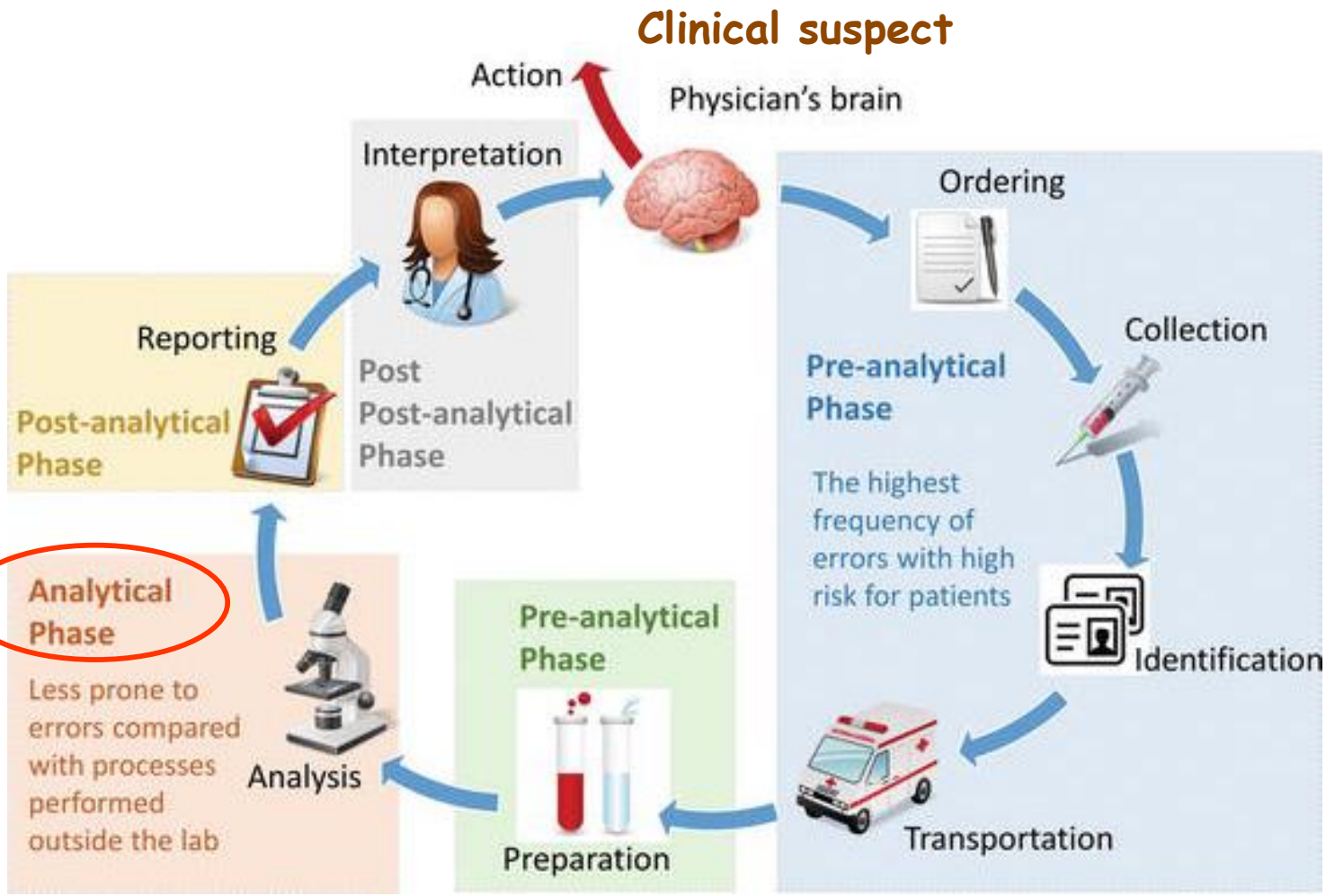
Diagnosis



- Virus
- Bacteria
- Fungi
- Parasites

Microbial diagnosis

Analytical phase

Suspected infection

DIRECT methods

To detect microorganisms or their products in specimens collected from the patient

Blood, stool, urine, swab, etc.

INDIRECT methods

To detect evidence of the patient's immune response (production of antibodies) to infection

Serum

Direct methods

Sample

CULTURE
in enrichment broth,
non-selective and / or
selective solid medium

Rapid methods

BACTERIOSCOPIC EXAMINATION

ISOLATION
Pure culture

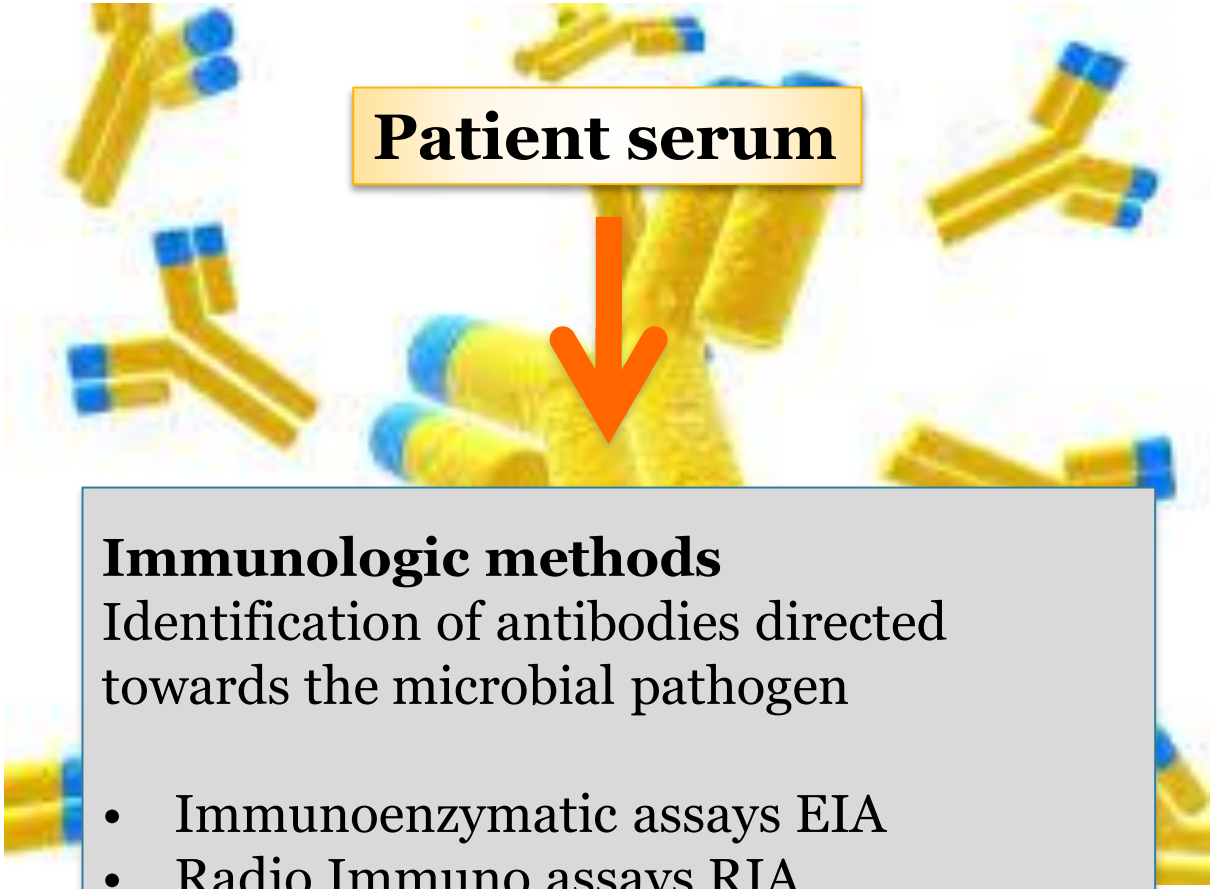
IDENTIFICATION
biochemical
immunologic
molecular

→ **ANTIBIOGRAM**

Immunological methods (antigen detection)
Latex agglutination assay
ELISA, IF
Immunochromatography

Molecular methods
PCR
NASBA
Hybridization
LCR

Indirect methods



Immunologic methods
Identification of antibodies directed towards the microbial pathogen

- Immunoenzymatic assays EIA
- Radio Immuno assays RIA
- Immunofluorescence assays IFA

Direct methods

detection of the pathogenic microorganisms

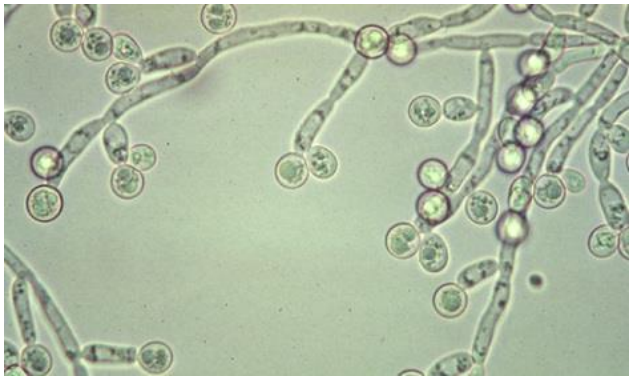
Microscopic examination (bacterioscopic exam)

- Culture (isolation)
- Antigen detection
- Molecular test

Direct methods: microscopy

Microscopy is an important first step in the examination of specimens

WET MOUNT
Allows the observation of microbial shape, arrangement and motility



STAINING
- **SIMPLE**
- **DIFFERENTIAL**

Allows the identification of microbial differential characteristics

Gram staining
Ziehl-Neelsen staining

Microscopy: staining

CLINICAL USE

Fixed specimens (Heat or chemical fixation)

Sample evaluation (sputum-neutrophils)
Number and percentage of PMN neutrophils
Presence or absence of microorganisms (*bacteria – fungi - parasites)*

Gram stained specimens

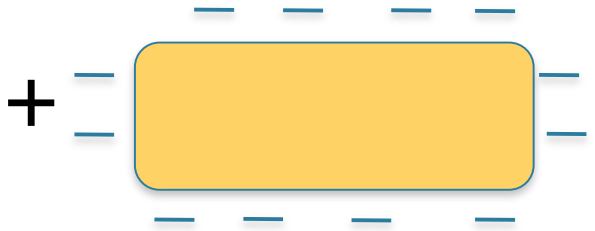
Morphology (cocci-bacilli-coccobacilli)
Arrangment (chains-clusters-diplococci)
Absolute quantity of bacteria
Relative percentage of Gram pos/neg
Intra- or extra-cellular localization

Other specific staining

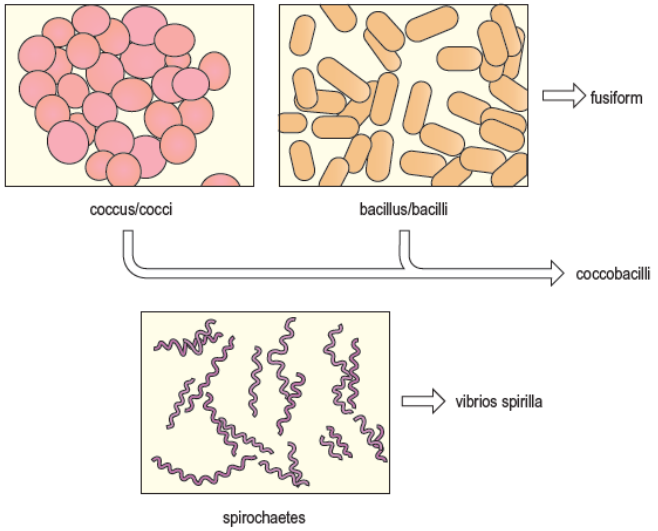
Acid-fast bacteria (Ziehl Neelsen)

Simple staining

A **BASIC DYE** binds to the acidic components of the bacterial cell wall (surface, proteins, nucleic acids)



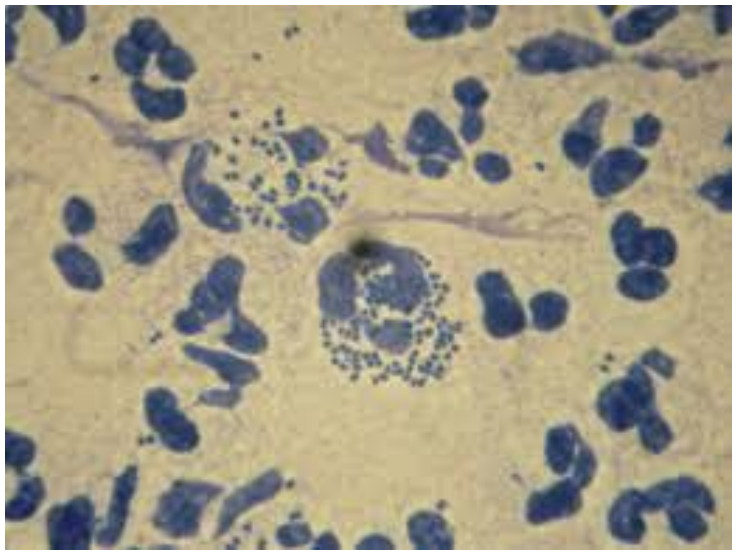
The basic dye is applied to the specimen for a variable time. The dye excess is washed with a water rinse.



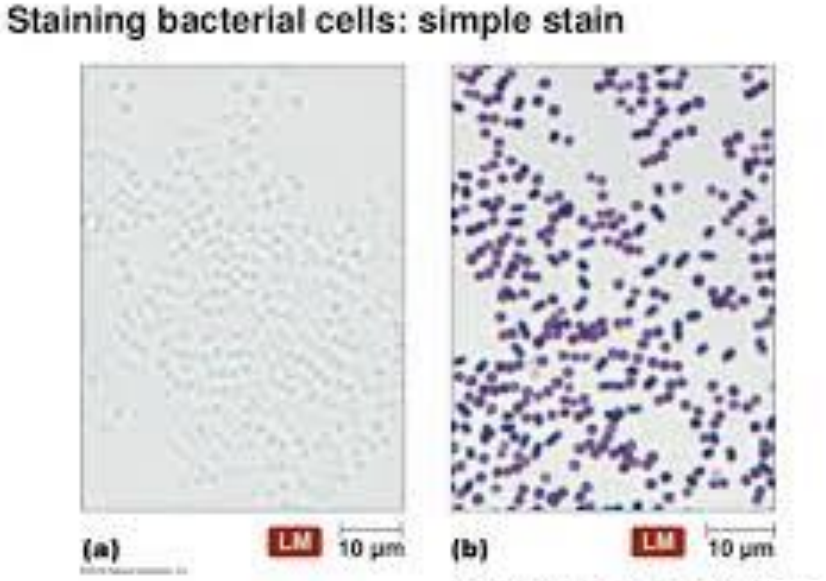
- Observation of cell **morphology** and **arrangment**
- Identification of intracellular bacteria

Examples: Crystal violet, Basic fuchsine, Methylene blue

Simple staining

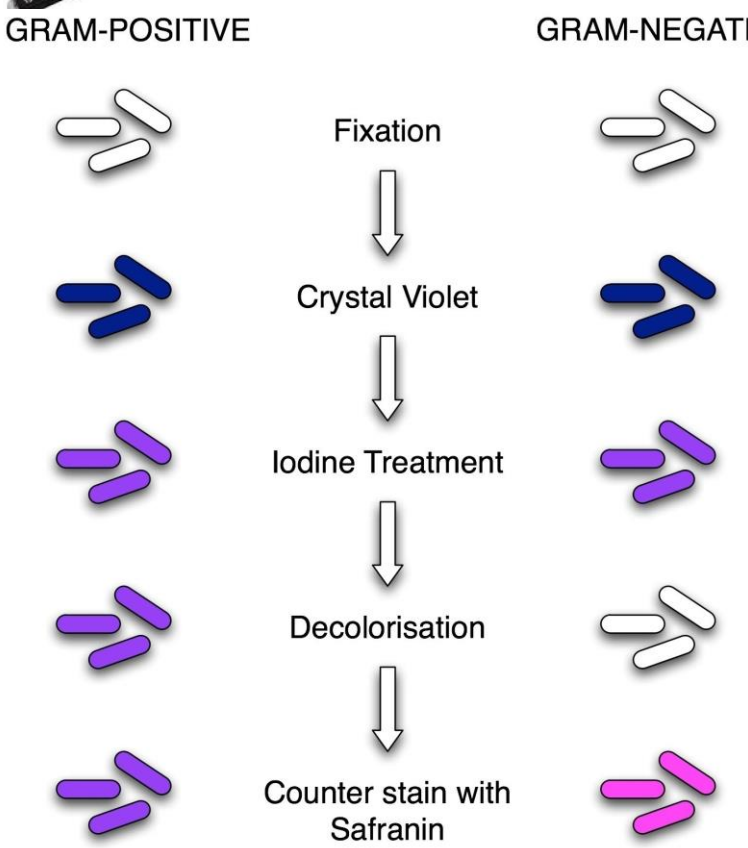


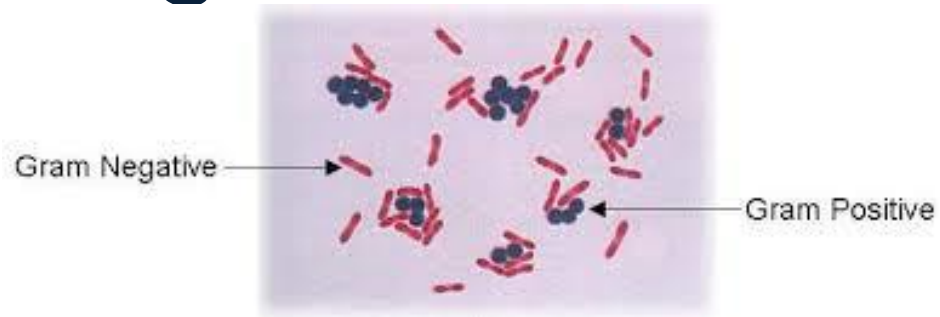
Methylene blue

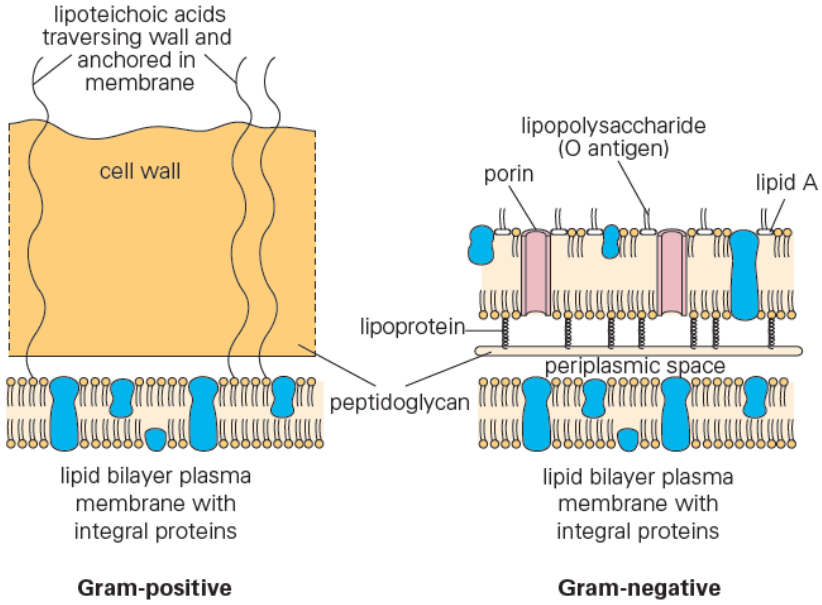


Crystal violet

Differential staining: GRAM





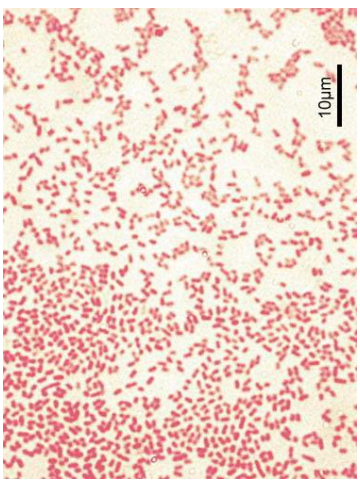


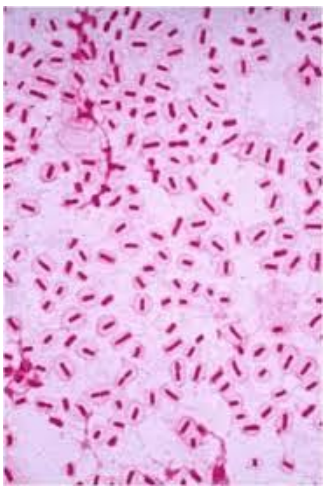
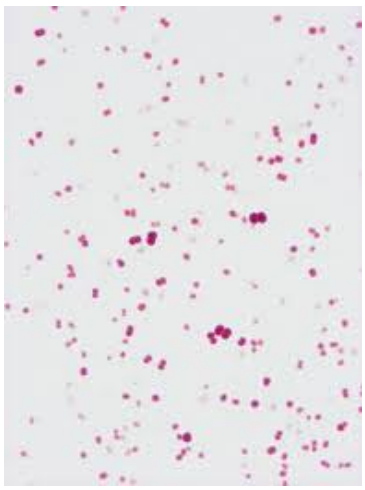
The difference in staining between Gram- and Gram+ is related to differences in the structure of the cell walls

Differential staining: GRAM

NEGATIVE

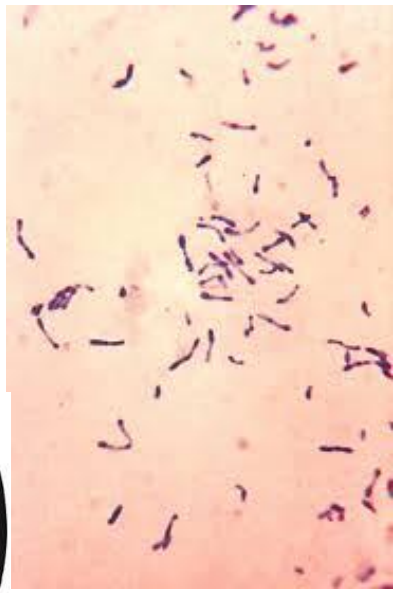
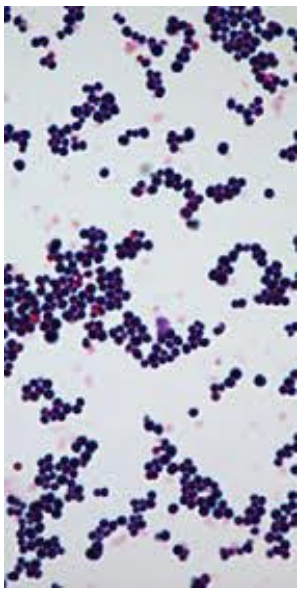
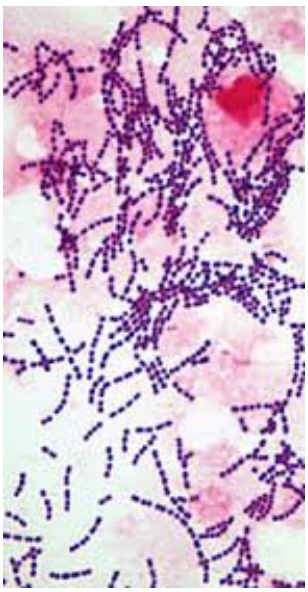
Pseudomonas, Klebsiella, Neisseria, E. coli



POSITIVE

Coryneform bacteria Staphylococcus Streptococcus

Differential staining: GRAM

CLINICAL USE

- **Sample evaluation** before culture
- **Presumptive identification**
  bacterial meningitis and pneumonia, bacteriuria, gonorrhea, pyogenic infections
- **Hint for the use of particular culture methods**
  anaerobic bacteria, fungi
- **Help in the interpretation of the culture isolation**
  patient treated with antibiotics
- **Information on the nature of the infection**
  Poli/mono-microbic infections

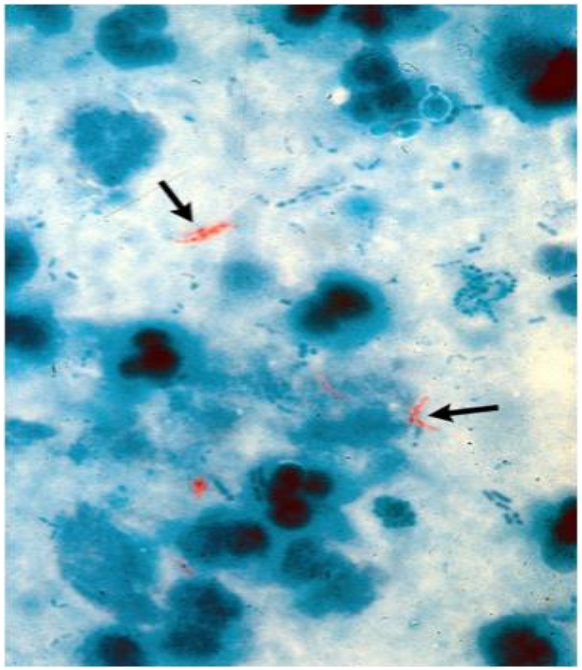
Performing a Gram stain may, in some cases, save the patient's life

Differential staining: Ziehl-Neelsen

Some organisms, particularly mycobacteria such as ***M. tuberculosis*** (tubercolosis) and ***M. leprae*** (leprosy, Hansen's disease), which have waxy cell walls, do not readily take up the Gram stain. Special staining techniques are used which rely on the ability of such organisms to retain the stain in the presence of 'decolourizing' agents such as acid and alcohol

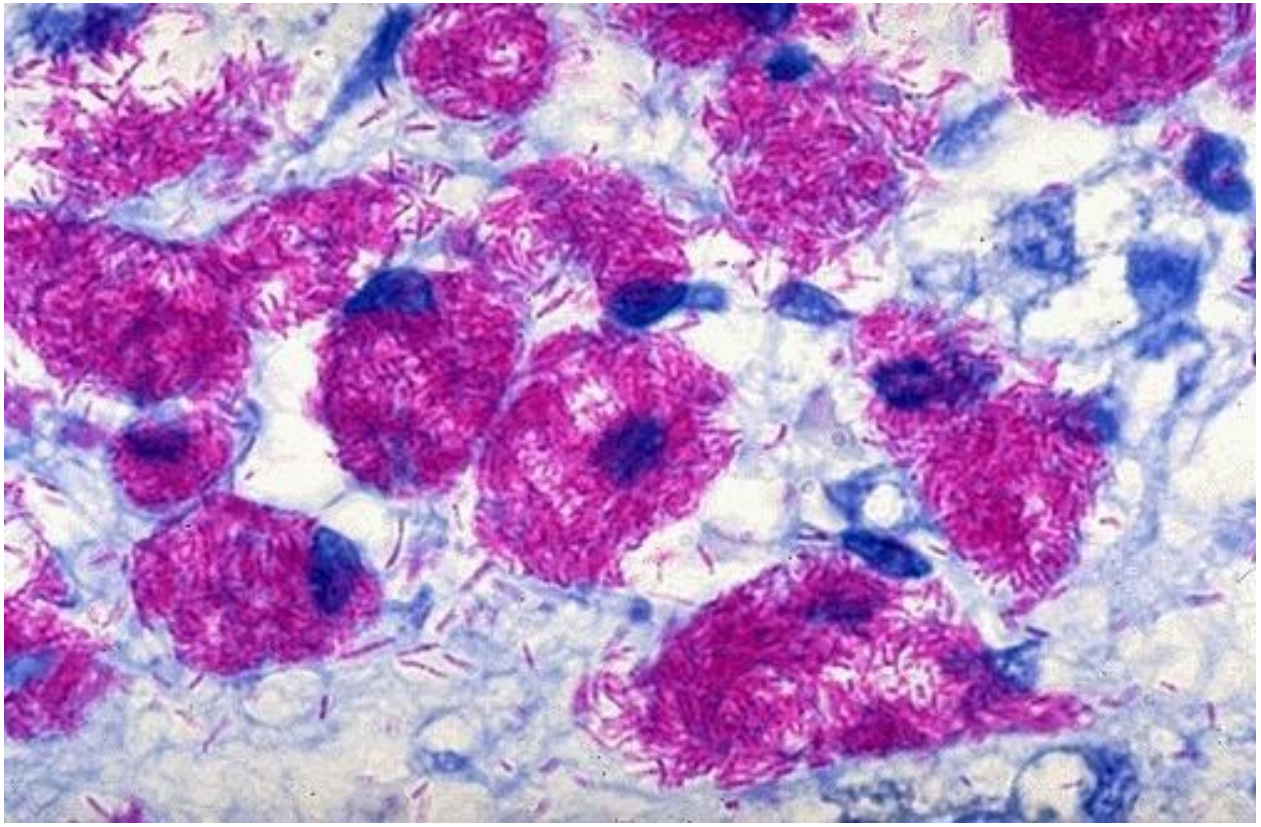
The Ziehl–Neelsen stain uses heat to drive the fuchsin stain into the cells

→ mycobacteria stained with fuchsin withstand decolourization with acid and alcohol and are therefore known as **acid-fast bacteria (AFB)**, whereas other bacteria lose the stain after acid and alcohol treatment



Acid-fast bacteria appear red coloured, other bacteria and cells are blue coloured

Differential staining: Ziehl-Neelsen



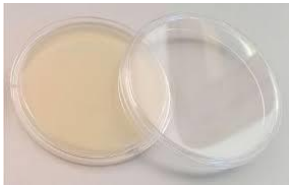
Smear on sputum slide of a patient with pulmonary tuberculosis.

Acid-fast bacilli colored in red. Polymorphonuclear leukocytes colored in blue

Direct methods

detection of the pathogenic microorganisms

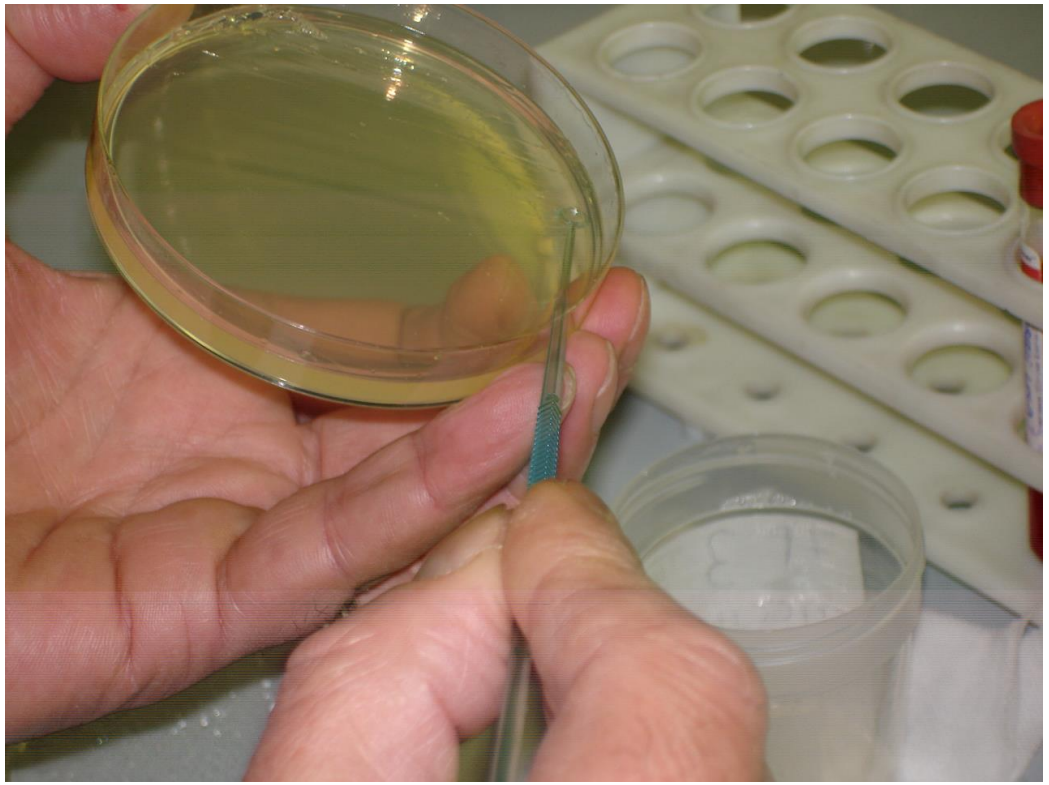
- Microscopic examination (bacterioscopic exam)
- **Culture (isolation)**
- Antigen detection
- Genetic test

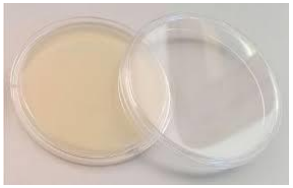


Culture

The microorganisms' cultivation in laboratory is a necessary condition for their study

For this purpose, the knowledge of nutritive substances and physical conditions required for growth is important

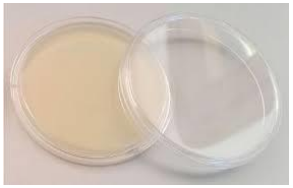




Culture: media

Culture media (liquid or solid) contain all the organic and inorganic substances required for the microbial growth

The chemical composition of the different culture media depends upon the nutritional needs of the cultivated microorganisms



Culture: media

MAIN COMPONENTS

Amino-acids	peptone
Growth factors	blood, serum, yeast extract
Energy sources	sugars, carbohydrates
Buffering agents	phosphates, citrate
Minerals	calcium, magnesium, iron
Selective agents	Antibiotics, chemicals
Indicators for pH change	Phenol red, neutral red, exc.
Gelling agents	agar

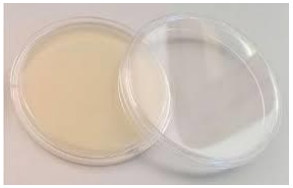
Culture: media

General-purpose media: rich in nutrients, often enriched with horse or sheep blood, allow the growth of almost all bacterial species of medical interest

Selective media: promote the growth of only selected microorganisms thanks to the presence of factors that inhibit the growth of other species

Differential media: distinguish one microorganism type from another growing on the same medium, allowing the presumptive identification of the isolated species

Enrichment media: allow to increase the growth of the microorganism of interest, including some of the more **fastidious** ones, thanks to factors inhibiting the growth of contaminating species



Culture

INCUBATION

Aerobic

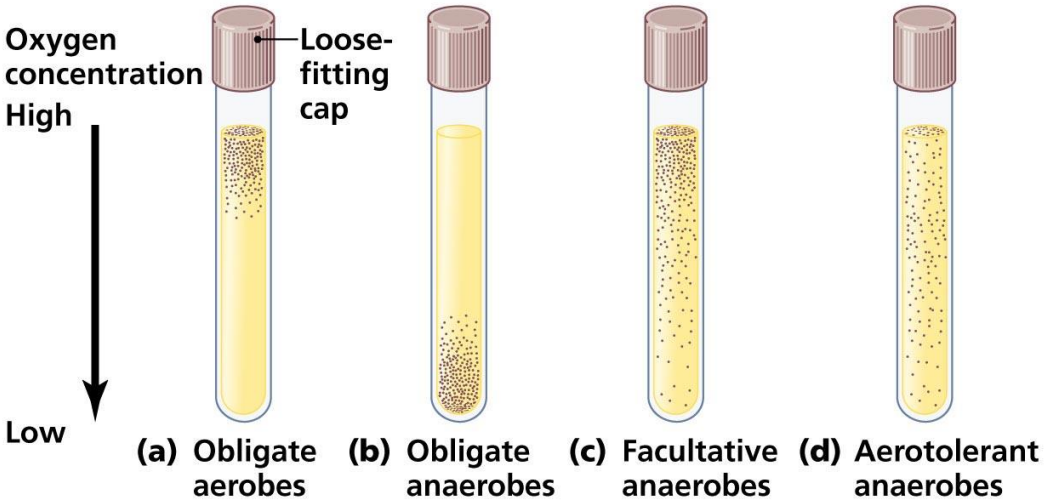
37° C

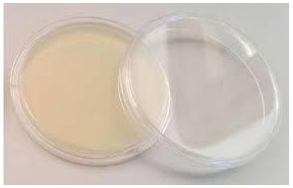
5-8° C

24 h

48 h

Anaerobic





Culture: identification

Macroscopic characteristics of the colonies

Blood agar

staphylococci **streptococci**

Mac Conkey Agar

No lactose fermentation
Pseudomonas

Lactose fermentation
enterobacteria

Chromogenic media

Identification
species determination

PRESUMPTIVE ID

- **Microscopic features** (staining, shape, arrangment)
- **Macroscopic features** (appearance of the colony)

FINAL ID (DEFINITIVE)

- **biochemical**
- **immunological**
- **molecular**

Biochemical ID

EVALUATION OF THE MICROBIAL METABOLIC PROPERTIES

Sugar metabolism through the oxydative pathway (aerobic) or the fermentation pathway (anaerobic)

Production of specific enzymes and/or metabolic products

“Manual”or “automatic” methods

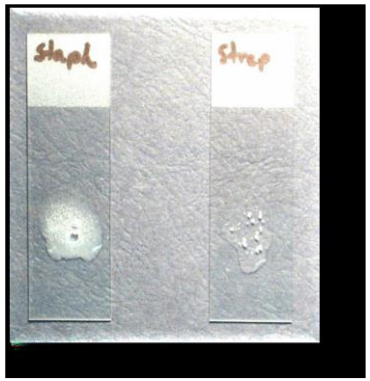
Rapid identification (4-6 h)

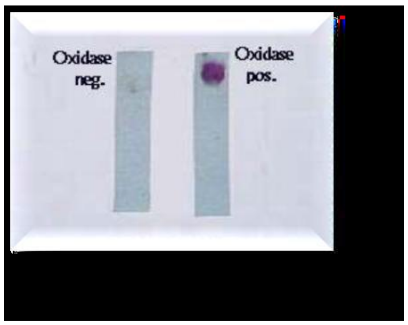
Manual Biochemical ID

Catalase test

$2H_2O_2 \rightarrow 2H_2O + O_2$

POS: Stafilococci
NEG: Streptococci

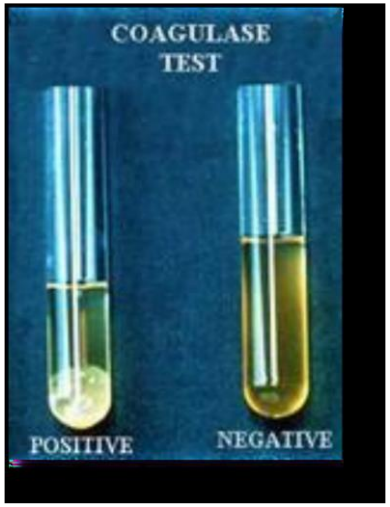




Oxydase test

distinguish enterobacteria from non fermenting Gram negative bacteria

Bacitracin sensitivity test



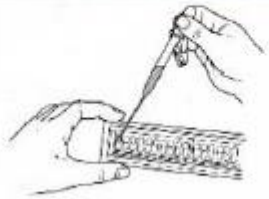
Coagulase Test

POS: Staphylococcus aureus
NEG: Staphylococcus epidermidis

Manual ID

API (bioMérieux)
Identification System

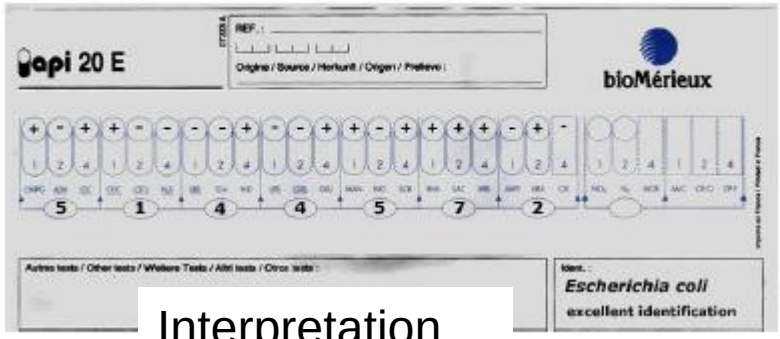
Sugar fermentation and metabolization of other substrates (urease, indole, etc) are detected by a pH indicator producing a colorimetric reaction



Inoculation

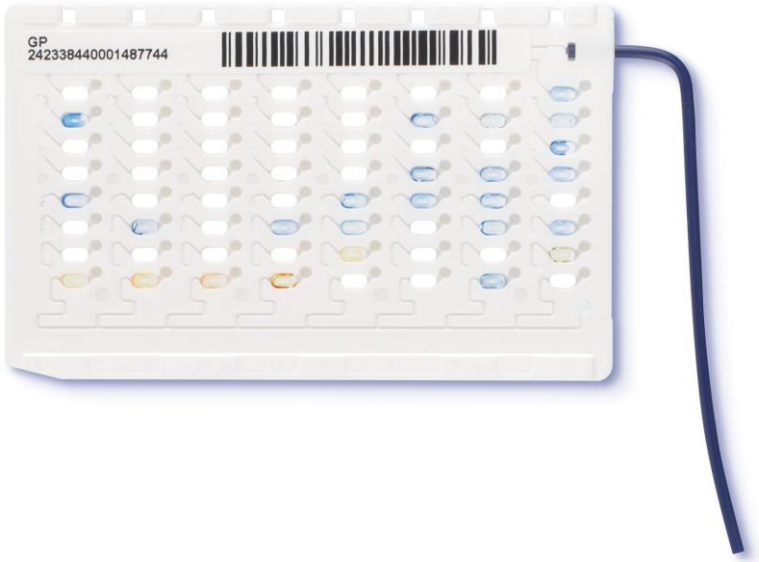
Incubation

Results



Interpretation

Automatic ID:
(Biomerieux System)

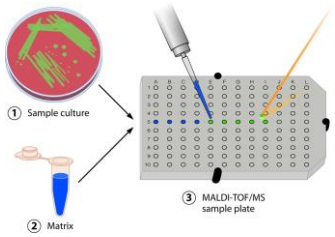


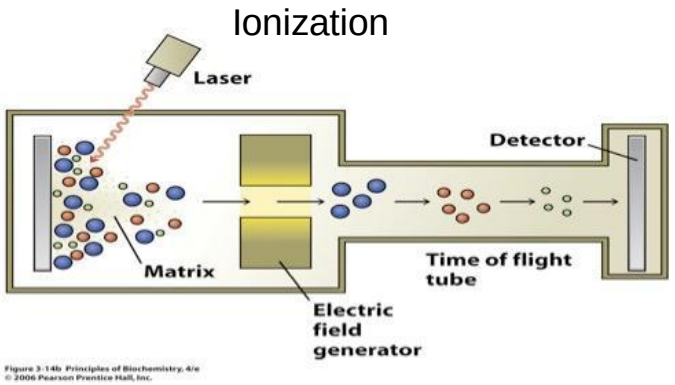
The system involves the use of a card containing a series of wells (approximately 30) containing biochemical (ID) or antibiotic (AST) substrates in dehydrated form.
Two types of cards:

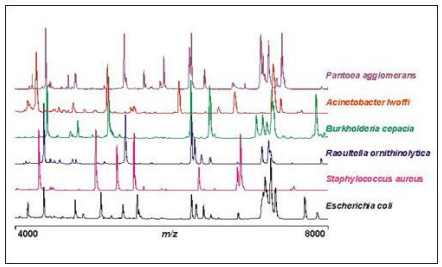
- for identification (ID)
- for antibiogram (AST)

Gram-positives (GP)
Gram-negatives (GN)
Yeasts (YST)
Bacillus spp. (BCL)
Anaerobi, *Corynebacterium* (ANC)
Neisseria, Haemophilus (NH)

ID MALDI-TOF







Mass spectrometry

Microbial identificaton is achieved through the production of mass spectra

The mass spectra generated are analyzed by dedicated software and compared with stored profiles of known species, genus or family to allow the microbial identification

1946 species can be identified

Direct methods

detection of the pathogenic microorganisms

- Microscopic examination (bacterioscopic exam)
- Culture (isolation)
- **Antigen detection**
- Molecular test

SEROLOGICAL REACTIONS

Immuno-Precipitacion
Agglutination
Complement fixation
Neutralization

→ The reaction can be observed at macroscopic level

Do not allow the identification of the Ab classes

Immunoenzymatic assay
Immunofluorescence
Immunoblotting
Radioimmunoassay
Chemiluminescent immunoassay

→ Use of Ag (or Ab) bound to an «indicator»

Allow the identification of the Ab classes

Antigen test

Detection of soluble carbohydrate antigens by agglutination of antibody-coated latex particles or red blood cells

- *Legionella* in urine
- *C. difficile* in stools
- Group A *Streptococcus* in throat swabs
- *Streptococcus pneumoniae* in CSF and urine
- *Haemophilus influenzae* type B in CSF and urine
- *Cryptococcus neoformans* in CSF and urine

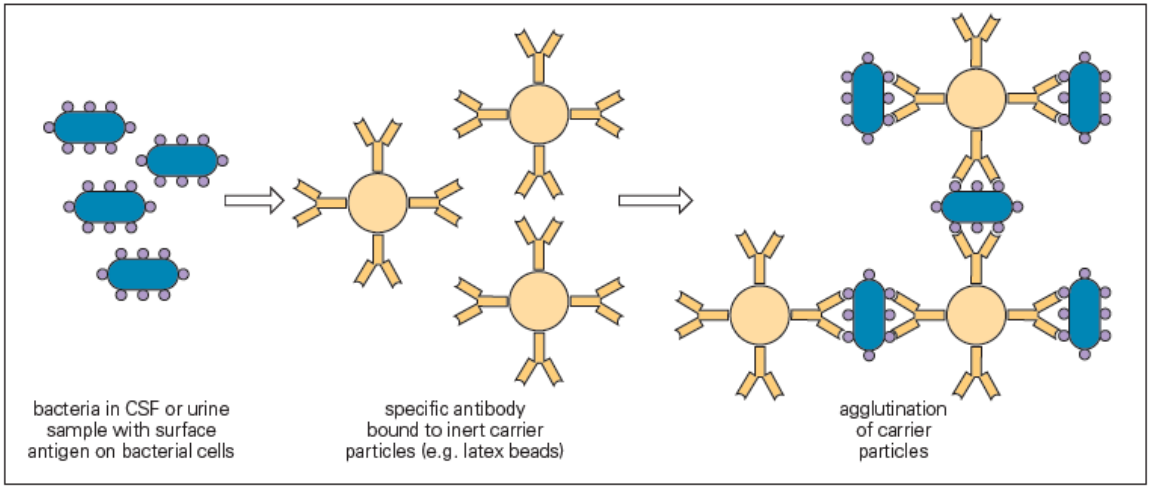
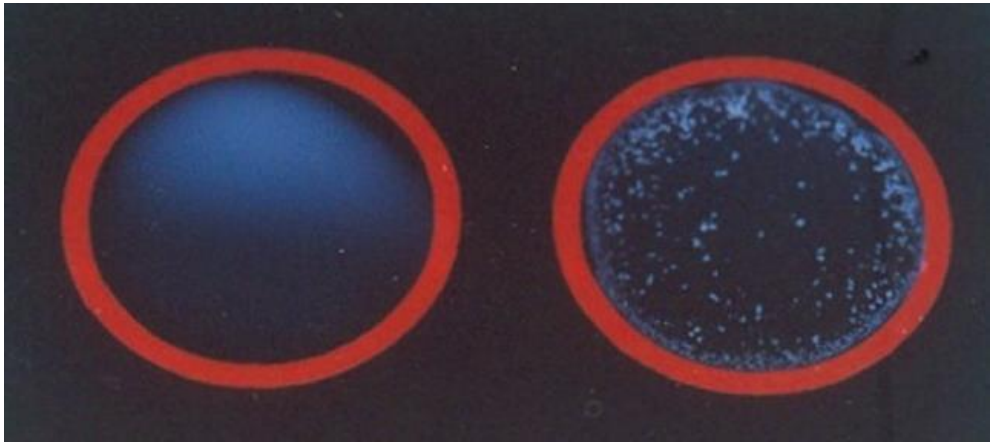
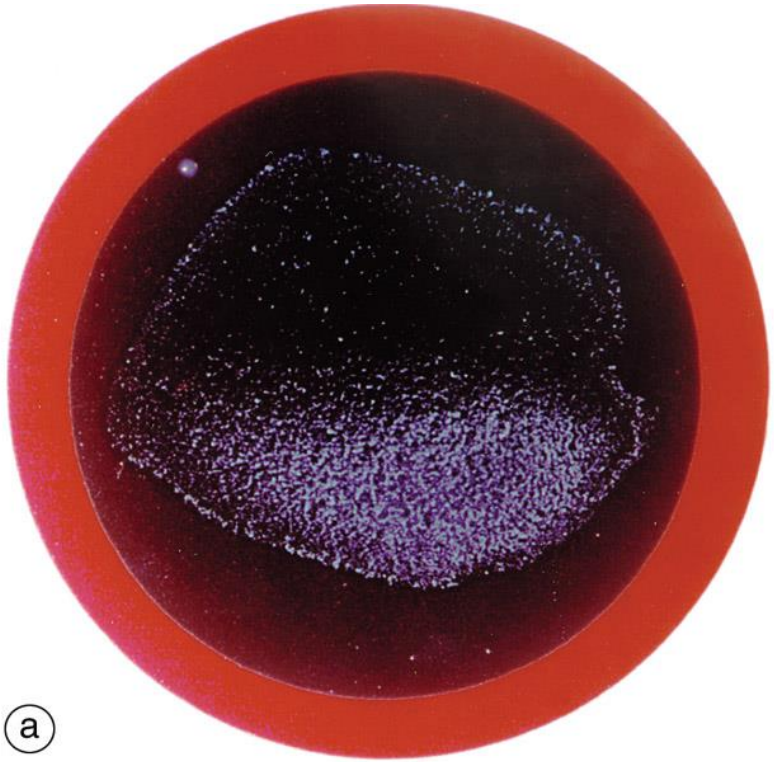


Figure 32.9 When a specimen of cerebrospinal fluid (CSF) containing bacteria (e.g. *Haemophilus influenzae*) is mixed with a suspension of latex particles coated with specific antibody (e.g. *H. influenzae* anticapsular antibodies), the interaction between antigen and antibody causes an immediate agglutination of particles, which is visible to the naked eye.





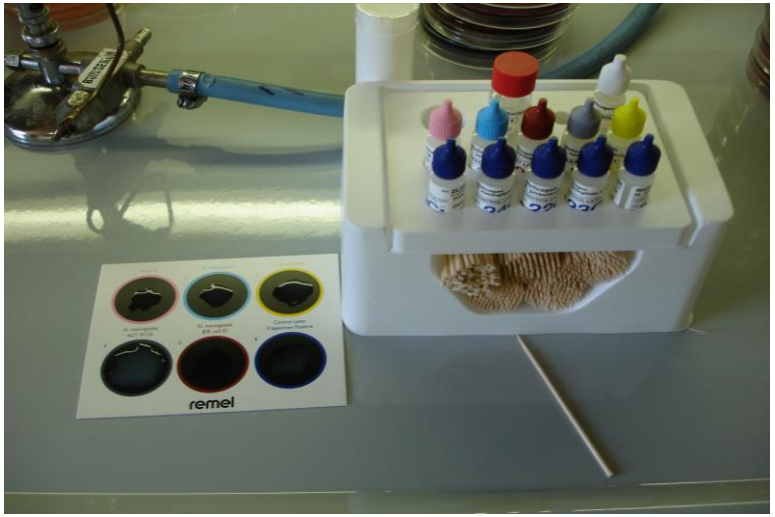
Latex agglutination tests can be taken by collecting a sample containing the specific antigen, or antibody, which is later mixed with an antibody, or antigen, which is coated on latex beads in serial dilutions with normal saline. If the suspected substance is present, the latex beads will clump together. This clumping is called agglutination.

Positive agglutination test

(a)

Detection of soluble antigens by LATEX AGGLUTINATION → meningitis

- used when no bacterial cells are observed during microscopy examination, even in the presence of numerous neutrophil leukocytes. Bacteria may have been lysed by the presence of proteolytic enzymes produced by neutrophils
- especially useful when the patient has received antibiotics and organisms may appear morphologically unidentifiable in the CSF and fail to grow in culture



The main bacterial pathogens causing meningitis are tested:

N. meningitidis
S. pneumoniae
S. agalactiae
H. influenzae

Urinary Antigens of *Legionella* and *Pneumococcus*

Rapid diagnosis is allowed

Positivity is detected from only 1 day to many months after the onset of symptoms

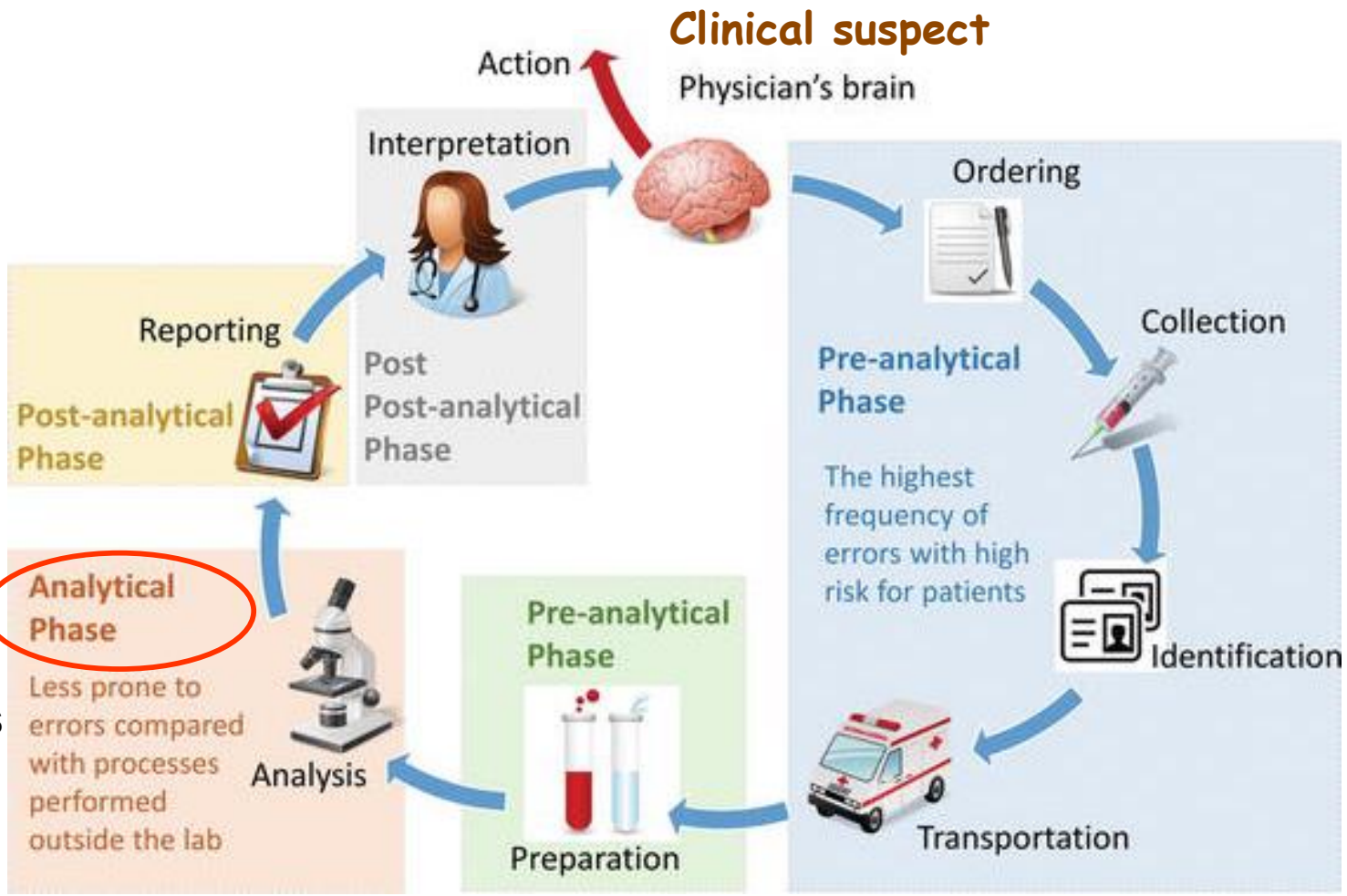
Immunochromatographic test

Urinary antigen positivity is sufficient for the microbiological case definition

Sensitivity 70-100 %
Specificity 100%

Not all serotypes are detected

Diagnosis



Virus
Bacteria
Fungi
Parasites