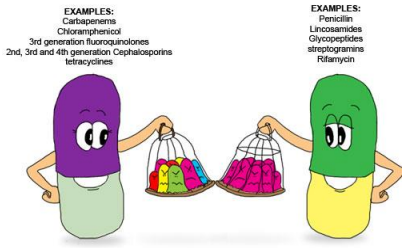
Antibiotics_1

Definitions

- The term: Antibiotic is used mainly, but not exclusively, as antibacterial agents
- Antibiotics are either naturally occurring microbial products or synthetic/semi-synthetic compounds
- **Antibacterial spectrum**: a range of antimicrobial activity against bacteria
  - A broad-spectrum antibacterial drug: inhibit a wide variety of gram-positive and –negative bacteria
  - A narrow-spectrum antibacterial drug: active only against a limited variety of bacteria



Cont. Definitions

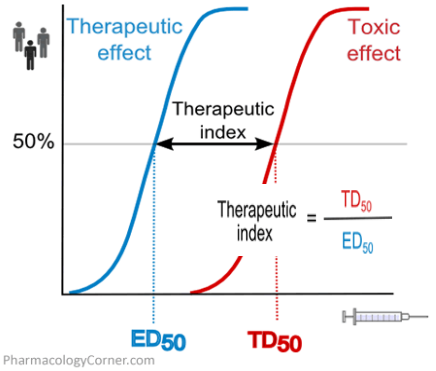
➢ Selectivity

kills harmful microbes without damaging the host

- Many chemicals are useful in restricting bacterial growth.
  - Inherently toxic.
  - Non-selective
  - Cannot be used therapeutically.
- Many antibiotic molecules are toxic if administered at high concentrations.

➢ Therapeutic index

The ratio between the toxic dose and the therapeutic dose of a drug, used as a measure of the relative safety of the drug for a particular treatment.



➢ **Categories of antibiotics:**

- Bacteriostatic (inhibit – reversible)
- Bactericidal (kill – irreversible)

❖ Bacteriostatic *Vs.* Bactericidal (immunocompromised patients)

❖ Distinction B/W **Bacterio-static** and **-cidal**

The sources of antibiotics

- Bacteria

most prolific source is the *Streptomycete* group

e.g. streptomycin, tetracycline, gentamicin, bacitracin, chloramphenicol, rifamycin.

- Moulds (filamentous fungi)

e.g. penicillins from *Penicillium* spp., cephalosporins from *Cephalosporium* spp.

- Synthesis

e.g. chloramphenicol

- Semi-synthesis

Part of molecule is produced by a microorganism but part of it is modified/altered chemically, usually to *improve* it.

e.g. many penicillins - ampicillin, carbenicillin, cloxacillin

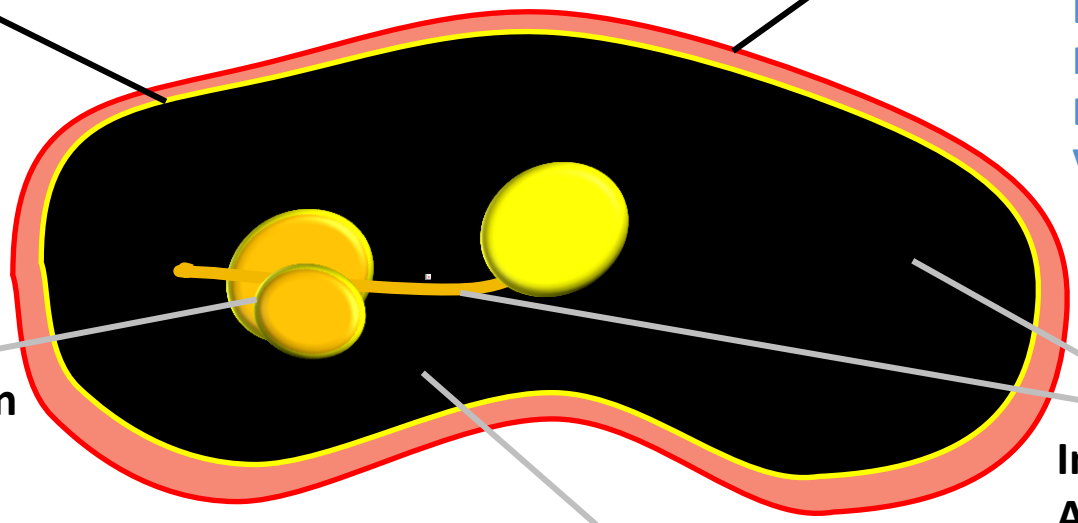
Main properties of typical antibiotics

- Unlike sterilization, disinfection and antiseptic techniques
  1. Selectively toxic for bacteria (**competence**)
  2. Destroy or inhibit structures present in bacteria but not in the host
  3. Mostly have little side effects on patients but not toxic
  4. Work along with the host immune system
  5. Slow emergence of resistance
  6. Narrow spectrum of activity
  7. Cidal activity

Other properties of typical antibiotics

- **Pharmacological properties:**
- ✓ Long plasma half-life
- ✓ Good tissue distribution including CSF
- ✓ Low plasma-protein binding
- ✓ Oral and parenteral dosing forms
- ✓ No interference with other drugs
- **Other properties:**
- ✓ Easy and unexpansive to produce
- ✓ Short treatment duration
- ✓ Appropriate administration

Modes of action



Alteration of Cell Membrane

Polymyxin
Bacitracin

Inhibition of Cell wall synthesis

Beta-lactams
Isoniazid
Ethambutol
Vancomycin

Inhibition of Protein Synthesis

Aminoglycoside
Tetracycline
Clindamycin

Antimetabolite Activity

Sulfonamides
Para-aminosalicyclic acid

Inhibition of Nucleic Acid Synthesis

Quinolone
Rifampin
Metronidazole

Cont. Modes of action

Classification of antibiotics by target site:

1. Inhibition of cell wall synthesis (most common mechanism)
2. Inhibition of protein synthesis (translation) (second largest class)
3. Alteration of cell membranes
4. Inhibition of nucleic Acid synthesis
5. Antimetabolite activity

Inhibitors of cell wall synthesis

A. Beta-lactams

- ✓ Contain a beta-lactam ring
- ✓ Inhibit cell wall synthesis by binding to penicillin-binding proteins (PBPs)
- ✓ Resistance involve alteration of the target site (e.g. MRSA) or alteration of the uptake or through drug inactivation
- ✓ Resistance by production of beta-lactemases
- ✓ Cause mild rashes and immediate hypersensitivity reaction

Inhibitors of cell wall synthesis

B. Glycopeptides

- ✓ Large molecules include vancomycin and teicoplanin
- ✓ Are bactericidal agent
- ✓ Act at an earlier stage than beta-lactams
- ✓ Active only against Gram-positive organisms
- ✓ Must be given by injection for systemic infections
- ✓ Gram-negative are intrinsically resistant to glycopeptides
- ✓ Organisms may acquire resistance to glycopeptides (e.g. vancomycin-resistant enterococci, VRE)
- ✓ Resistance in the staphylococci occurs by mutation or by acquisition from enterococci
- ✓ Potentially ototoxic and nephrotoxic

Inhibitors of cell membrane

Polymyxins

- ✓ Are bacteriocidal cyclic polypeptides that disrupt the structure of cell membranes
- ✓ Active against Gram-negative organisms except *proteus* spp.
- ✓ Primarily used topically and have also been used for gut decontamination, wound irrigation and as bladder washout
- ✓ Resistance due to chromosomally mediated alteration in membrane structure or antibiotic uptake

Inhibitors of protein synthesis

A. Aminoglycosides

- ✓ Family of related molecules with bacteriocidal activity
- ✓ Acts by binding to specific proteins in the 30S ribosomal subunit
- ✓ Must be given intravenously or intramuscularly for systemic treatment
- ✓ Gentamycin and the other aminoglycosides (e.g. tobramycin, amikacin and netilmicin) are important for the treatment of serious Gram-negative infections
- ✓ Active against staphylococci but not streptococci and anaerobes
- ✓ Aminoglycosides-modifying enzymes is the main cause of resistance to aminoglycosides
- ✓ Potential nephrotoxic and ototoxic

Inhibitors of protein synthesis

B. Tetracyclines

- ✓ Bacteriostatic compounds
- ✓ Bind to the small ribosomal subunit thus preventing aminoacyl transfer RNA from entering the acceptor sites on the ribosome
- ✓ active against a wide variety of bacteria
- ✓ The use is restricted due to widespread resistance
- ✓ Usually administered orally
- ✓ Should be avoided in pregnancy and in children under 8 years of age
- ✓ May cause liver damage, teeth brown staining and encouraging overgrowth by resistant and undesirable bacteria and fungi

Inhibitors of protein synthesis

C. Chloramphenicol

- ✓ Bacteriostatic
- ✓ Binds to the large (50S) ribosomal subunit where it blocks the action of peptidyl transferase thus preventing peptide bond synthesis
- ✓ active against a wide variety of bacteria
- ✓ Cause dose-dependent bone marrow suppression and may cause aplastic anemia
- ✓ The common resistance mechanism is the inactivation of the drug by a plasmid mediated enzymatic mechanism

Inhibitors of protein synthesis

D. Macrolides, Lincosamides and streptogramins

- ✓ Share overlapping binding sites on ribosomes
- ✓ Resistance to macrolides confers resistance to the other two groups
- ✓ The clinically important drugs are the macrolide erythromycin and the lincosamide clindamycin
- ✓ Erythromycin is an alternative to penicillin for streptococcal infections but resistant strains of streptococci are common
- ✓ Erythromycin is relatively free of serious toxic side effects
- ✓ Clindamycin active against anaerobes both Gram-positive and –negative
- ✓ Pseudomembranous colitis caused by *Cl. difficile* was first noted following clindamycin treatment

Inhibitors of protein synthesis

E. Fusidic acid

- ✓ Bacteriostatic
- ✓ Active against a wide range of Gram-positive cocci and is important for staphylococcal infections resistant to beta-lactams
- ✓ Should be used with other antistaphylococcal drugs to prevent emergence of resistance

Inhibitors of nucleic acid synthesis

A. Quinolones

- ✓ Bacteriocidal Synthetic agent
- ✓ Interfere with replication of the bacterial chromosome through inhibiting the activity of bacterial DNA gyrase and topoisomerases
- ✓ Resistance to quinolones is chromosomally mediated
- ✓ Used as alternatives to beta-lactam antibiotics for treating a variety of infections
- ✓ Safe and tolerable agents
- ✓ Administrated orally
- ✓ e.g. trovafloxacin is the fourth generation of quinolones compounds that cover Gram-negative/–positive and anaerobes
- ✓ Fluoroquinolones are not recommended for children or pregnant or lactating women because of possible toxic effects on cartilage development

Inhibitors of nucleic acid synthesis

B. Rifamycins

- ✓ Rifampicin is clinically the most important rifamycin and blocks the synthesis of mRNA
- ✓ Are bacteriocidal agents
- ✓ Rifampicin is administrated orally
- ✓ Rifampicin is used in the treatment of mycobacterial infection
- ✓ Rifampicin is used as prophylaxis of close contacts of meningococcal and *Haemophilus meningitis*
- ✓ Resistance is provided by chromosomal mutations that alter the RNA polymerase target
- ✓ Rashes and jaundice are side effects of rifampicin treatment

Antimetabolite agents

A. Sulfonamides

- ✓ Bacteriostatic compounds
- ✓ Structural analogues of para-aminobenzoic acid (PABA)
- ✓ Act in competition with PABA for the active site of dihydropteroate synthetase, an essential enzyme in the synthetic pathway of tetrahydrofolic acid (THFA) for the nucleic acid synthesis
- ✓ Useful in the treatment of urinary tract infection, but resistance is widespread
- ✓ Rarely, cause *Stevens-Johnson syndrome*

Antimetabolite agents

B. Trimethoprim (and co-trimoxazole)

- ✓ Structural analogues of the aminohydroxy-pyrimidine moiety of folic acid
- ✓ Prevents the synthesis of THFA by inhibiting dihydrofolate reductase
- ✓ Trimethoprim is often given in combination with sulfamethoxazole as co-trimoxazole
- ✓ co-trimoxazole is active against wide range of urinary tract pathogens
- ✓ Resistance to Trimethoprim is provided by plasmid-encoded dihydrofolate reductases

Antimicrobial resistance

- Resistant microorganism cause infections that fail to respond to current treatment
- Threat to global stability
  - Prolonged illness
  - Greater risk of death
  - Higher medical and social costs
- **Superbugs**
- High percentages of resistant tuberculosis strains, MRSA and multidrug-resistant Gram-negative bacteria
- Ongoing problem, new resistance mechanisms have emerged, making the latest generation of antibiotic virtually ineffective

Inappropriate antibiotic use

- Use of antibiotics with no clinical indication (e.g. for viral infections)
- Use of broad spectrum antibiotics when not indicated
- Inappropriate choice of empiric antibiotics
- Inappropriate dose or/and route lead to ineffective concentration of antibiotics at site of infection
- Inappropriate duration of drug regimen

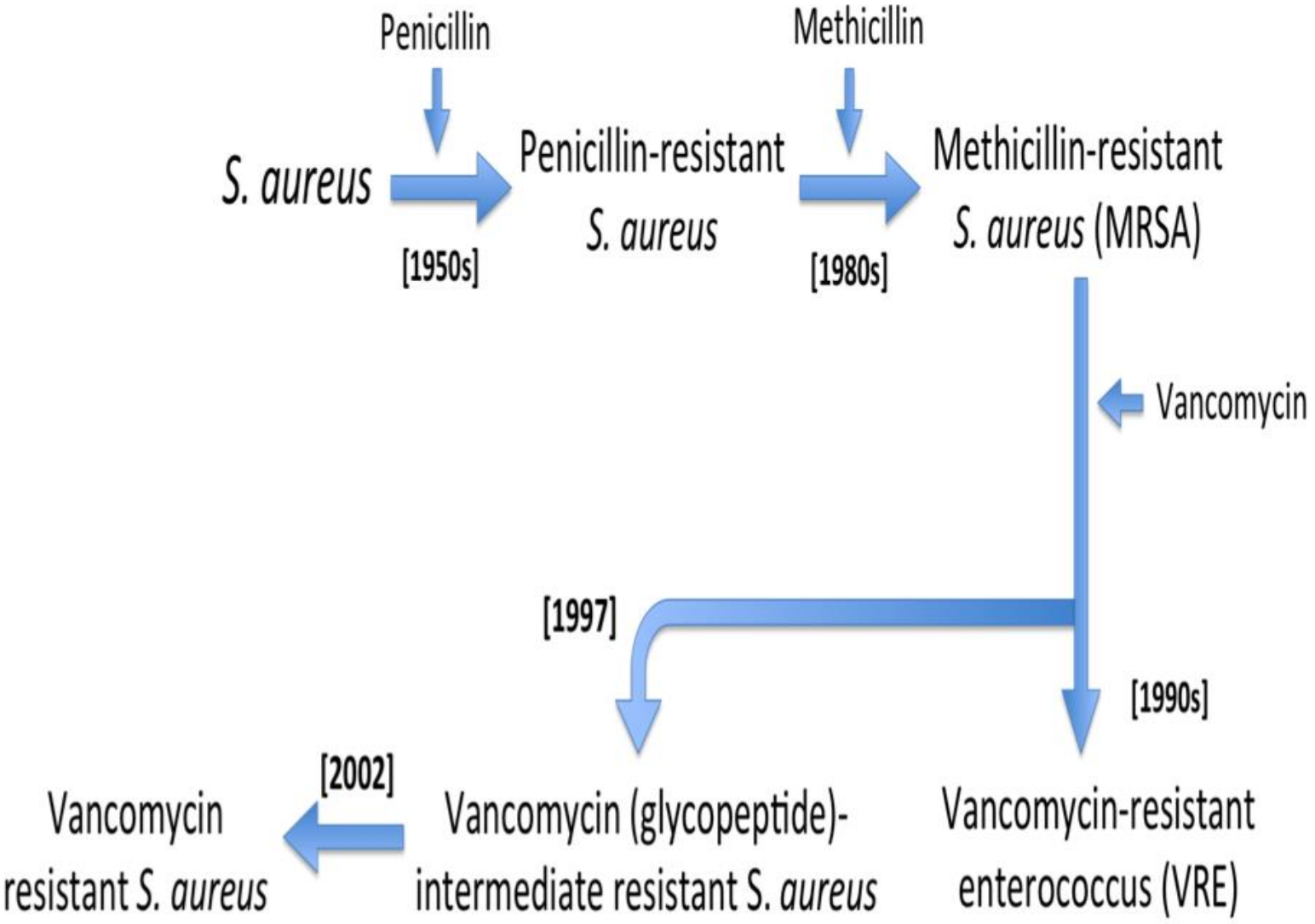
Resistance to antibacterial agents

- Resistance to antibacterial agents is a matter of degree
- Resistant organism is a one that will not be inhibited or killed by an antibacterial agent at concentration of the drug achievable in the body after normal dosage
  - **Intrinsic resistance**
  - **Acquired resistance**
- Some organisms are innately resistant to some families of antibiotic
- Innately resistant organisms could be either because they lack a susceptible target or because they are impermeable to the antibacterial agent
- Within innately susceptible species, there are strains that develop or acquire resistance

Mechanisms of Antibiotic Resistance

1. Altered target site
   - ✓ Alteration of the target site lead to lowering the affinity for the antibacterial
   - ✓ Normal metabolism of the target may proceed
2. Altered uptake (decreased entry)
   - By decreasing the permeability of the cell wall
3. Enzymatic degradation
   - e.g. Beta-lactamases
   - e.g. Aminoglycoside-modifying enzymes
   - e.g. Chloramphenicol acetyl transferases
4. Bypass pathway
   - By pumping the drug out of the cell (efflux mechanism)

The genetics of resistance



The genetics of resistance

A. Chromosomal mutation

may result in resistance to a class of antimicrobial agents (cross-resistance)

- Resistance may arise from:
  1. **A single chromosomal mutation in one bacterial resulting in the synthesis of an altered protein**
     - e.g. single amino acid change in the enzyme dihydropteroate synthetase resulting in a lowered for sulfonamides
  2. **A series of mutations**
     - e.g. changes in penicillin-binding proteins (PBPs) in penicillin-resistant pneumococci

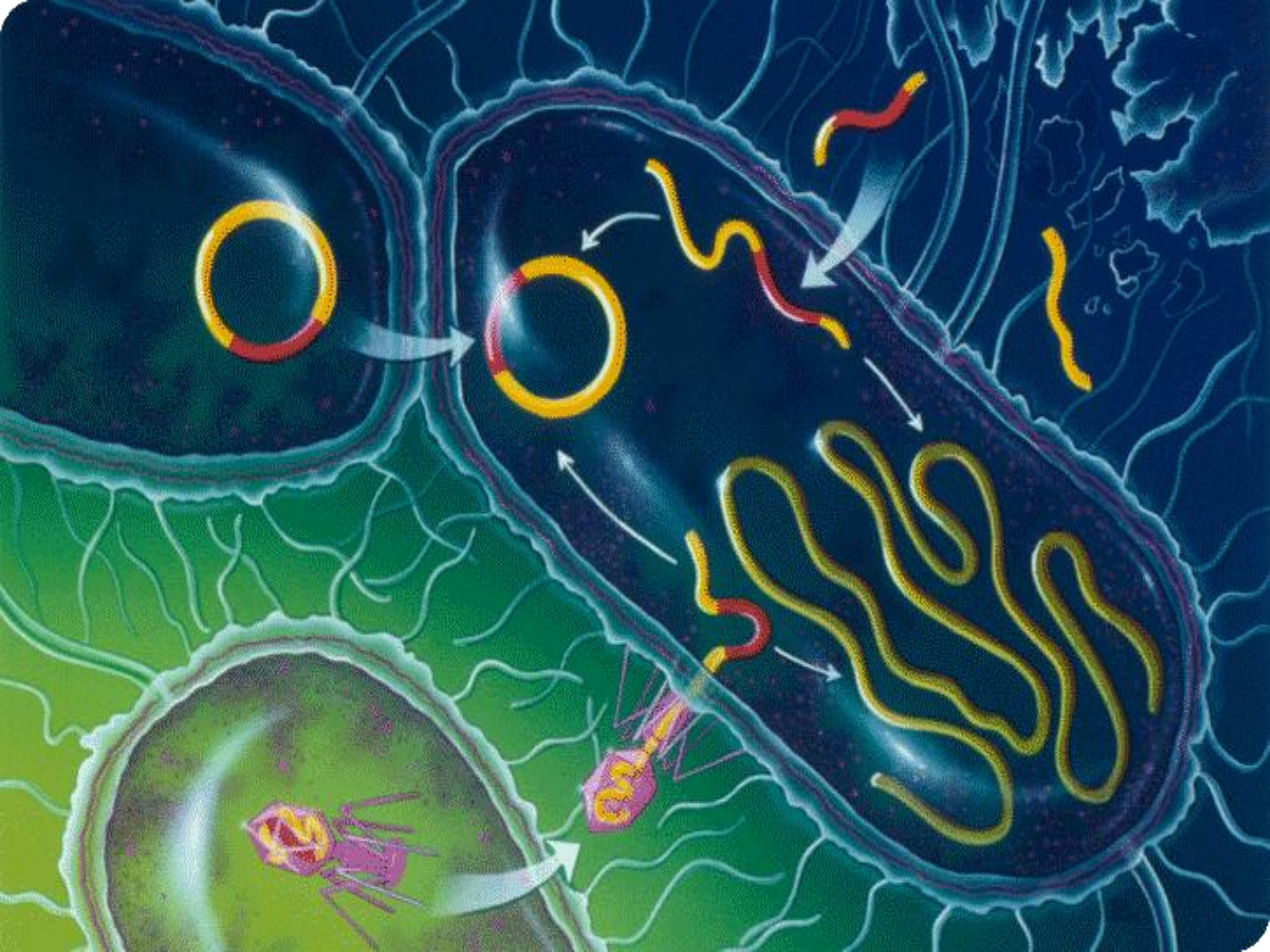
The genetics of resistance

B. Genes on transmissible plasmids

may result in resistance to different classes of antimicrobial agents (multiple resistance)

Plasmids often codes for resistance determinants to several unrelated families of antibacterial agents at once

- e.g. TEM-1 plasmid is the most common plasmid-mediated beta-lactamase in gram negative bacteria (widespread in *E. coli* and enterobacteria) also accounts for penicillin resistance in *Neisseria gonorrhoeae* and ampicillin resistance in *H. influenzae*



The genetics of resistance

C. Genes on transposable elements (transposons)

Resistance genes may also occur on transposons (jumping genes)

Transposons by a replicative process are capable of generating copies that may integrate into the chromosome or into plasmids

Antibiotic combination

- Provide a synergistic effect
- Prevent or delay emergence of persistent organisms
- Treat polymicrobial infections
- Treat serious infections in the stage before the infectious agent is identified

  – **Synergism *vs* Antagonism**

Methods of evaluation

- **Antimicrobial susceptibility testing (AST)**

➢ Susceptibility tests examine the interaction between antibiotics and bacteria in an isolated and artificial manner in order to determine the drugs to which microorganisms are susceptible and resistant

➢ Categories:

A. Agar dilution method

B. Disk diffusion method (Kirby-Bauer assay)

C. Gradient diffusion method Epsilometer test (Etest)

B. Dilution tests

- Macrodilution method
- Microdilution method

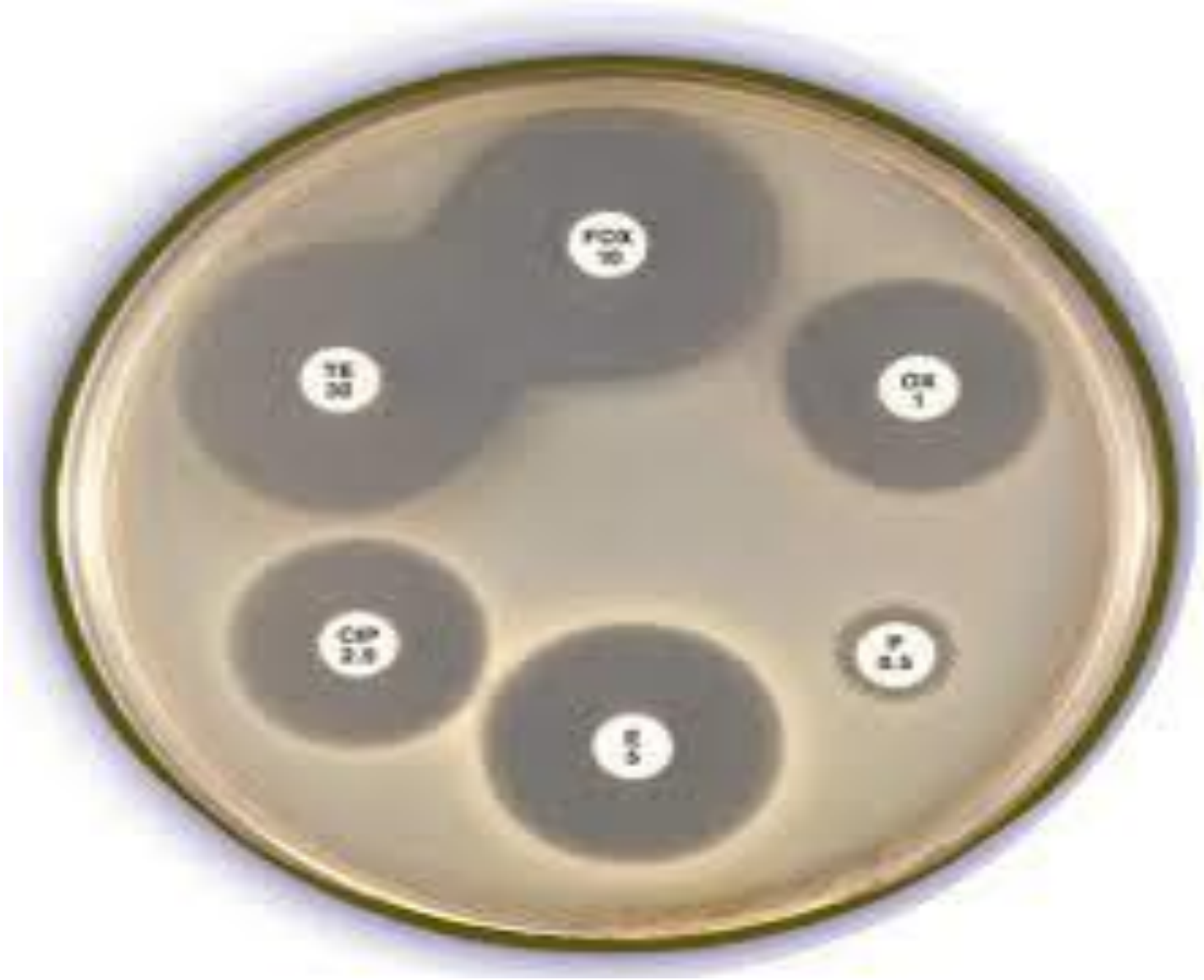
✓ **Minimum Inhibitory Concentration (MIC)**

✓ **Minimum Bactericidal Concentration (MBC)**

Disk diffusion test (Kirby-Bauer assay)

- Provides qualitative interpretive category results of susceptible, intermediate, and resistant bacterial isolates
- **Zone of inhibition**: If the bacteria are susceptible to a particular antibiotic, an area of clearing surrounds the wafer where bacteria are not capable of growing

Kirby-Bauer assay



Disk diffusion method

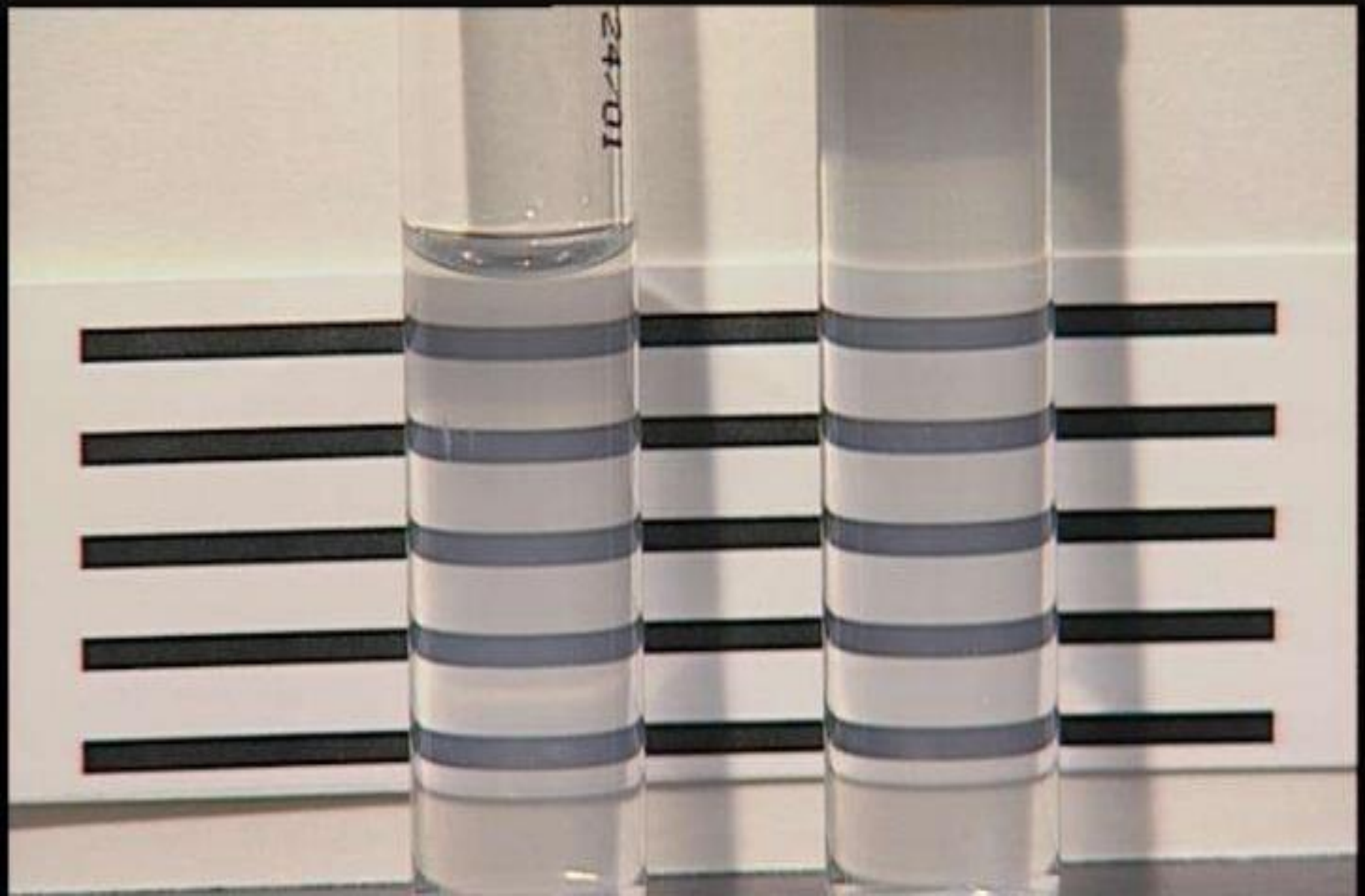
- General standard properties:

Medium	Mueller Hinton, 4 mm thickness, pH 7.2 – 7.4
Antibiotic disks	Store at -20°C minimum
Inoculum	McFarland 0.5, 10^8 cfu/ml
Incubator	35°C - 37°C
Atmosphere	Ambient air

Cont.. Agar disk diffusion method

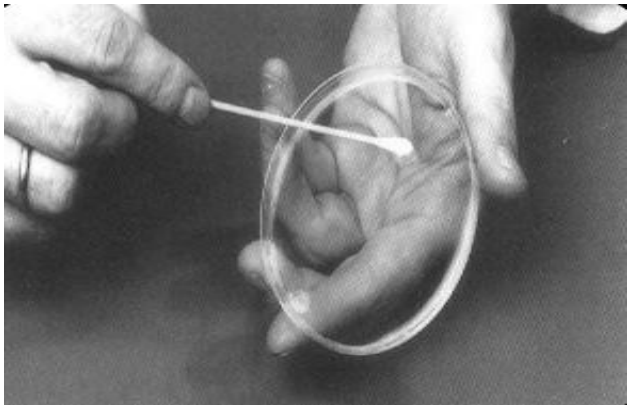
1. Prepare a pure culture of the sample on a non selective medium
2. Select at least 4-5 well-isolated colonies of the same morphological type from an agar plate
3. Touch the top of each colony with a wire loop and transfer them to a tube containing 4-5 ml of a suitable broth medium
4. Incubate the culture at 35°C -37°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard

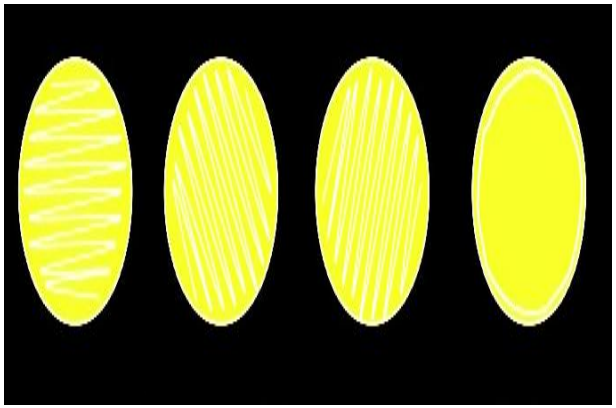
ADJUSTED TEST ORGANISM



Cont.. Agar disk diffusion method

5. Inoculate the plate with uniformity by streaking the swab over entire sterile agar surface
6. Repeat this procedure two more times and rotate the plate 60° each time to ensure an even distribution of inoculum
7. Allow the plate 3-5 minutes to dry, but no longer than 15 min for any excess surface moisture
8. The surface should be moist but without droplet of moisture (warm to room temp.)



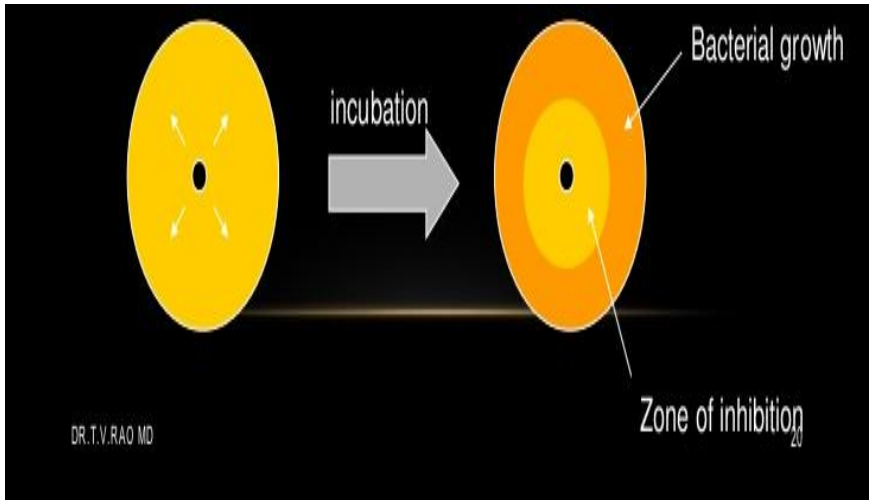


Cont.. Agar disk diffusion method

9. Apply antibiotic impregnated disks on the bacterial lawn
10. Place the appropriate disks evenly (no closer than 24 mm from center to center)
11. Invert the plates and incubate them for 16-18 h at 35°C -37°C
12. After incubation, observe for a clearing on the bacterial lawn (zone of inhibition)

Interpretation

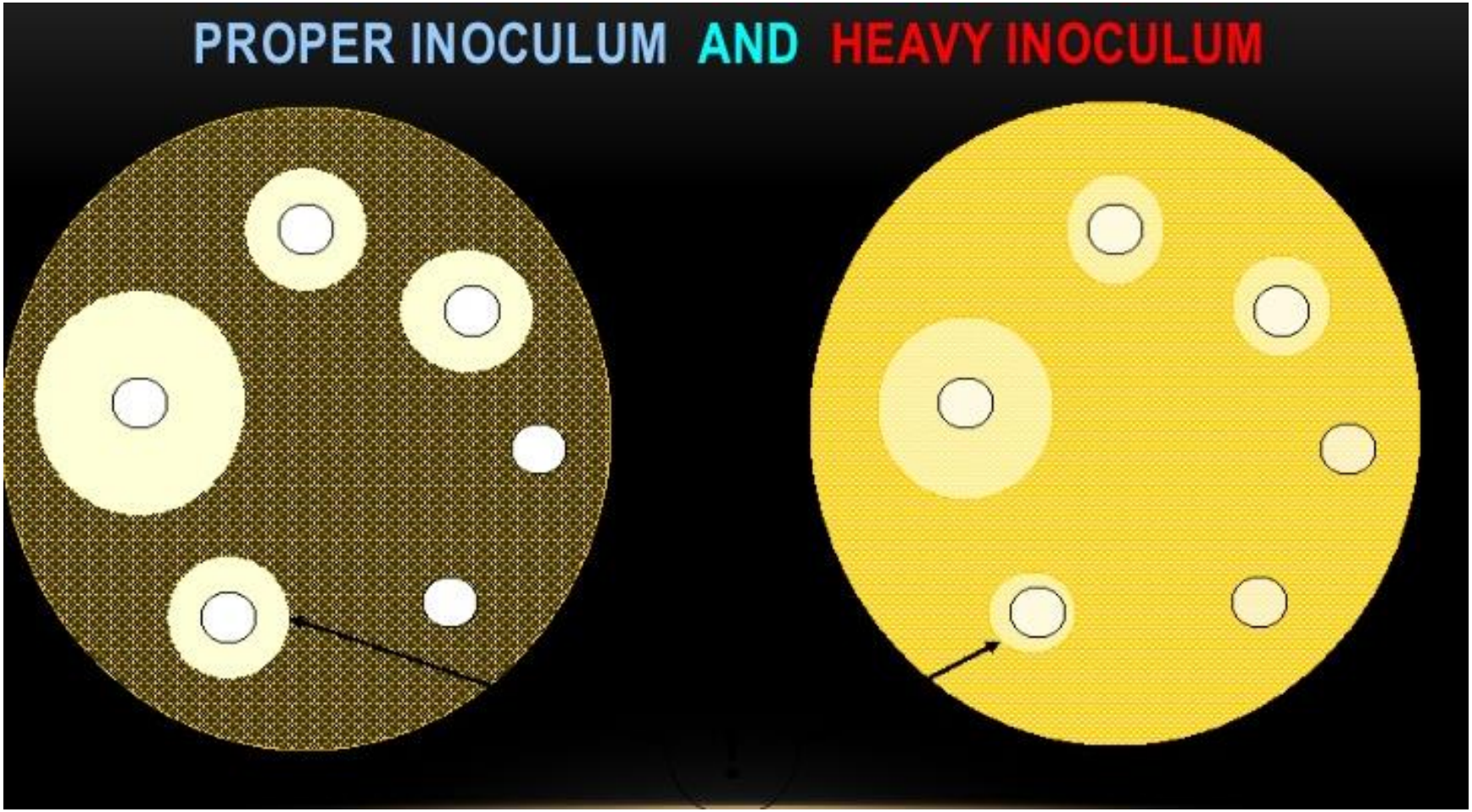
- ✓ Antibiotic diffuse out onto the agar
- ✓ Concentration of antibiotics decrease as they diffuse further away from the disks
- ✓ Examine each plate and measure the diameters of the zones of complete inhibition, including the diameter of the disk



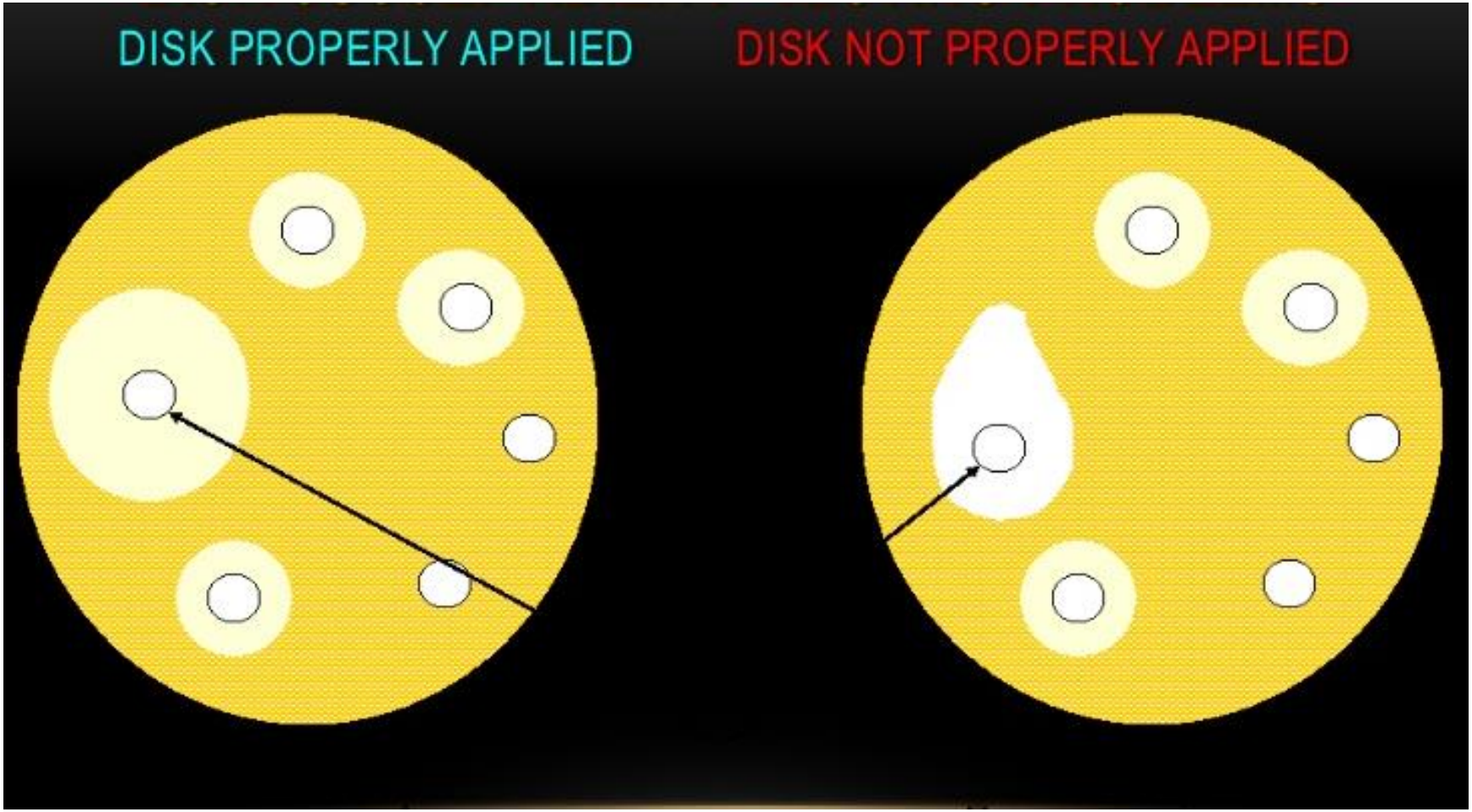
Interpretation

- Interpret the results as “resistant” or “susceptible” according to the guidelines provided by the CLSI

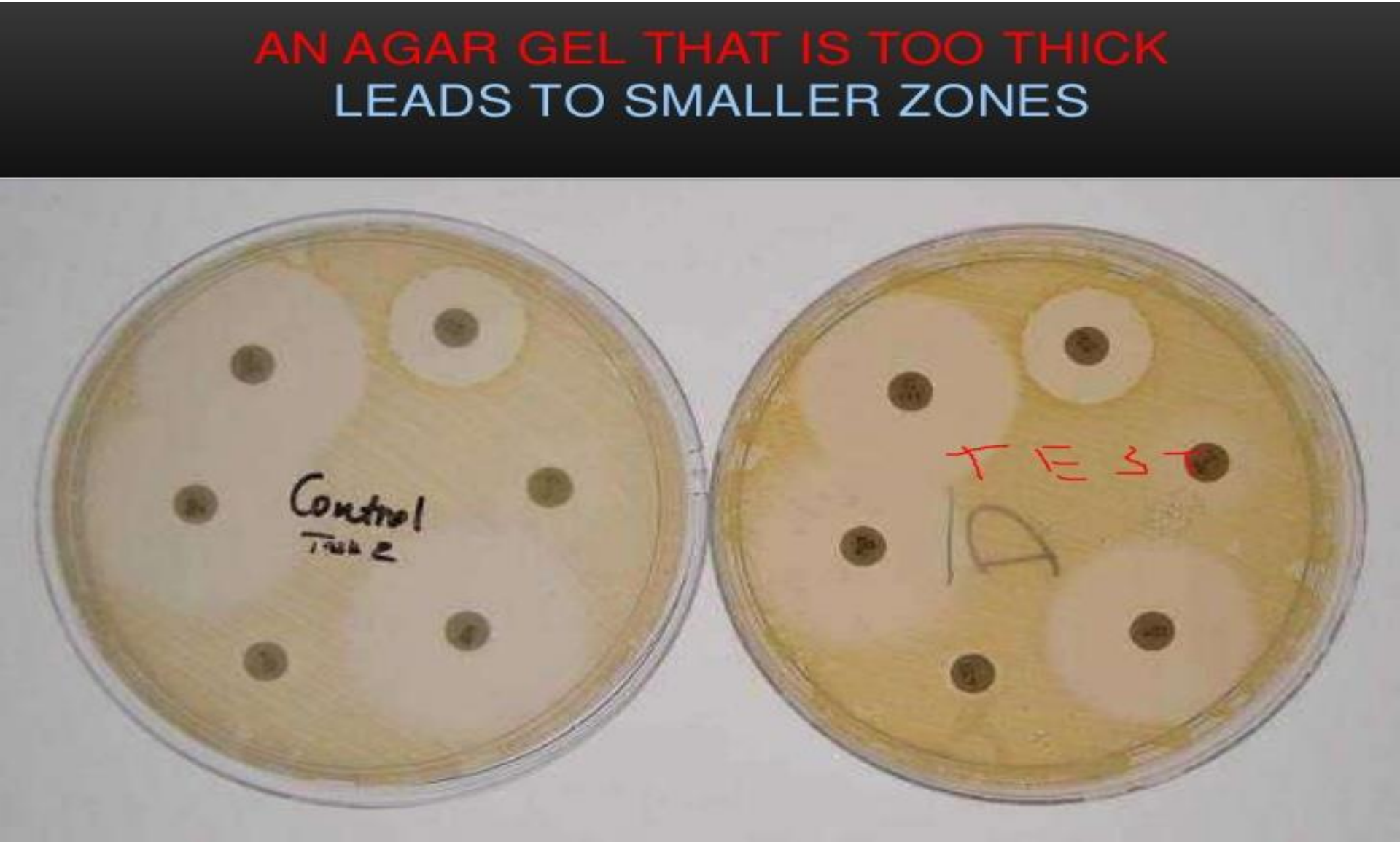
Disk susceptibility testing problems



Disk susceptibility testing problems

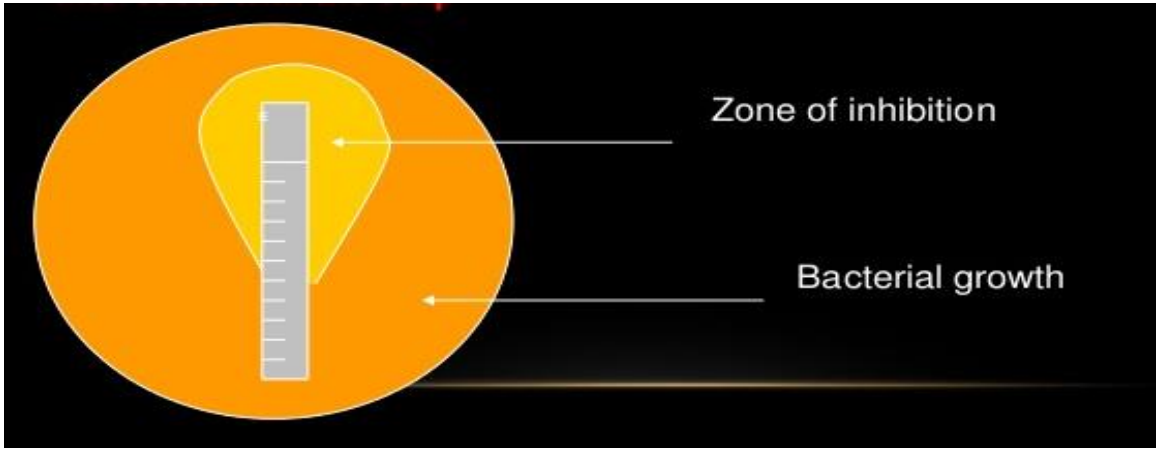


Disk susceptibility testing problems

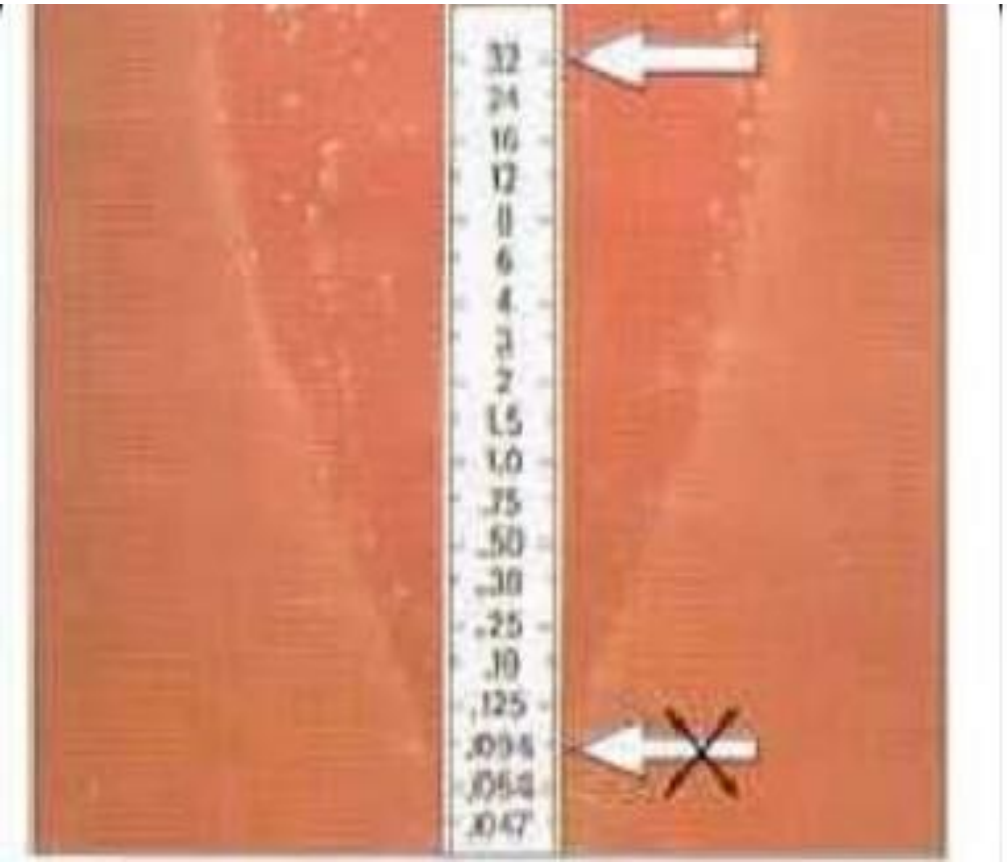


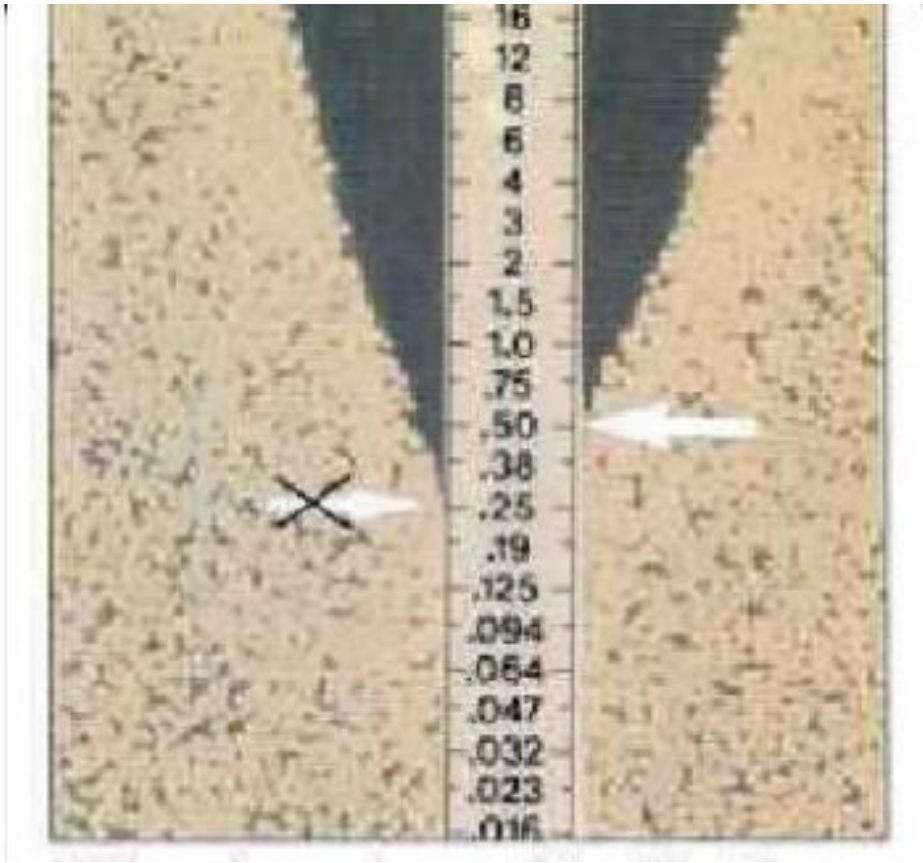
E-Epsilometer test (E-test)

- Combine the principles of the kirby-bauer and MIC tests
- A plastic strip with a predefined gradient of antibiotic concentration
- Results are read directly on the strip where the zone of inhibition intersects with the strip



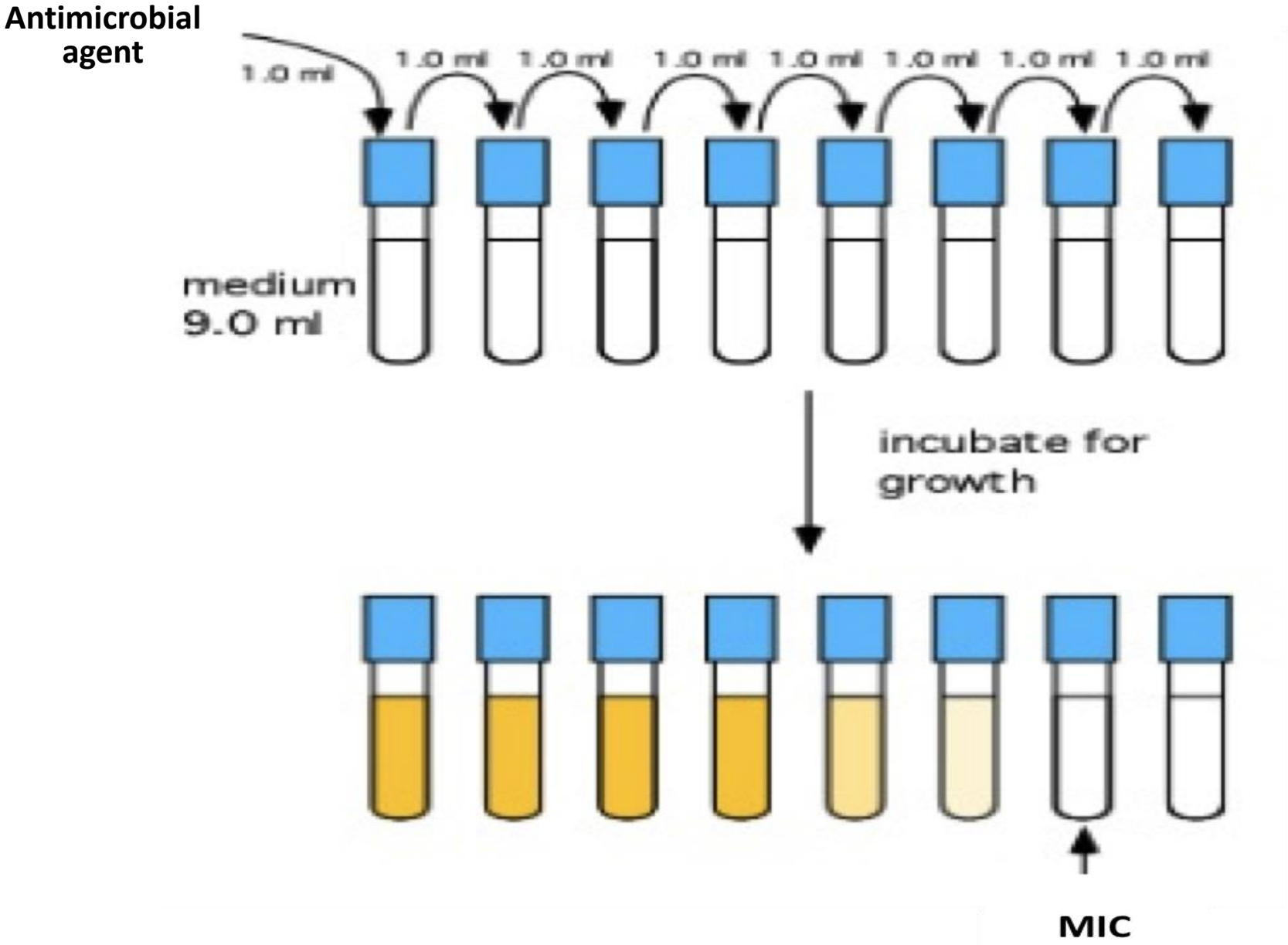
Common interpretation problems of E-test



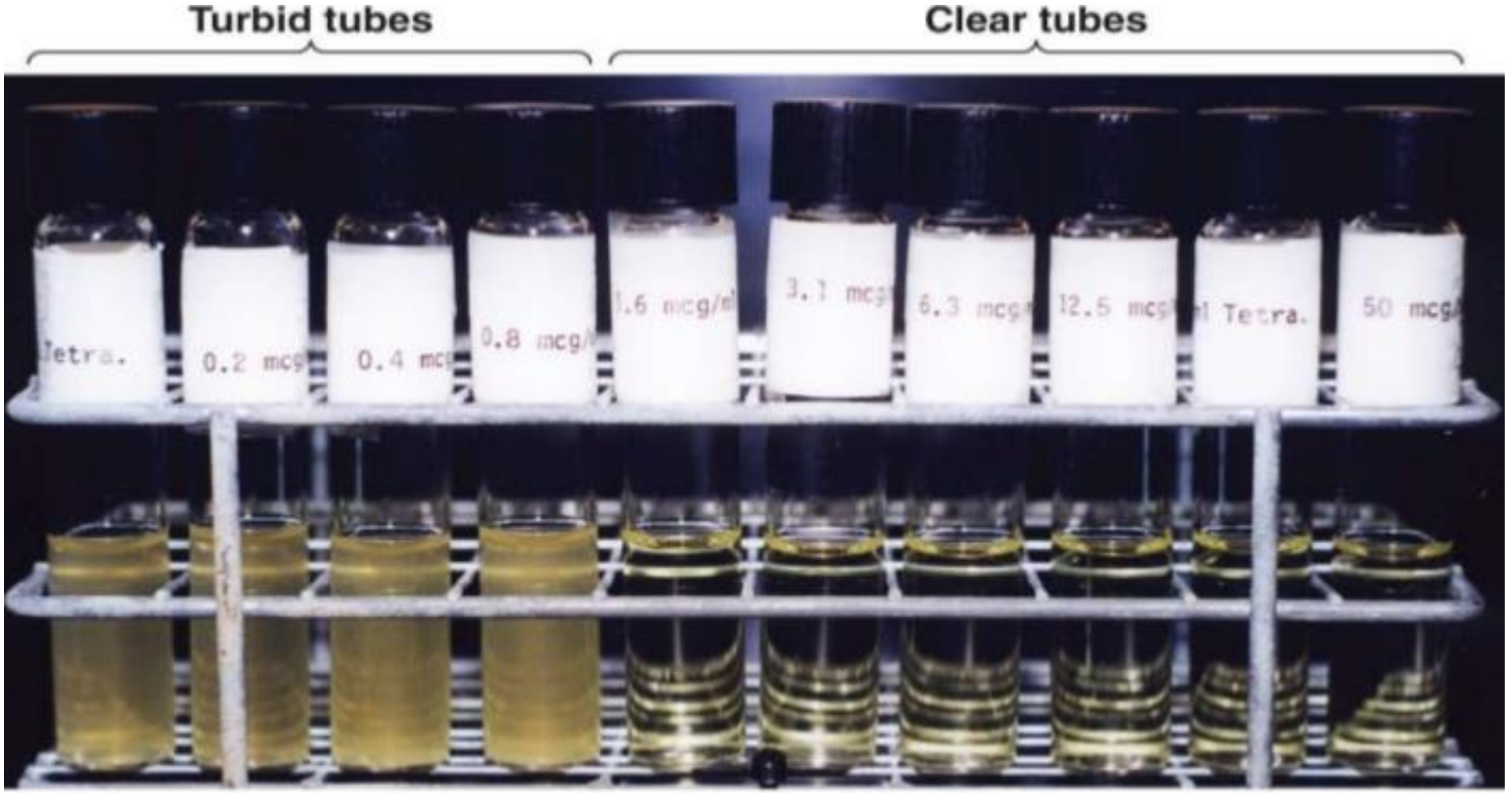


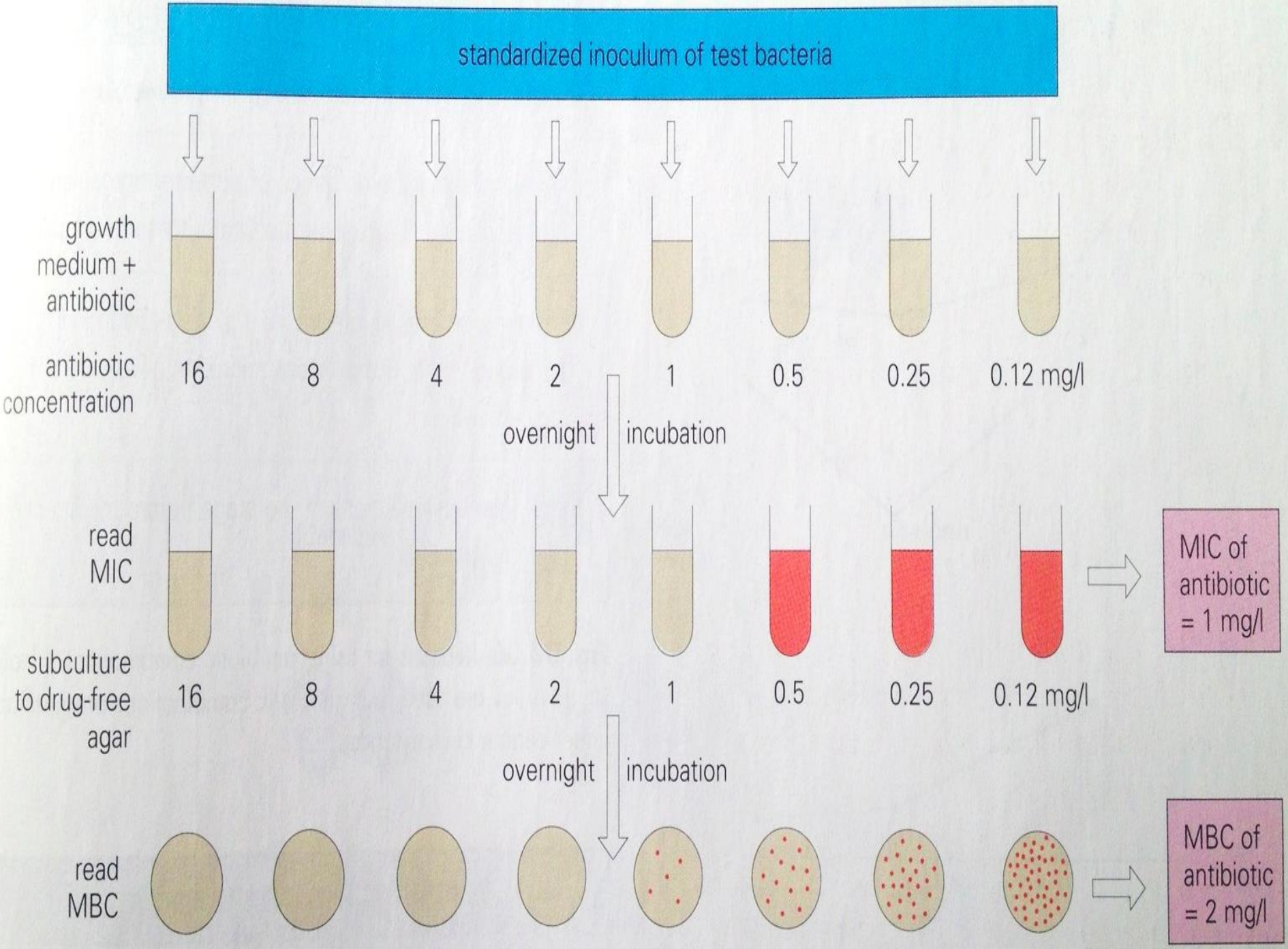
Minimum Inhibitory Concentration (MIC)

- Is the lowest concentration that will inhibit visible growth of the bacterial isolate *in vitro*
- Serial dilutions of the test antibiotic are prepared in broth or agar medium and inoculated with a suspension of the test organism
- Variable that affect MIC:
  1. Inoculum size
  2. The growth medium
  3. The interpretation of the result


Antimicrobial agent
1.0 ml
1.0 ml
1.0 ml
1.0 ml
1.0 ml
1.0 ml
1.0 ml
medium 9.0 ml
incubate for growth
MIC

Minimum Inhibitory Concentration test




standardized inoculum of test bacteria
growth medium + antibiotic
antibiotic concentration
16
8
4
2
1
0.5
0.25
0.12 mg/l
overnight incubation
read MIC
MIC of antibiotic = 1 mg/l
subculture to drug-free agar
16
8
4
2
1
0.5
0.25
0.12 mg/l
overnight incubation
read MBC
MBC of antibiotic = 2 mg/l

Minimum Bactericidal Concentration (MBC)

- Is the lowest concentration of an antibiotic required to kill the organism
- The test dilutions are subcultured onto a fresh drug-free medium and incubated for a further 18-24 h
- The antibacterial agent is considered to be bactericidal if the MBC is equal to or not greater than four-fold higher than MIC

Cont. dilution tests

➢ Advantages of dilution tests

- Could be used for large drug screening program
- Could be automated using Microtiter plates
- MIC test can be extended to determine the MBC

➢ Disadvantages of dilution tests

- More costly than diffusion test
- Required for every isolate from every patient

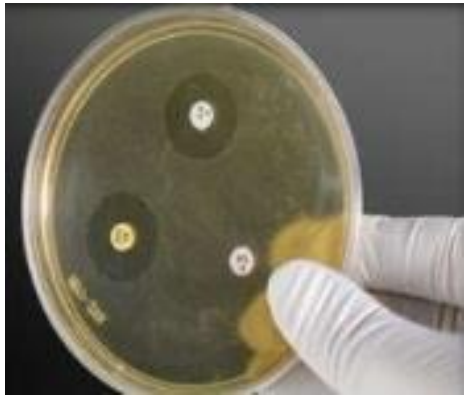
Standard strains for quality assurance

- Precision and accuracy ensured through control strains
- Known susceptibility to antimicrobial agents
- Standard strains include:
  - ✓ *Staphylococcus aureus* ATCC 25923
  - ✓ *Escherichia coli* ATCC 25922
  - ✓ *Pseudomonas aeruginosa* ATCC 27853

Ambiguous results

Are referred to the provided reading guide for:

- ✓ Organism related effects
- ✓ Drug related effects
- ✓ Resistance mechanism related effects
- ✓ Technical and handling effects
  - Lack of standardization of the inoculums
  - Thickness and quality of the culture media
  - Condition and duration of incubation



Incorrect patient results

- Misidentifying of the organism
- Clerical errors
- Examining and reporting the inappropriate choice of antibiotic
- Examining the wrong patient’s sample
- Improper method of antimicrobial disks preservation

Antibiotic assays

- Such as high performance liquid chromatography and direct assays for biological activity (bioassay)
- The importance of antibiotic assays is seen when:
  1. Antibiotic has a narrow therapeutic index, e.g. aminoglycosides
  2. The normal route of excretion of antibiotic is impaired
  3. The absorption of the antibiotic is uncertain
  4. The penetration of antibiotic is irregular or unknown
  5. Patients receiving prolonged therapy for serious infections
  6. Patients fail to respond to apparently appropriate therapy
  7. Neonates have serious infections