



BAILEY & SCOTT'S
**DIAGNOSTIC
MICROBIOLOGY**



TWELFTH EDITION



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2014

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Diagnostic Microbiology

320 MIC

Lecture: 10 Diagnosis of Bacterial Infections II

Non-cultural bacterial diagnostic methods

- **Antigen detection.**

e.g. latex agglutination

- **Antibody detection.**

e.g. agglutination tests, complement fixation tests, indirect immunofluorescence

- **Molecular methods.**

e.g. Polymerase Chain Reaction (PCR)

Two SEROLOGICAL METHODS:

1. Identification of an organism with known antiserum:

- **Capsular swelling (Quelling) reaction:** The capsule swells up when comes in touch with specific antiserum. Reaction is positive with: *Streptococcus pneumonia*, *Haemophilus influenza*, *Niesseria meningitides*.
- **Slide agglutination test:** Used to identify *Salmonella* & *Shigella*, looking for O, H, & Vi antigens.
- **Latex agglutination test:** Latex beads are coated with specific antibody, and agglutinated by homologous antigen. The test is used in diagnosis of *H. influenzae* , *N. meningitidis* , *Cryptococcus neoformans*.
- **Counter immunoelectrophoresis test:** The unknown bacterial antigen and the known specific antibody move towards each other and form a precipitate. The test is used to diagnose CSF pathogens, e.g.: *H. influenzae* , *N. meningitidis* , *S. pneumoniae*.
- **ELISA:** an enzyme is linked to the known antibody and used to detect the homologous antigen.
- **Fluorescent – antibody test:** the known antibody is labeled with a fluorescent dye & detected by an U.V.microscope, either directly or indirectly when antibody unites with antigen.

Two SEROLOGICAL METHODS:

2. Identification of serum antibodies with known antigens:

- **Slide & tube agglutination test:** Serial dilution is made for patient serum and then bacterial antigen is added. Highest dilution of serum with agglutination shows the titre.
- This test is to diagnose: **enteric fever, brucellosis, plague and rickettsial diseases.**
- **Cold agglutinin test:** Patients infected with *Mycoplasma pneumoniae* will develop autoimmune antibodies that agglutinate human RBC at 4°C but not at 37°C.
- **Serological tests for syphilis: Include:**
 - 1- **Non-treponemal tests:**
using cardiolipin antigen: Rapid plasma regain (RPR) and VDRL tests.
 - 2- **Treponemal tests:**
such as immobilizing test.

Classical bacterial identification can only be performed on pure cultures of bacteria

• Isolation of Individual Bacteria

- Specimen is “streaked”, using a sterile loop, onto solid media.
- The agar plates (media) are incubated at appropriate temperature and atmosphere.
 - Often at 35 °C.
 - Often at 5% CO₂.
 - Usually first examined after 24 hours.



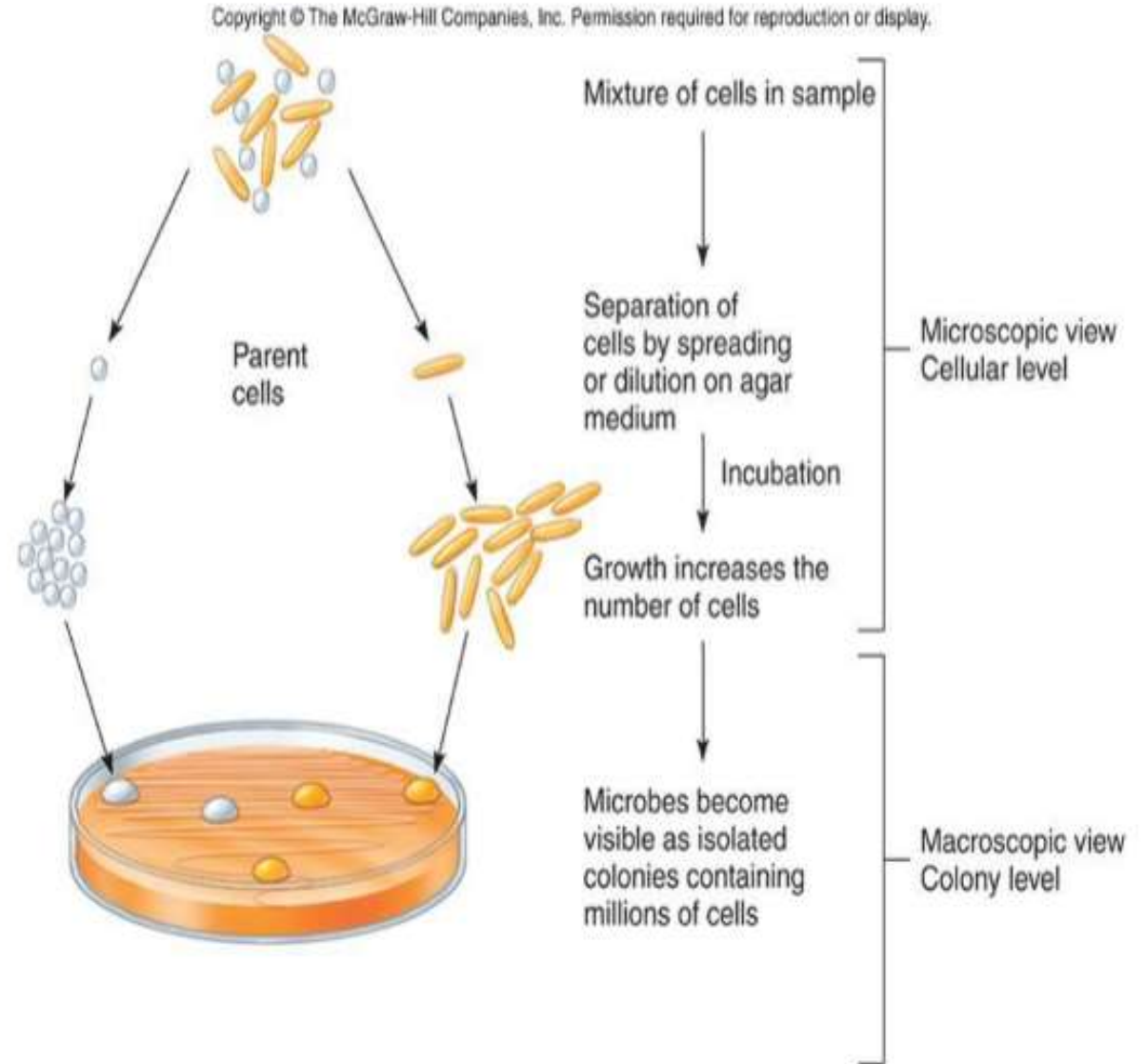
Isolation techniques

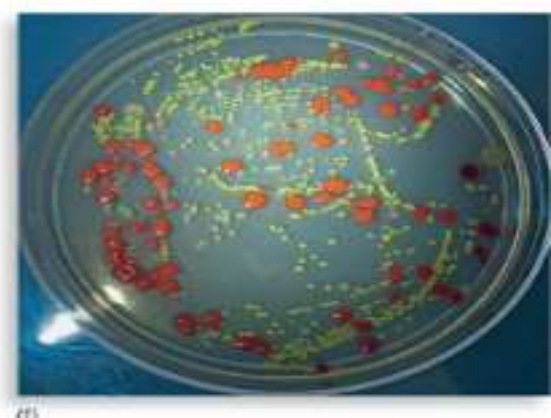
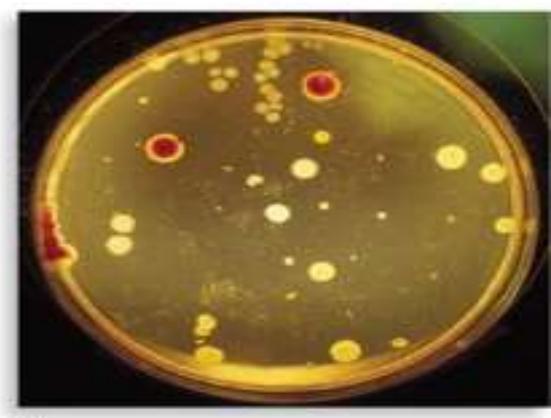
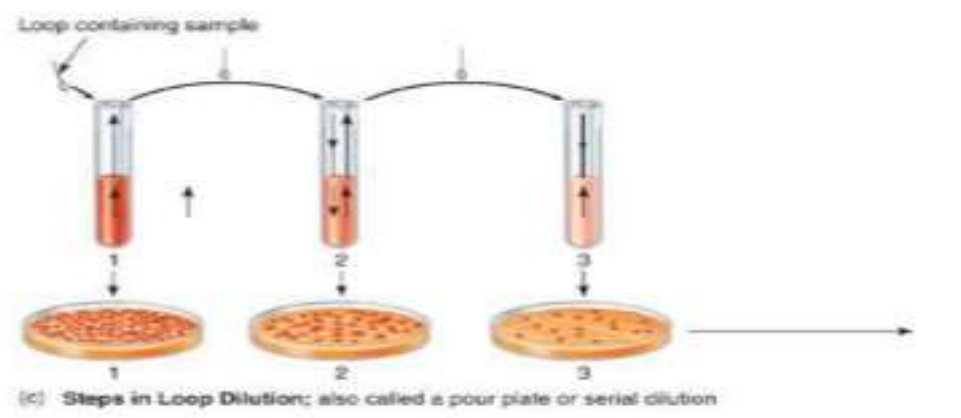
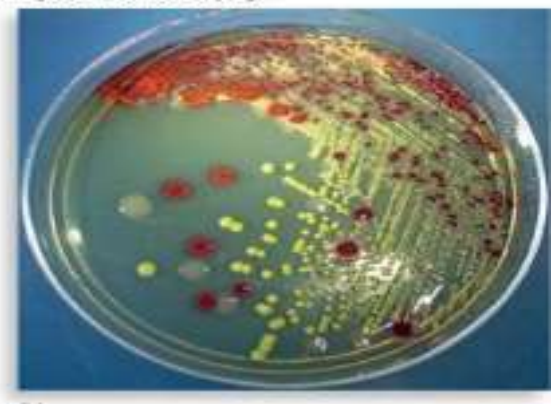
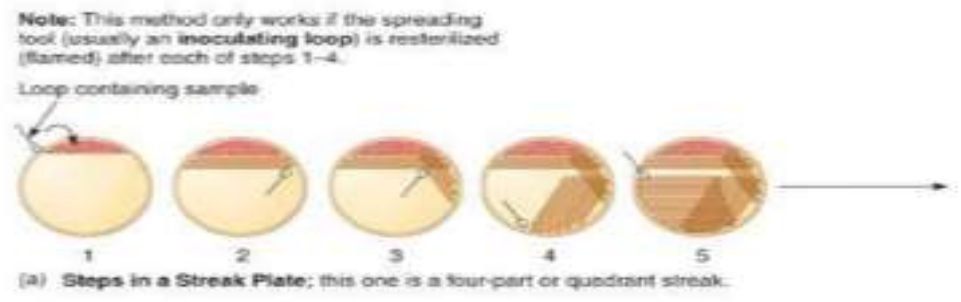
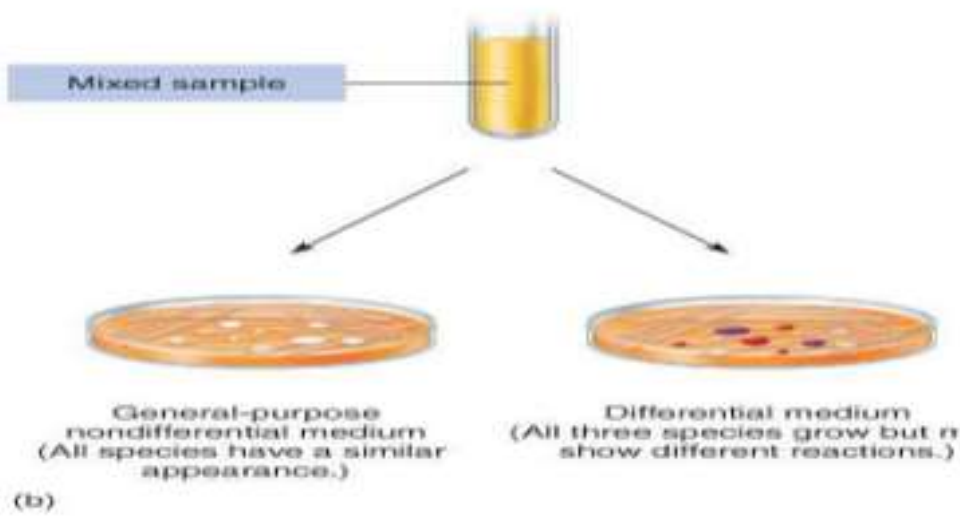
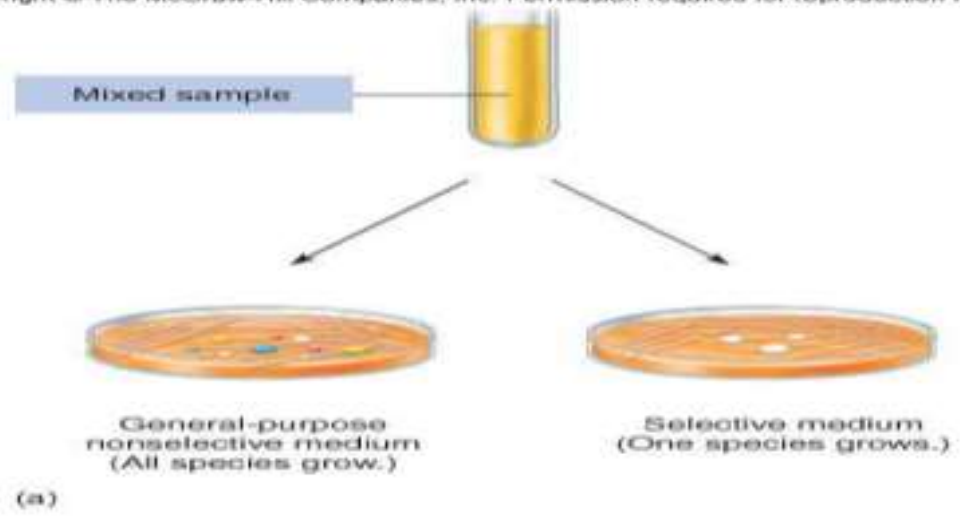
- Include:

- **Streak plate technique**

- **Pour plate technique**

- **Spread plate technique**





Growth of Colonies

Bacterial Colony

- Result of one bacterium being isolated from others during “streaking procedure”
- That bacterium grows in numbers exponentially
- Many bacteria have a generation time of 20 minutes



Pure Culture of
Francisella tularensis
Colonies After Growth

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Pure Culture of
Yersinia pestis
Colonies on Blood Agar After 48 hours of Growth

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Colony “Picking”



- Sterile needle or loop is touched to surface of colony and transferred to fresh, sterile media.
- Incubation for 24 hours.

Now we have a pure culture of bacteria:

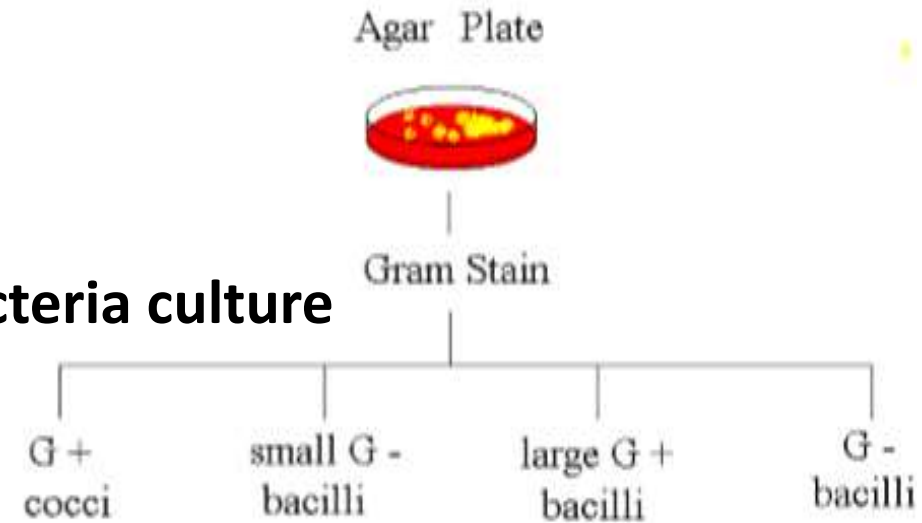
Testing is now done to confirm the identification of the bacteria culture

Stains

Biochemical tests

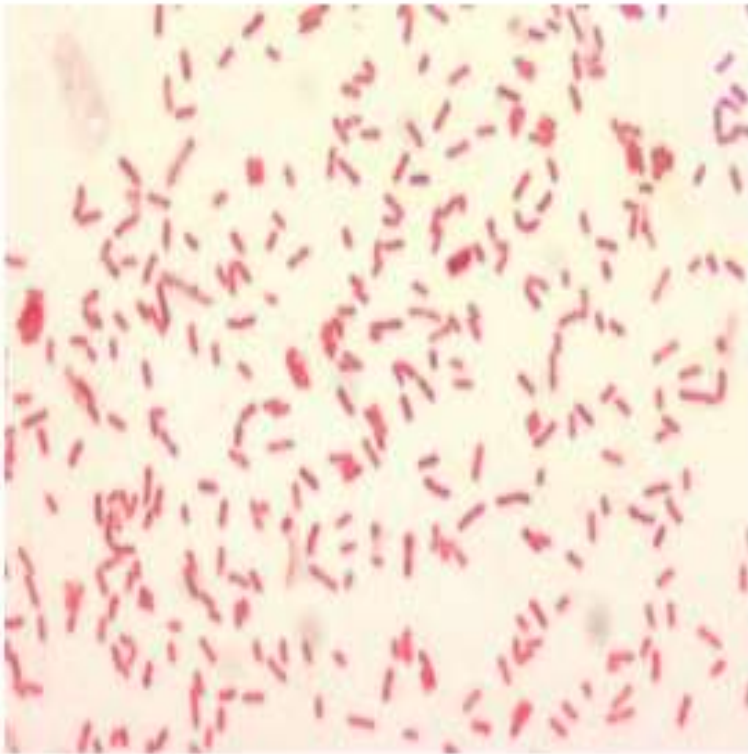
Serological tests (using known antibodies)

Molecular tests (nucleic acid probes)



Yersinia pestis Gram stain

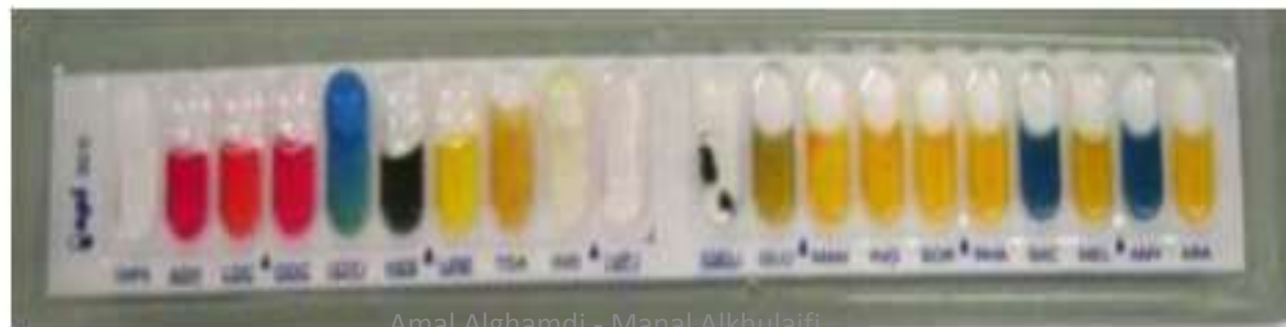
Antimicrobial Sensitivity Test



Examples of Biochemical Tests



API 50 Test

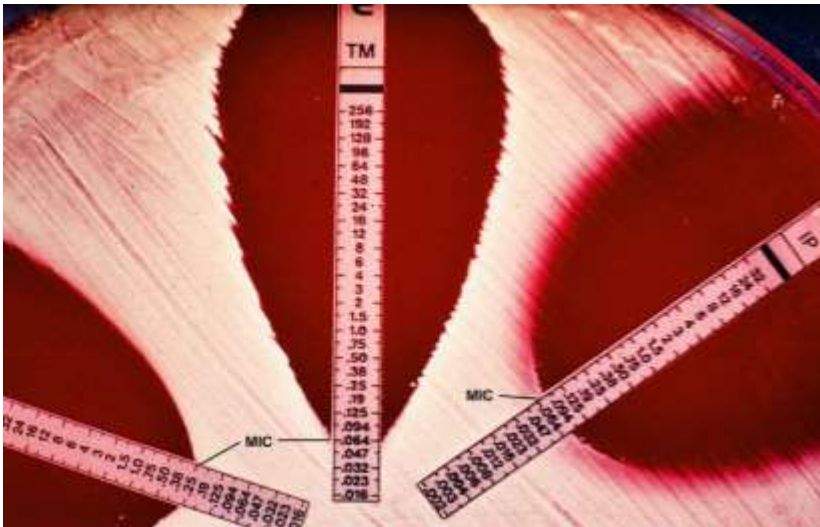


Factors limiting usefulness of bacteriological investigations

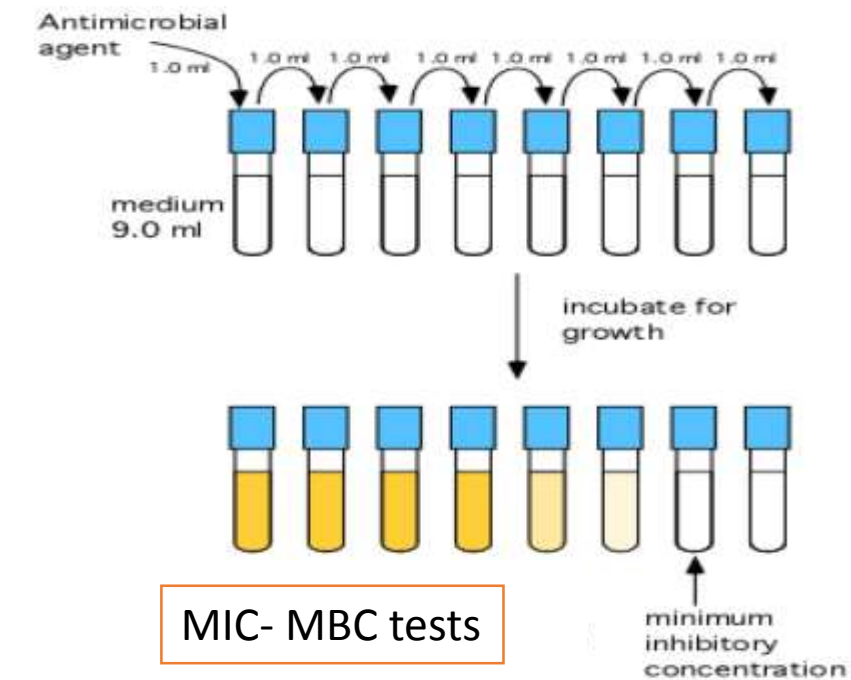
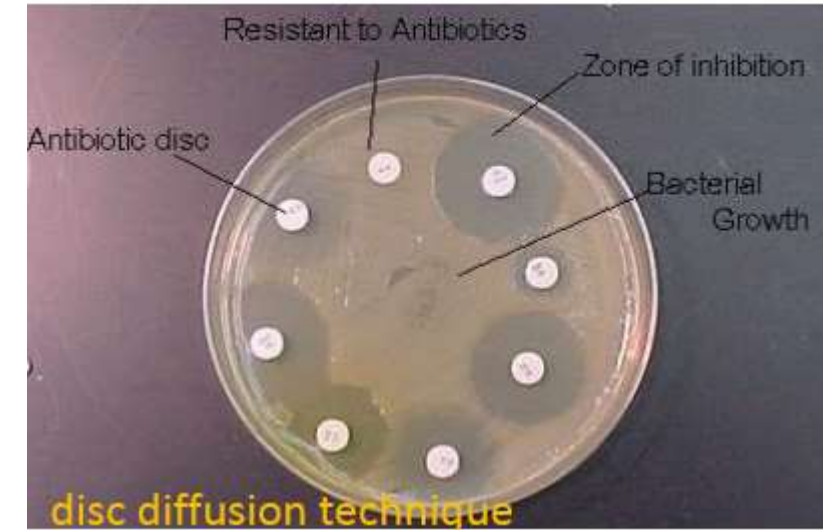
- **wrong sample** (e.g. saliva instead of sputum)
- **delay in transport / inappropriate storage** (e.g. CSF)
- **overgrowth by contaminants** (e.g. blood cultures)
- **insufficient sample / sampling error** (e.g. in mycobacterial disease)
- **patient has received antibiotics**

Sensitivity tests

- On solid media:
disc diffusion technique.
- In liquid media:
minimum inhibitory concentration (MIC)- (MBC) tests.
- Breakpoint methods (E-test).



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