret control and test lines according to the specific manufacturer’s instructions.

Point-of-care molecular assays

From sampling to an actionable result





A closed cartridge can integrate extraction, amplification and detection.

Microscopy: what can we learn?

An early presumptive result may guide the next diagnostic step.

Bright field

Stained bacteria and cellular elements: morphology, arrangement and Gram reaction.

Wet mount / phase contrast

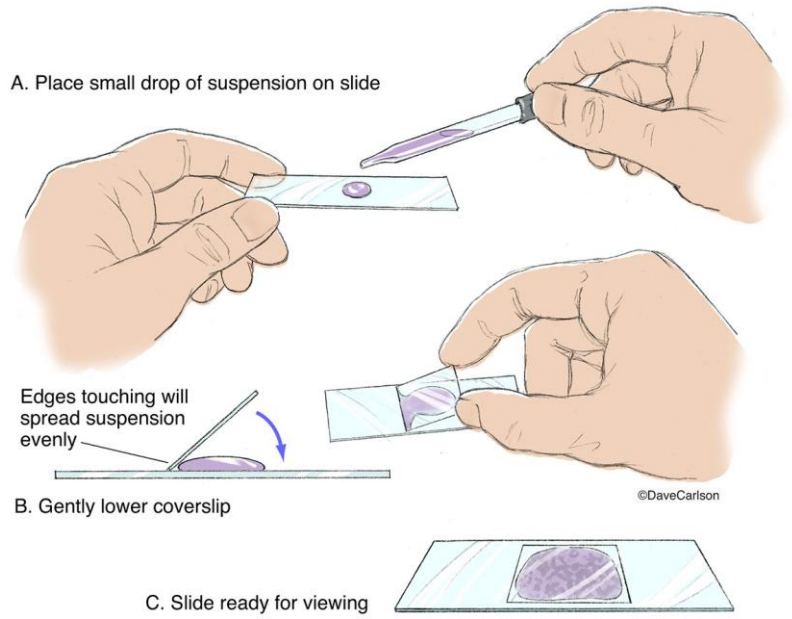
Fresh preparations can reveal motility, parasites, fungi and other structures.

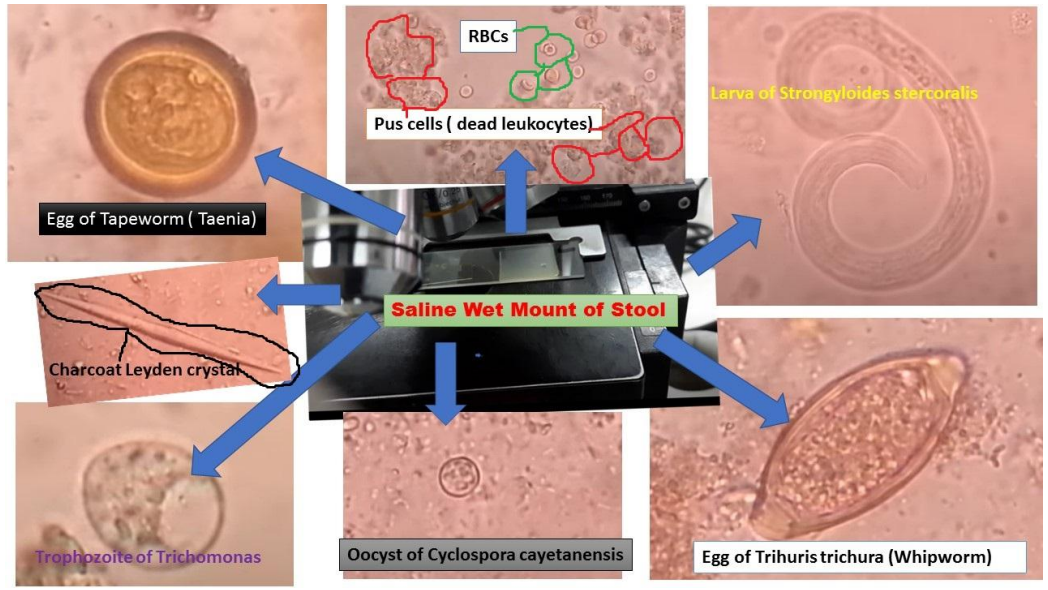
Fluorescence

Fluorophores or labeled antibodies improve contrast or target specificity.

Wet mounts and direct examination

Observe structures without assuming that morphology alone identifies a pathogen.

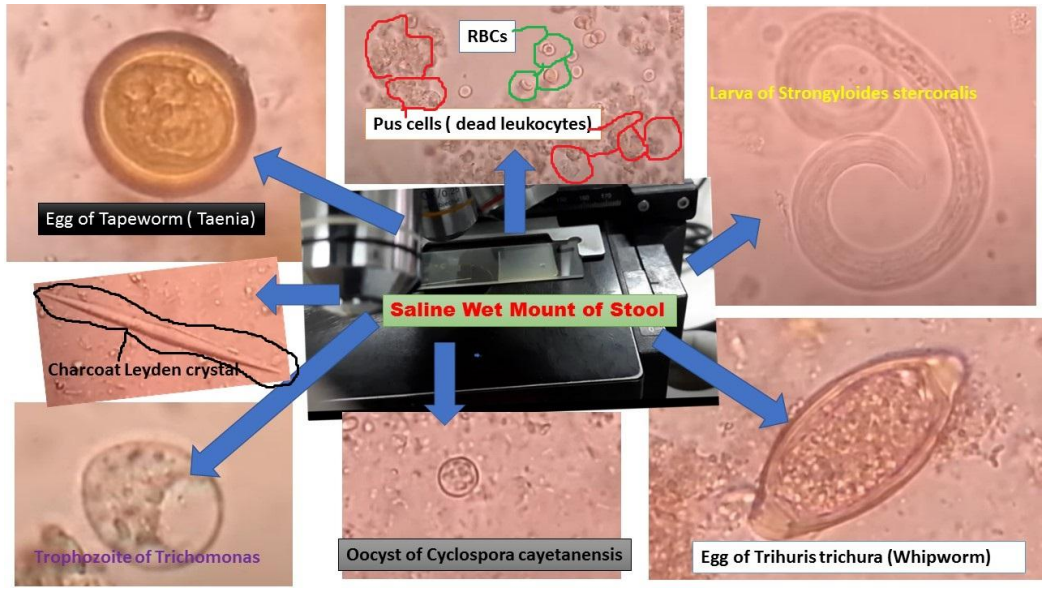




Examples from the original lecture: wet preparation and Charcot–Leyden crystals.

Microscopy of parasites

Connect microscopic appearance to specimen type and clinical context.





Eggs, larvae and diagnostic structures require morphological expertise and suitable samples.

Major staining strategies

Choose the stain based on the organism or structure of interest.

Simple and differential

Single dyes describe morphology; Gram stain separates bacterial cell-wall phenotypes.

Acid-fast

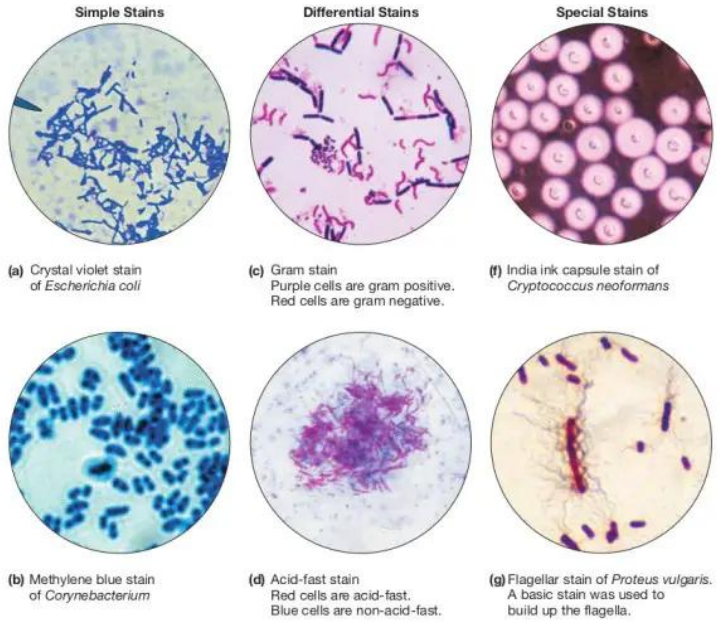
Ziehl–Neelsen or fluorochrome methods can reveal acid-fast bacilli.

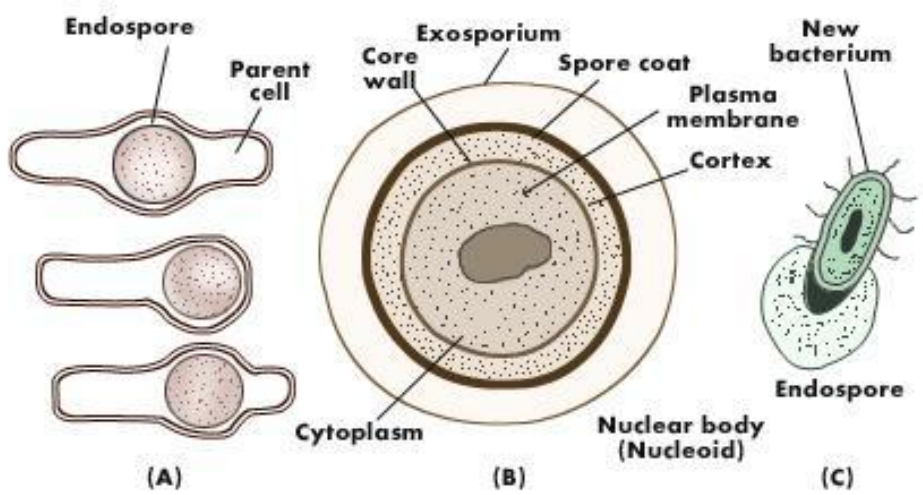
Special staining

Capsules, endospores, fungal structures or other microbial features.

Gram and acid-fast staining

Differential stains are powerful but do not replace species-level identification.



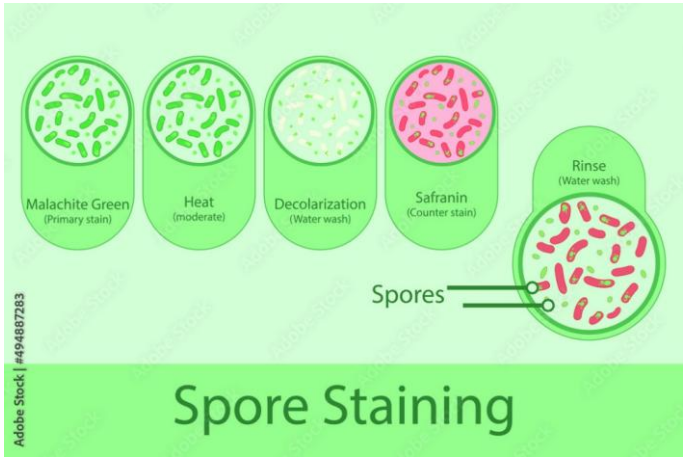


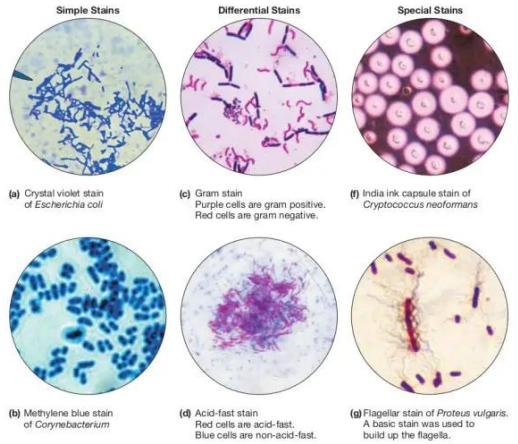
Endospore formation. A, Endospores according to their position in parent cells. B, An edospore in cross-section. C, Germination of endospore

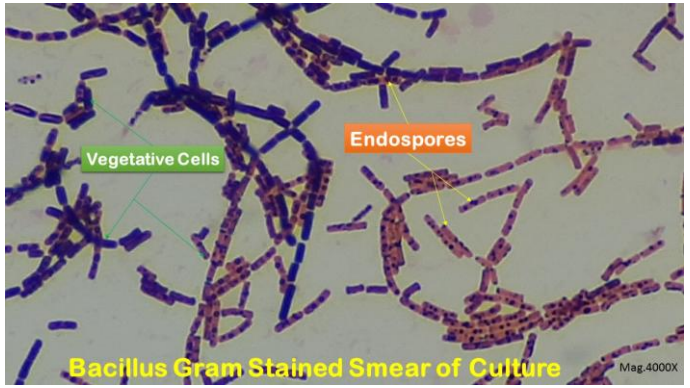
Interpret together with specimen quality, morphology and clinical information.

Special stains and spores

Capsules and endospores can require dedicated approaches.





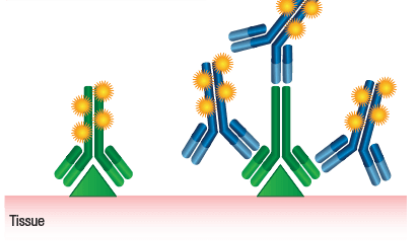


Examples of bacterial structures from the original lecture.

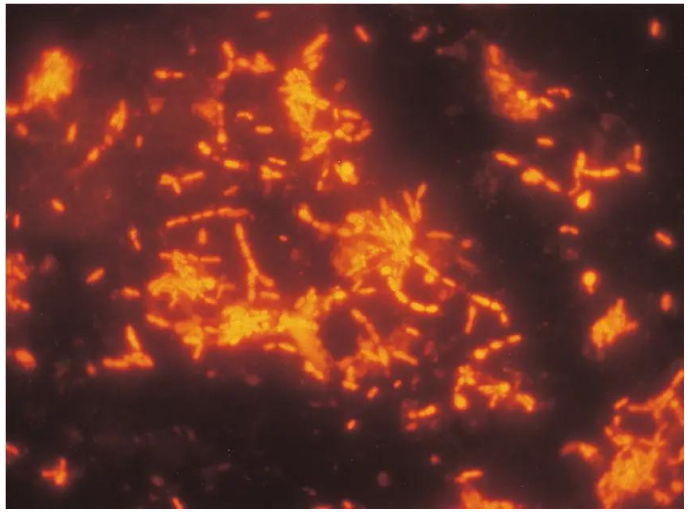
Negative and fluorescent staining

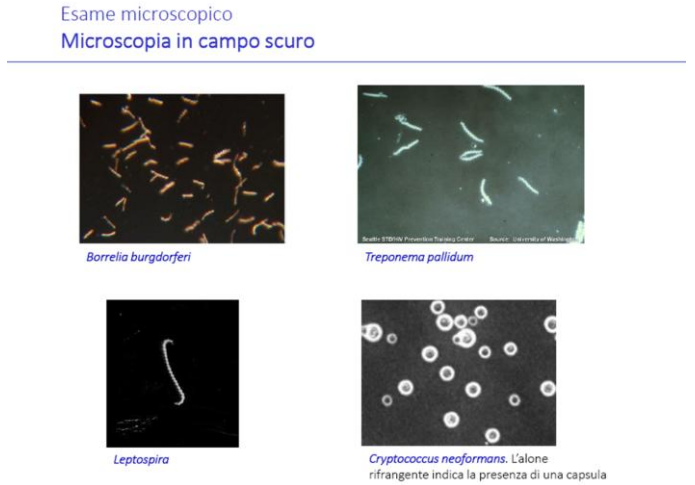
Contrast can result from staining the background or the target.





Antigen Detection	Direct immunofluorescence with primary antibody conjugated to a fluorophore	Indirect immunofluorescence with secondary antibody conjugated to a fluorophore
Protocol	Parallel staining	Parallel staining
Primary Antibody	Same host species can be used for multiple targets	Different host species or isotype for each target
Seconday Antibody	No	Yes
Signal Amplification	None	Moderate

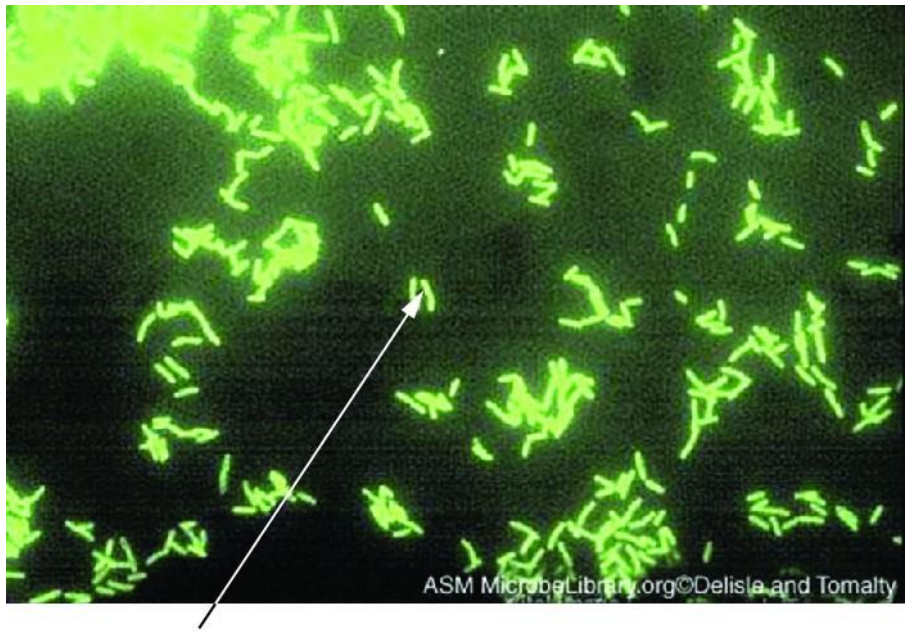




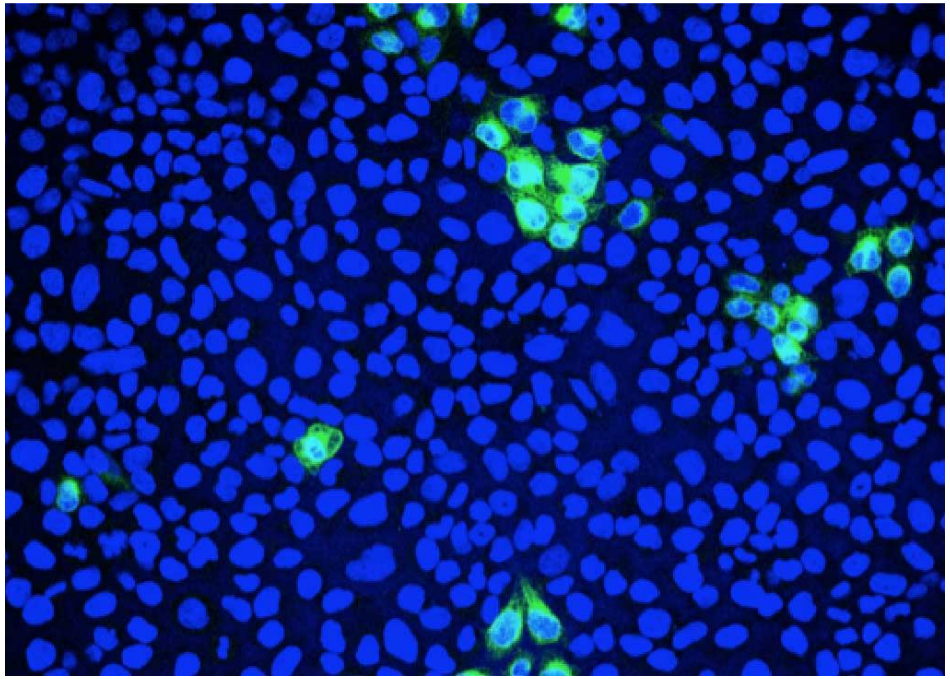
Acridine orange binds nucleic acids; DAPI preferentially labels DNA.

Detection in infected cells

Microscopic changes may be suggestive, not necessarily diagnostic.



Fluorecein-labeled antibody attached to *Legionella* bacilli



Cytopathic effects and immunostaining require contextual interpretation and controls.

Microscopy usually provides a presumptive diagnosis

Especially valuable when speed matters.

A visual finding ≠ definitive identification

Example: a Gram stain from cerebrospinal fluid may support urgent clinical decisions while confirmatory testing proceeds.

Why culture remains fundamental

Isolation provides material for additional tests.

Identification

Growth and colony properties support downstream biochemical or proteomic identification.

AST

Isolates permit phenotypic antimicrobial susceptibility testing when appropriate.

Surveillance

Isolates support typing, outbreak investigation and reference-laboratory work.

From specimen to isolate

The process must match the organism, specimen and clinical question.

- Inoculation on appropriate solid or liquid media
- Incubation with suitable atmospheric and temperature conditions
- Examine colony morphology and isolate suspicious colonies
- Identify the isolate and perform indicated susceptibility testing
- Validate, interpret and report the result

Liquid versus solid media

A physical-state distinction.

Liquid medium

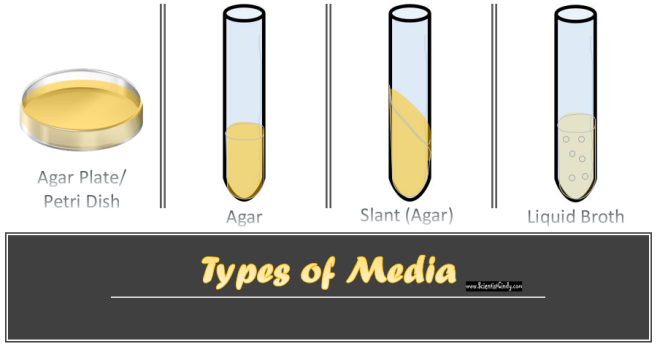
Supports suspended growth and enrichment; visible turbidity does not prove purity.

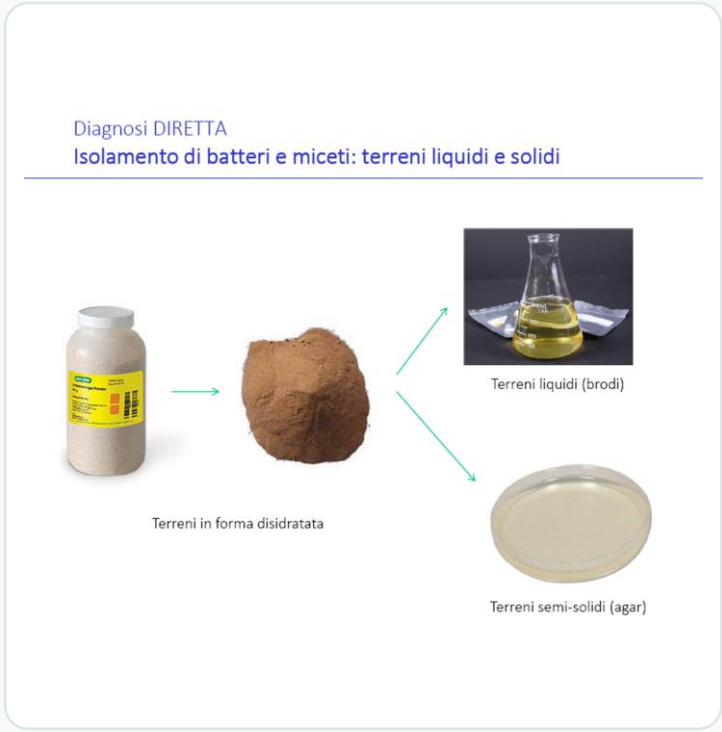
Solid medium

Agar provides a surface for colony isolation, morphological assessment and enumeration where appropriate.

Liquid and solid media

Examples and laboratory appearance.





Esame colturale (isolamento)

Terreni NON SELETTIVI

Privi di inibitori, consentono la crescita della maggior parte delle specie microbiche. Tra i più frequentemente impiegati ricordiamo:

- Agar sangue: contiene il 5% sangue montone/cavallo, evidenzia la attività emolitica:
  - α-emolisi = emolisi parziale, dà luogo alla formazione di un alone verde intorno alla colonia per degradazione della bilirubina a biliverdina (streptococchi viridanti; *S. pneumoniae, E. faecalis*);
  - β-emolisi = emolisi completa, dà luogo alla formazione di un alone trasparente intorno alla colonia (*S. pyogenes, S. agalactiae, S. aureus*);
  - γ-emolisi = mancanza di emolisi (streptococchi viridanti, *E. faecalis*).
- Agar cioccolato: contiene emoglobina (emina o fattore X), NAD (fattore V) e vitamine, per l'isolamento di germi "esigenti" dal punto di vista nutritivo (*Haemophilus* spp., *Neisseria* spp., *S. pneumoniae*). Il caratteristico color cioccolato deriva dal trattamento termico del sangue che ne causa la emolisi e, quindi, il rilascio dei fattori di crescita.
- Brodi nutrienti: a composizione chimica ricca, consente la crescita della maggior parte dei batteri (es. brodo di soia-caseina, infusione cuore-cervello, etc.)

Agar is principally a gelling agent, not a general nutrient source.

Classification by chemical composition

Composition is distinct from selective or differential function.

Defined media

Precisely specified components; useful for controlled physiological studies.

Complex media

Peptones or extracts with incompletely defined molecular composition; widespread in routine culture.

Minimal media

Provide essential requirements for organisms suited to their formulation.

Functional classification of culture media

A single medium may belong to several categories.

Non-selective / enriched

Support broad growth or demanding organisms (e.g., blood and chocolate agar).

Selective / enrichment

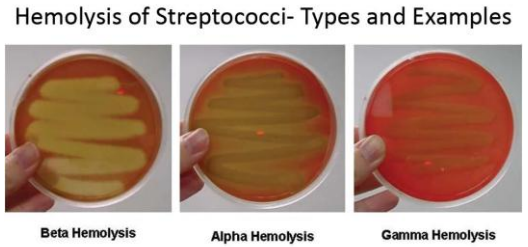
Favor recovery of target organisms, by selective inhibition or growth enrichment.

Differential

Include indicators that distinguish metabolic or phenotypic properties.

What does a plate tell us?

Observe the medium and the colony phenotype together.



Esame colturale (isolamento)

Terreni SELETTIVI

Questi terreni consentono la crescita di particolari specie microbiche grazie alla presenza di agenti selettivi, tra cui:

- coloranti: cristalvioletto, verde brillante, fucsina basica
- antibiotici: con attività batteriostatica/battericida vs "contaminanti"

Vengono utilizzati per l'isolamento di microrganismi patogeni presenti in campioni prelevati da siti caratterizzati da una da flora microbica residente (ossia «contaminati»: cute, faringe, naso, intestino, vagina). Tra i più frequentemente utilizzati:

- Cetrimide agar: contiene cetrimide, composto dell'ammonio quaternario, che inibisce la crescita di tutti i microrganismi eccetto *P. aeruginosa*.
- Thayer-Martin agar (agar cioccolato addizionato di vancomicina, colistina, trimethoprim lattato e nistatina): selettivo per *Neisseriaceae* patogene.
- MacConkey agar: contiene sali biliari e cristalvioletto, che inibiscono la crescita dei Gram-positivi; selettivo per *Enterobacteriaceae*.
- Mannitol Salt agar: elevata concentrazione NaCl (7.5%), selettivo per stafilococchi.
- SS agar (sodio citrato, sali biliari, verde brillante): selettivo per *Salmonella* e *Shigella* spp.
- Terreni riducenti: solidi o liquidi, contengono composti chimici che si combinano chimicamente con O_2 atmosferico fino ad esaurirlo (agenti riducenti: tioglicolato di sodio, acido ascorbico, etc.). Selettivi per gli anaerobi.

Esame colturale (isolamento)

Terreni DIFFERENZIALI

Contengono ingredienti in grado di differenziare particolari specie (o gruppi) microbiche sulla base della capacità di fermentare specifici carboidrati:

- carboidrati (zuccheri);
- indicatori di pH (rosso fenolo, rosso neutro, blu di bromofenolo).

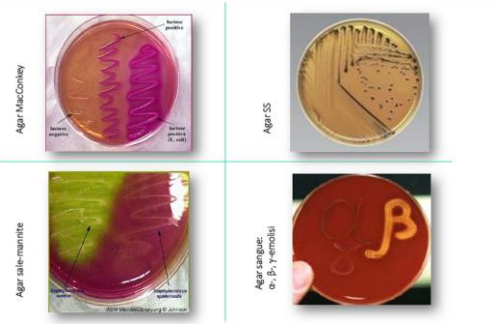
La fermentazione del carboidrato causa il rilascio di prodotti acidi della via fermentativa causando una acidificazione del terreno, prontamente indicata dal viraggio dell'indicatore di pH (e della colorazione delle colonie).

Esempi:

- Agar sangue (5% di montone oppure cavallo) per evidenziare la capacità di emolisi dei batteri: α-emolisi, β-emolisi, γ-emolisi.
- MacConkey agar (cristalvioletto, lattosio, rosso neutro), differenzia specie lattosio-fermentanti (*E. coli*) da quelle non fermentanti (*P. mirabilis, Salmonella* spp, *Shigella* spp.).
- Mannitol Salt agar (mannitolo, rosso fenolo), differenzia specie mannitolo-fermentanti (*S. aureus*) dalle non fermentanti (stafilococchi coagulasi-negativi).
- SS agar: i) tiosolfato di sodio e citrato ferrico consentono di evidenziare la produzione di solfuro di idrogeno da parte delle colonie di *Salmonella* con aree centrali nere; ii) lattosio, per evidenziare capacità fermentanti.

Esame colturale (isolamento)

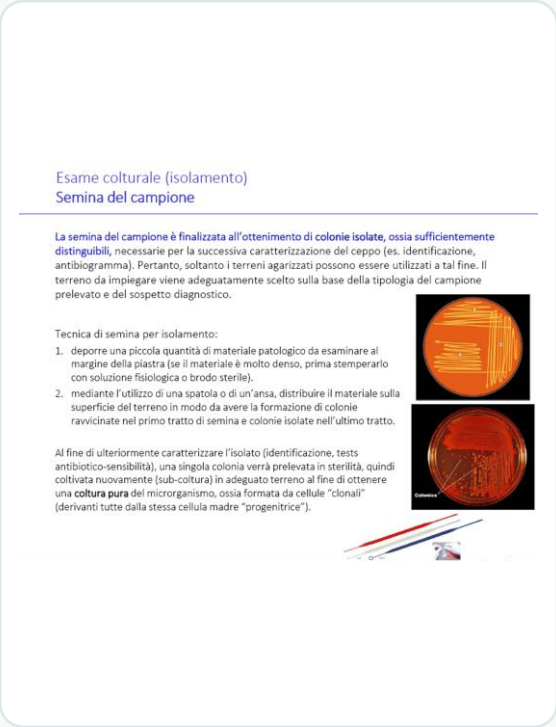
Terreni DIFFERENZIALI

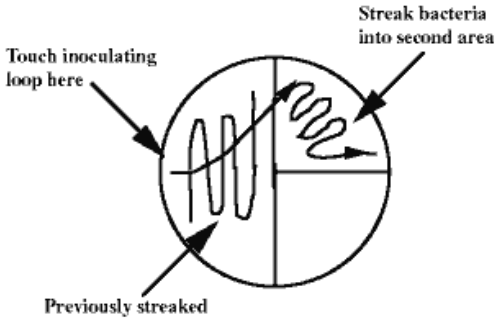


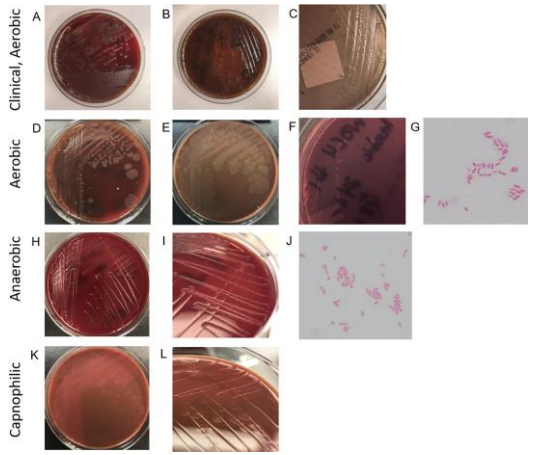
Identify the type of medium, relevant reaction and likely next confirmatory step.

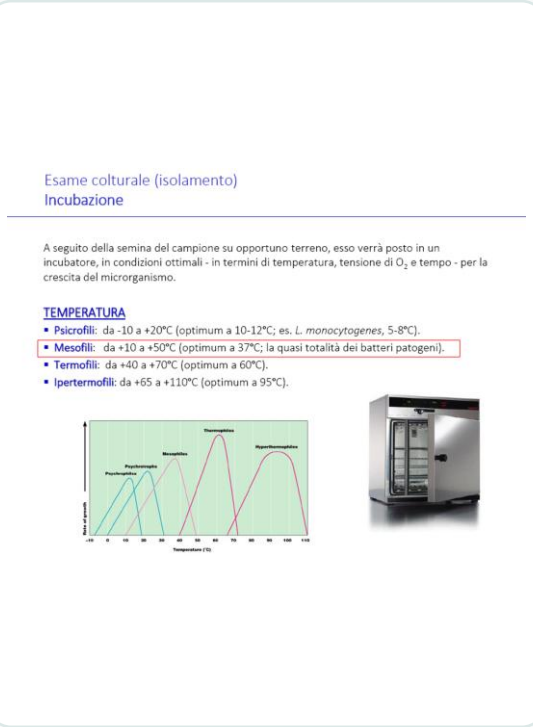
Comparing growth and reactions

Culturing is an iterative process of selection and confirmation.





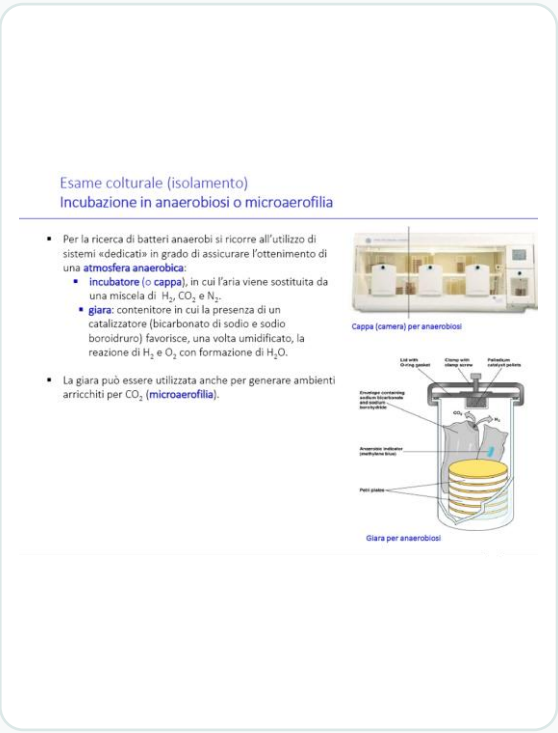


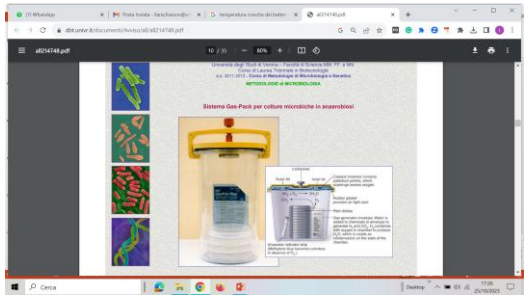


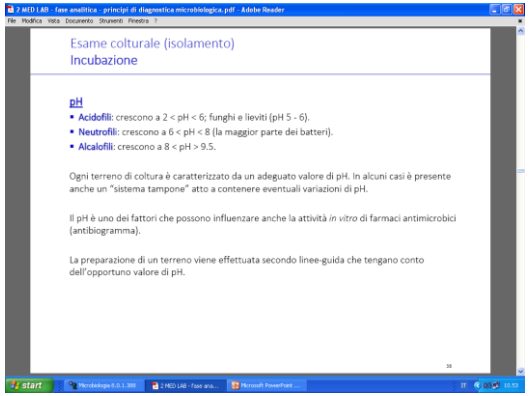
Media reactions are presumptive unless supported by appropriate confirmatory testing.

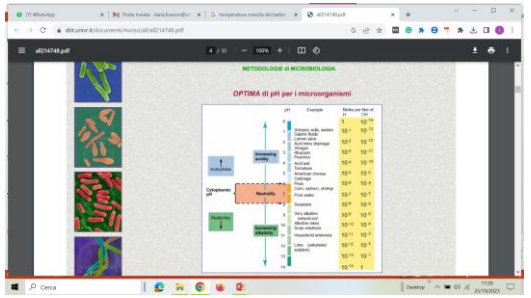
Additional culture appearances

A gallery from the original teaching material.





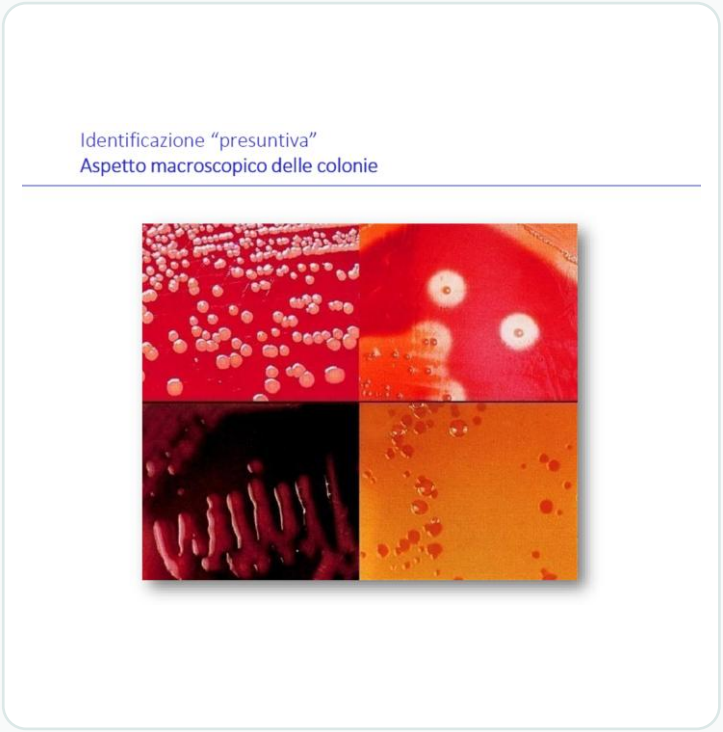


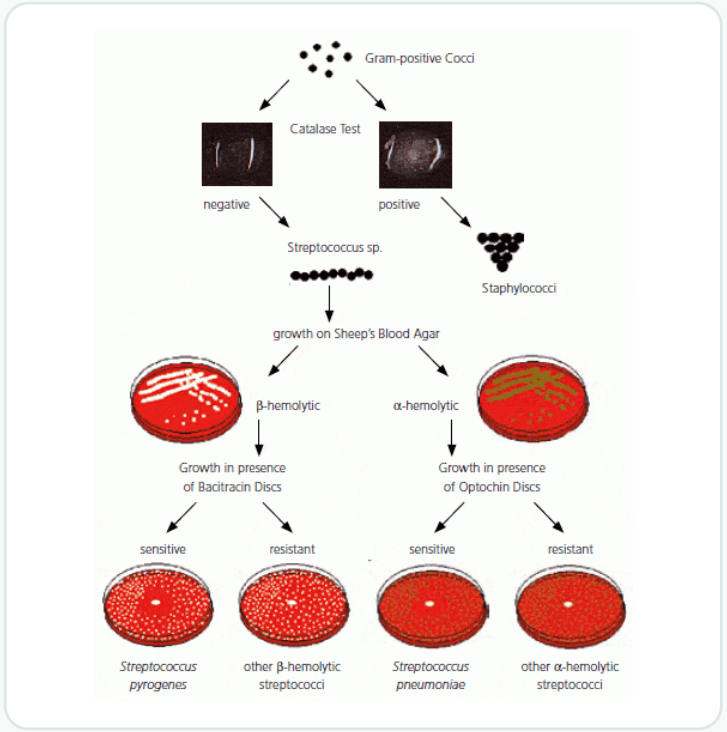


Use the examples to discuss selectivity, colony morphology and interpretation.

Presumptive identification

Phenotypic screening precedes confirmation.



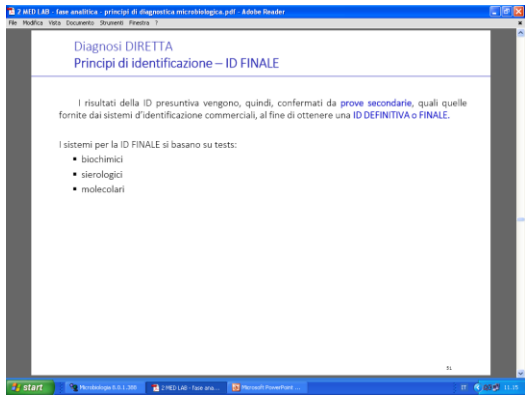


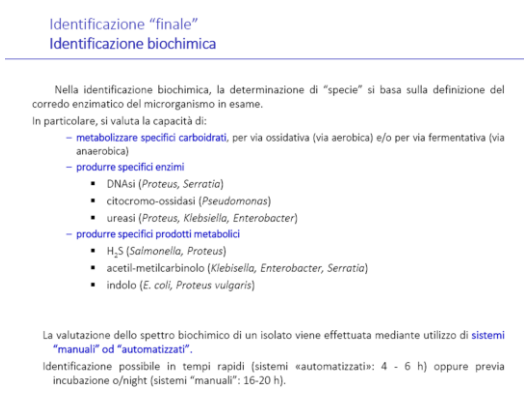


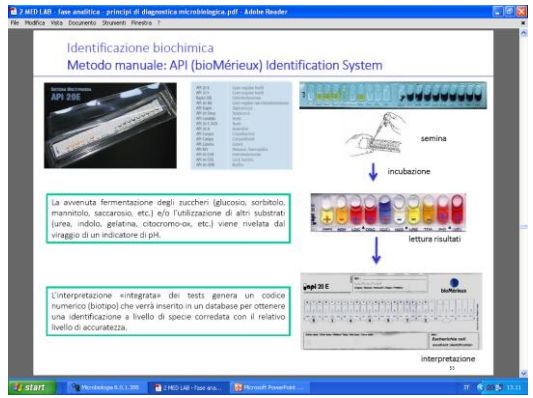
Example: presumptive identification of streptococci and related laboratory reactions.

Biochemical identification

Enzyme activity and substrate utilization create diagnostic profiles.









Interpret reaction profiles through validated identification systems.

MALDI-TOF MS: core principle

Matrix-assisted laser desorption/ionization, time-of-flight.

Sample and matrix

Material from a suitable colony is deposited on a target and mixed with matrix.

Ionization and flight

Laser exposure generates ions; their flight characteristics yield m/z signals.

Spectral matching

A software system compares the resulting fingerprint with validated reference spectra.

MALDI-TOF: interpretation

The database, platform and sample preparation determine performance.

What it does well

Rapid bacterial and fungal identification from suitable isolates; selected workflows can analyze positive blood cultures.

Important limits

A confidence score is platform-specific; discrimination may be limited for closely related taxa. Routine species ID is not equivalent to AST.

Serology detects the host response

Antibodies must be interpreted in relation to timing and clinical context.

IgM

May be associated with recent immune responses, but isolated IgM is not universally proof of acute infection.

IgG

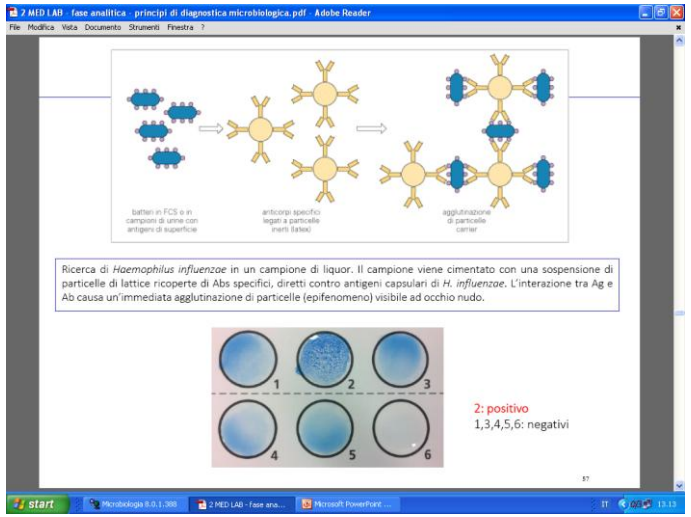
Can reflect past exposure or vaccination, and can also occur during active infection.

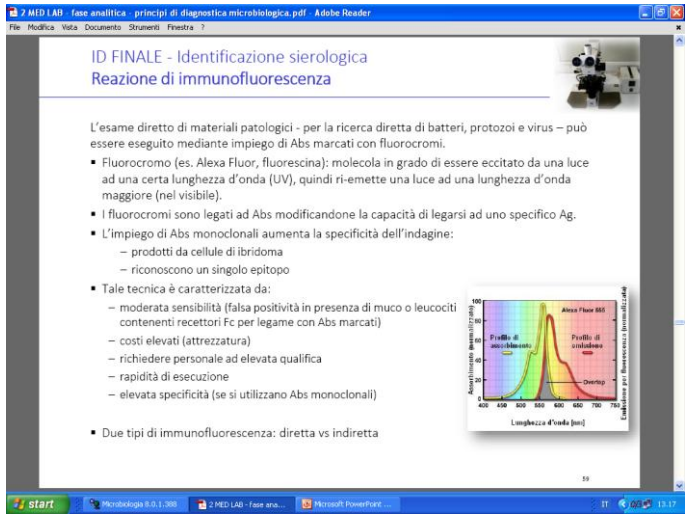
Paired sera / avidity

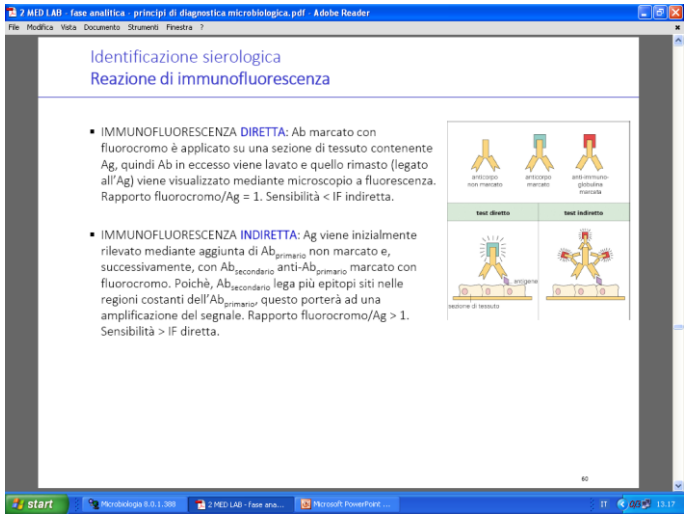
Seroconversion, rising titers or avidity testing may improve interpretation in selected diseases.

Antibody–antigen reactions

Agglutination and assay formats illustrated in the original lecture.



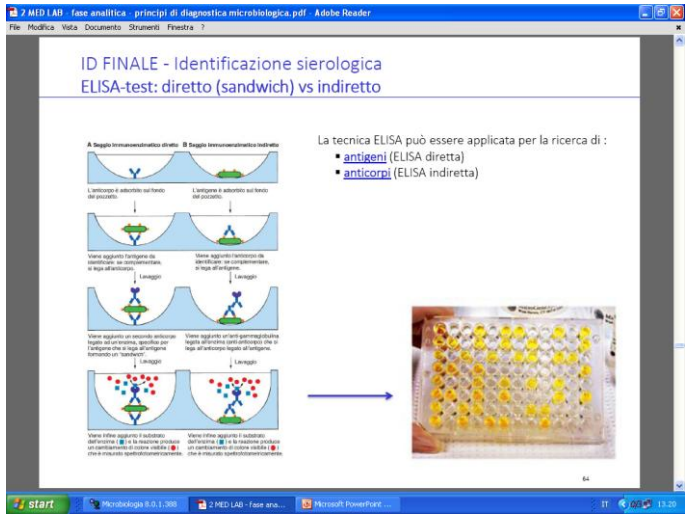


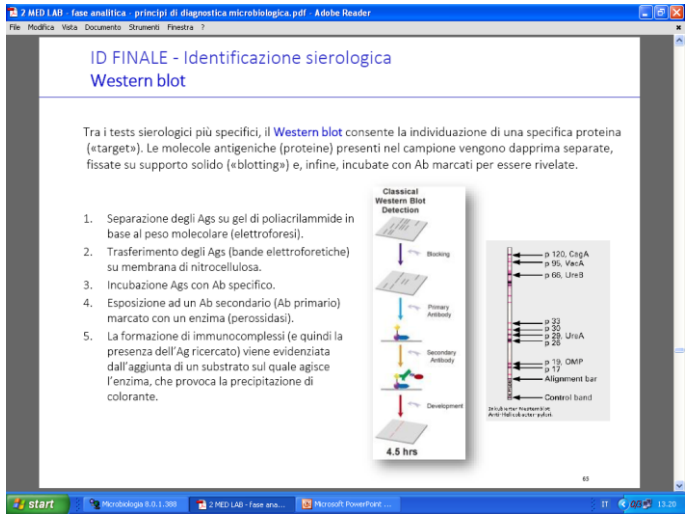


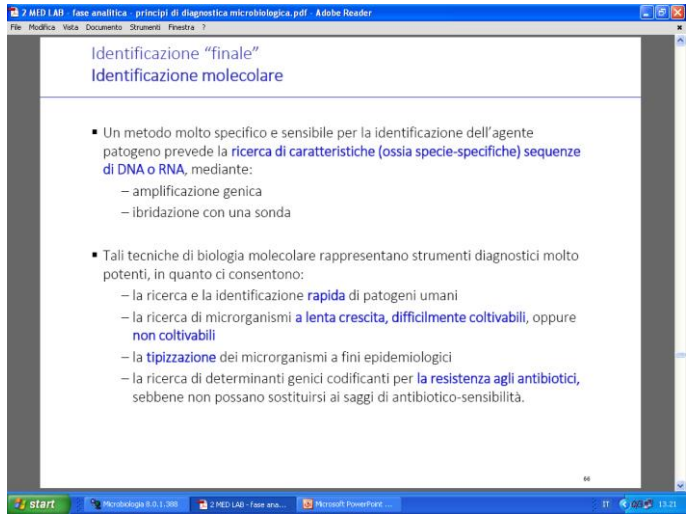
Detection of antigen and detection of antibodies answer different diagnostic questions.

ELISA assay formats

Direct, indirect and sandwich principles.



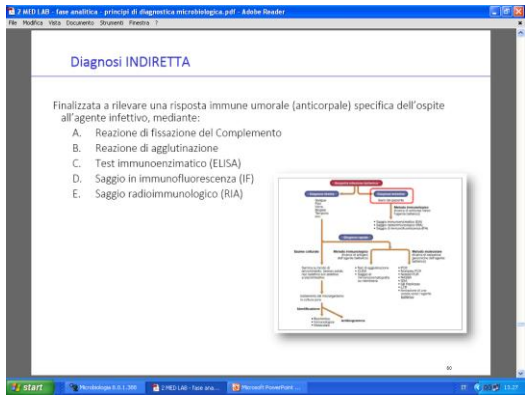


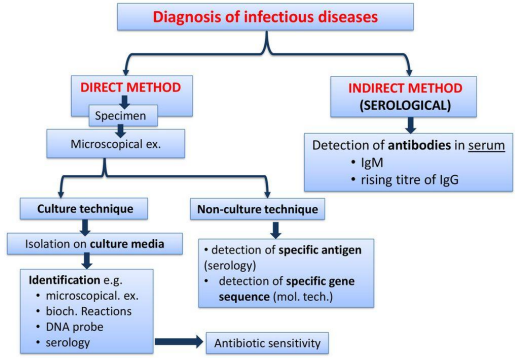


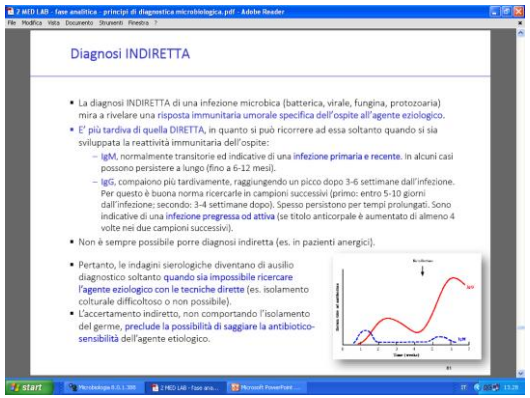
Signal direction and interpretation depend on assay format, analyte and controls.

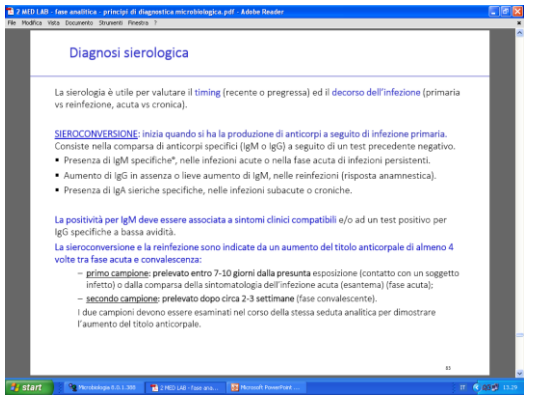
Immunoassay examples

The chosen assay depends on the analyte and intended use.





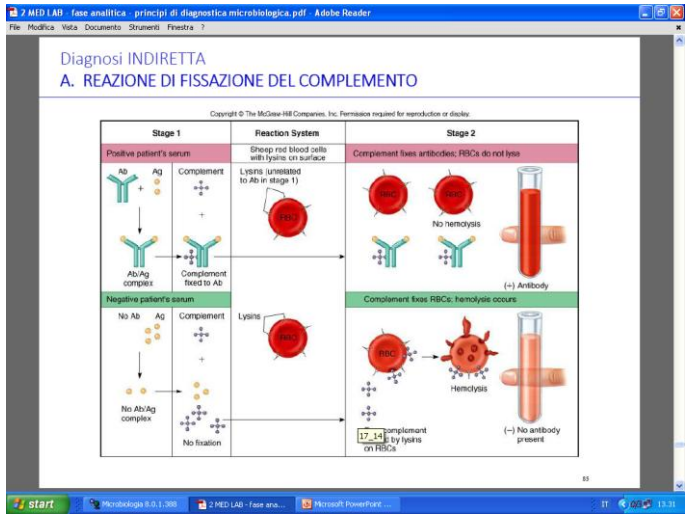


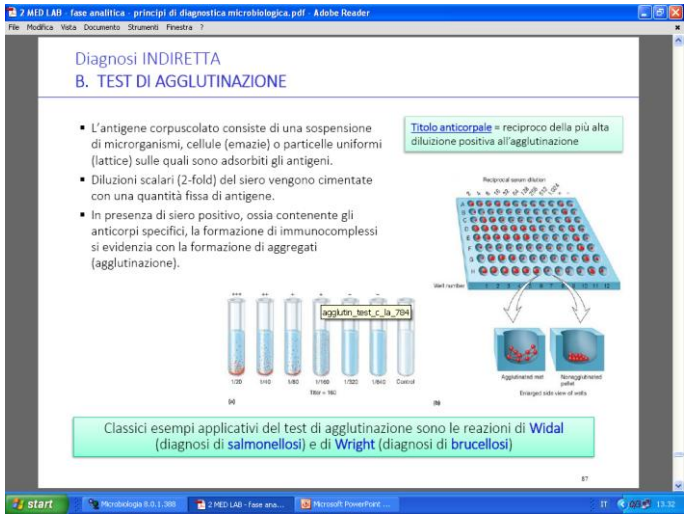


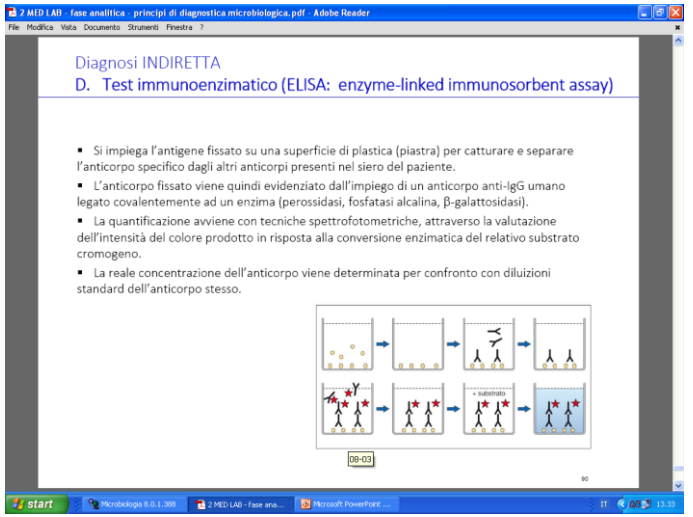
Always distinguish test validation from interpretation of a single patient sample.

Additional serological reactions

Examples retained from the original lecture.







Know the type of reaction and whether it measures antigen or antibody.

Controls throughout the diagnostic process

An interpretable result requires more than a working instrument.

Pre-analytical

Correct specimen, identity, collection time, transport, storage and rejection criteria.

Analytical

Positive/negative controls, contamination prevention, inhibition checks and instrument QC as relevant.

Post-analytical

Plausibility, critical-result communication, traceability and appropriate reporting.

Common interpretation traps

No single assay settles every clinical question.

Culture-negative

Prior antibiotics, organism viability, medium choice or sampling may affect recovery.

PCR-positive

Target nucleic acid may persist without demonstrating live organisms.

Serology-positive

Immune markers may reflect previous exposure or vaccination rather than current disease.

Choose complementary methods strategically

Integration of evidence increases confidence.

Clinical context + appropriate specimen + validated tests

The aim is not to run every available assay, but to select methods that answer the diagnostic question.

A laboratory decision exercise

A patient is suspected of having a serious bacterial infection.

Discuss

Which specimen and first-line direct tests would you prioritize?

What can be reported early as presumptive evidence?

Justify

When do culture, identification, AST and molecular methods add value?

What quality controls matter?

Five principles to remember

A method is useful only when its answer matches the clinical question.

1. Direct and indirect tests target different biological phenomena.
2. Specimen quality and disease stage change test performance.
3. Microscopy is fast; culture provides isolates for further analysis.
4. Molecular detection does not necessarily demonstrate viability.
5. Controls and contextual interpretation are essential.

Selected foundational references

Use current assay-specific guidelines and laboratory SOPs.

- WHO. Laboratory Biosafety Manual, 4th edition (2020).
- Clinical and Laboratory Standards Institute (CLSI): applicable microbiology standards.
- EUCAST: current antimicrobial susceptibility testing guidance.
- Manufacturer instructions for assays and MALDI-TOF identification criteria.