Sensitivity and Specificity

- The sensitivity of a technique usually depends on the number of microorganisms in the specimen.
- Its specificity depends on how morphologically unique a specific microorganism appears microscopically or how specific the antibody or genetic probe is for that genus or species.

For Example

- **The sensitivity of Gram stains smear of uncentrifuged urine is equivalent to the presence of $\geq 10^5$ CFU/ml of urine.**

- **The sensitivity of the Gram-stained smear for detecting Gram-negative coccobacilli in cerebrospinal fluid from children with *Haemophilus influenzae* meningitis is approximately 75 percent because in some patients the number of colony-forming units per milliliter of cerebrospinal fluid is less than 10^4.**

Another Example

- At least 10^4 CFU of tubercle bacilli per milliliter of sputum must be present to be detected by an acid-fast smear of decontaminated and concentrated sputum

An increase in the sensitivity of a test is often accompanied by a decrease in specificity

- Example

- Examination of a Gram-stained smear of sputum from a patient with pneumococcal pneumonia is highly sensitive but also highly nonspecific if the criterion for defining a positive test is the presence of any Gram-positive cocci.

- If, however, a positive test is defined as the presence of a preponderance of Gram-positive, lancet-shaped diplococci, the test becomes highly specific but has a sensitivity of only about 50 percent.

- Similar problems related to the number of microorganisms present affect the sensitivity of immunoassays and genetic probes for bacteria, chlamydiae, fungi and viruses.

- In some instances, the sensitivity of direct examination tests can be improved by collecting a better specimen.

For example

- The sensitivity of fluorescent antibody stain for Chlamydia trachomatis is higher when endocervical cells are obtained with a cytobrush than with a swab.
- The sensitivity may also be affected by the stage of the disease at which the specimen is collected.
- For example, the detection of herpes simplex virus by immunofluorescence, immunoassay, or culture is highest when specimens from lesions in the vesicular stage of infection are examined.

Finally

- sensitivity may be improved through the use of an enrichment or enhancement step in which microbial or genetic replication occurs to the point at which a detection method can be applied.

Techniques

- **For immunologic detection**
- Coagglutination, and enzyme-linked immuno sorbent assay (ELISA) are the most frequently used techniques in the clinical laboratory.

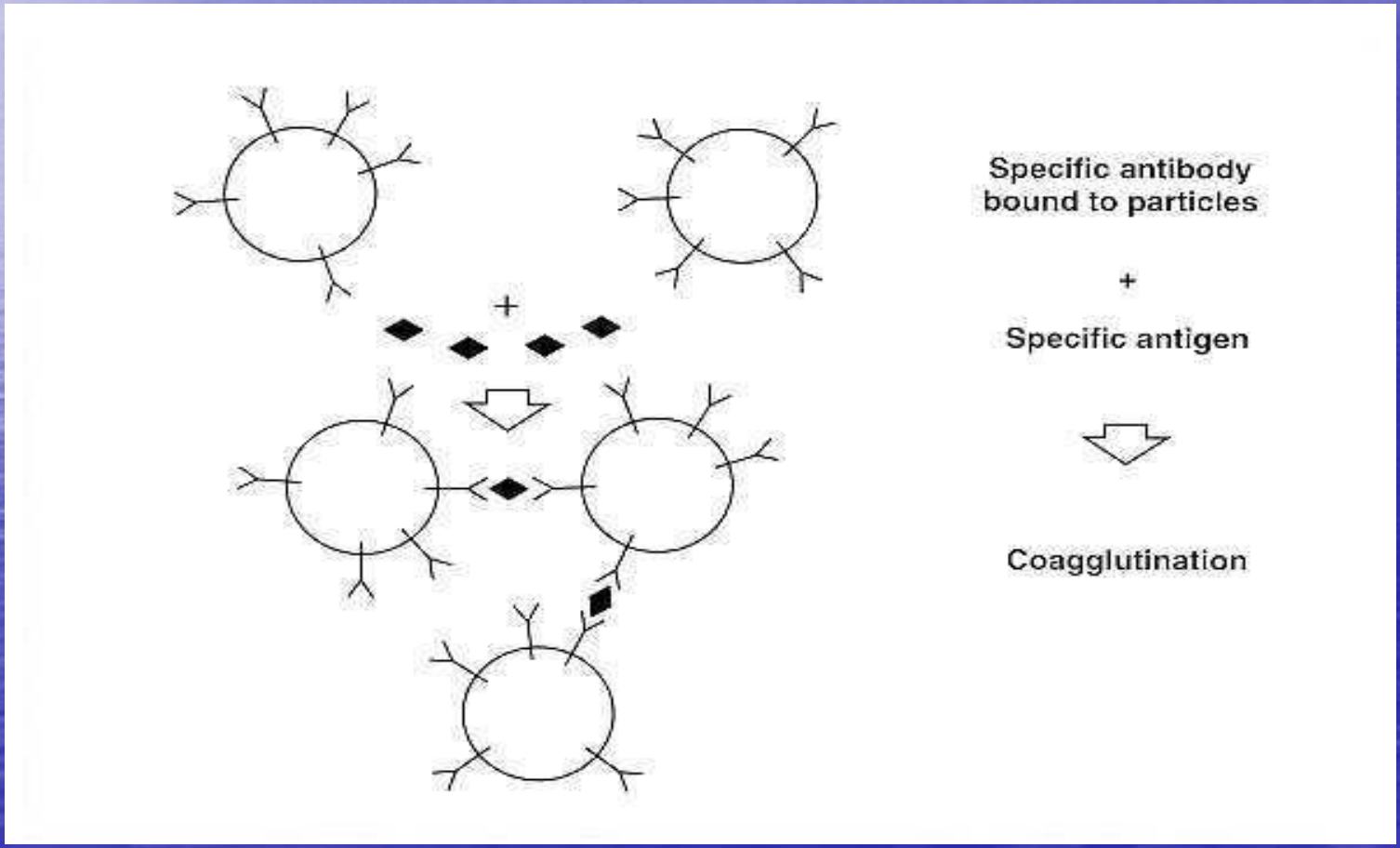
- There are several approaches to ELISA; the one most frequently used for the detection of microbial antigens uses an antigen-specific antibody that is fixed to a solid phase, which may be a latex or metal bead or the inside surface of a well in a plastic tray. Antigen present in the specimen binds to the antibody.

- The test is then completed by adding a second antigen-specific antibody bound to an enzyme that can react with a substrate to produce a colored product.

- When the enzyme-conjugated antibody is added, it binds to previously unbound antigenic sites, and the antigen is, in effect, sandwiched between the solid phase and the enzyme-conjugated antibody. The reaction is completed by adding the enzyme substrate.

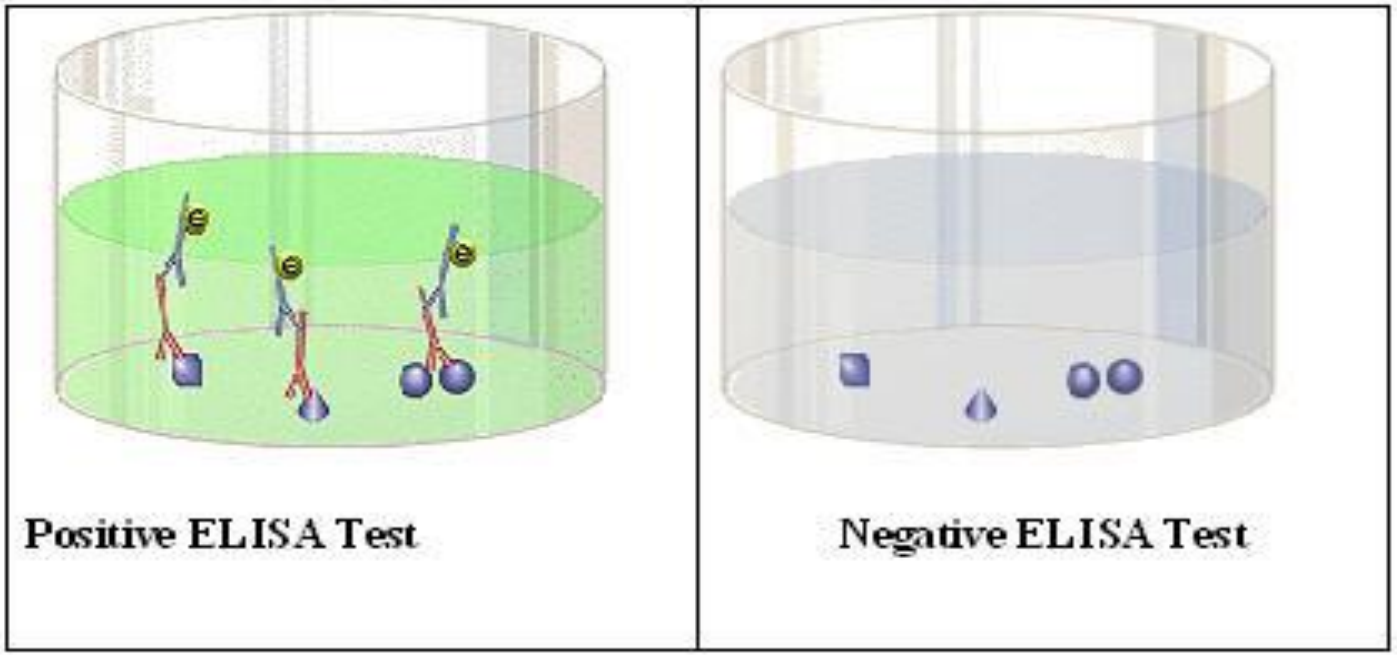
Agglutination test in which inert particles (latex beads or heat-killed *S. aureus* Cowan 1 strain with protein A) are coated with antibody to any of a variety of antigens and then used to detect the antigen in

specimens or in isolated bacteria



The ELISA Method

	Partially purified, inactivated HIV antigens pre-coated onto an ELISA plate
	Patient serum which contains antibodies. If the patient is HIV+, then this serum will contain antibodies to HIV, and those antibodies will bind to the HIV antigens on the plate.
	Anti-human immunoglobulin coupled to an enzyme. This is the second antibody, and it binds to human antibodies.
	Chromogen or substrate which changes color when cleaved by the enzyme attached to the seoond antibody.


Positive ELISA Test
Negative ELISA Test

- Genetic probes are based on the detection of unique nucleotide sequences with the DNA or RNA of a microorganism.

- Once such a unique nucleotide sequence, which may represent a portion of a virulence gene or of chromosomal DNA, is found, it is isolated and inserted into a cloning vector (plasmid), which is then transformed into Escherichia coli to produce multiple copies of the probe. The sequence is then reisolated from plasmids and labeled with an isotope or substrate for diagnostic use. Hybridization of the sequence with a complementary sequence of DNA or RNA follows cleavage of the double-stranded DNA of the microorganism in the specimen.

- The use of molecular technology in the diagnoses of infectious diseases has been further enhanced by the introduction of gene amplication techniques, such as the polymerase chain reaction (PCR) in which DNA polymerase is able to copy a strand of DNA by elongating complementary strands of DNA that have been initiated from a pair of closely spaced oligonucleotide primers. This approach has had major applications in the detection of infections due to microorganisms that are difficult to culture (e.g. the human immunodeficiency virus) or that have not as yet been successfully cultured (e.g. the Whipple's disease bacillus).

Culture

- In many instances, the cause of an infection is confirmed by isolating and culturing microorganism either in artificial media or in a living host.
- Bacteria (including mycobacteria and mycoplasmas) and fungi are cultured in either liquid (broth) or on solid (agar) artificial media

Liquid Media

- Liquid media provide greater sensitivity for the isolation of small numbers of microorganisms; however, identification of mixed cultures growing in liquid media requires subculture onto solid media so that isolated colonies can be processed separately for identification.

- Growth in liquid media also cannot ordinarily be quantitated. Solid media, although somewhat less sensitive than liquid media, provide isolated colonies that can be quantified if necessary and identified. Some genera and species can be recognized on the basis of their colony morphologies.

Differential Media

- In some instances one can take advantage of differential carbohydrate fermentation capabilities of microorganisms by incorporating one or more carbohydrates in the medium along with a suitable pH indicator. Such media are called differential media (e.g., eosin methylene blue or MacConkey agar) and are commonly used to isolate enteric bacilli. Different genera of the Enterobacteriaceae can then be presumptively identified by the color as well as the morphology of colonies.

Selective Media

- Culture media can also be made selective by incorporating compounds such as antimicrobial agents that inhibit the indigenous flora while permitting growth of specific microorganisms resistant to these inhibitors

Example

- is Thayer-Martin medium, which is used to isolate *Neisseria gonorrhoeae*.

This medium contains

- vancomycin to inhibit Gram-positive bacteria
- colistin to inhibit most Gram-negative bacilli
- Other antimicrobial agent to inhibit fungi.

The pathogenic *Neisseria* species, *N gonorrhoeae* and *N meningitidis*, are ordinarily resistant to the concentrations of these antimicrobial agents in the medium.

The number of bacteria in specimens may be used to define the presence of infection.

- For example
- there may be small numbers (< 10^3 CFU/ml) of bacteria in urine specimens from normal, healthy women; with a few exceptions, these represent bacteria that are indigenous to the urethra and periurethral region.

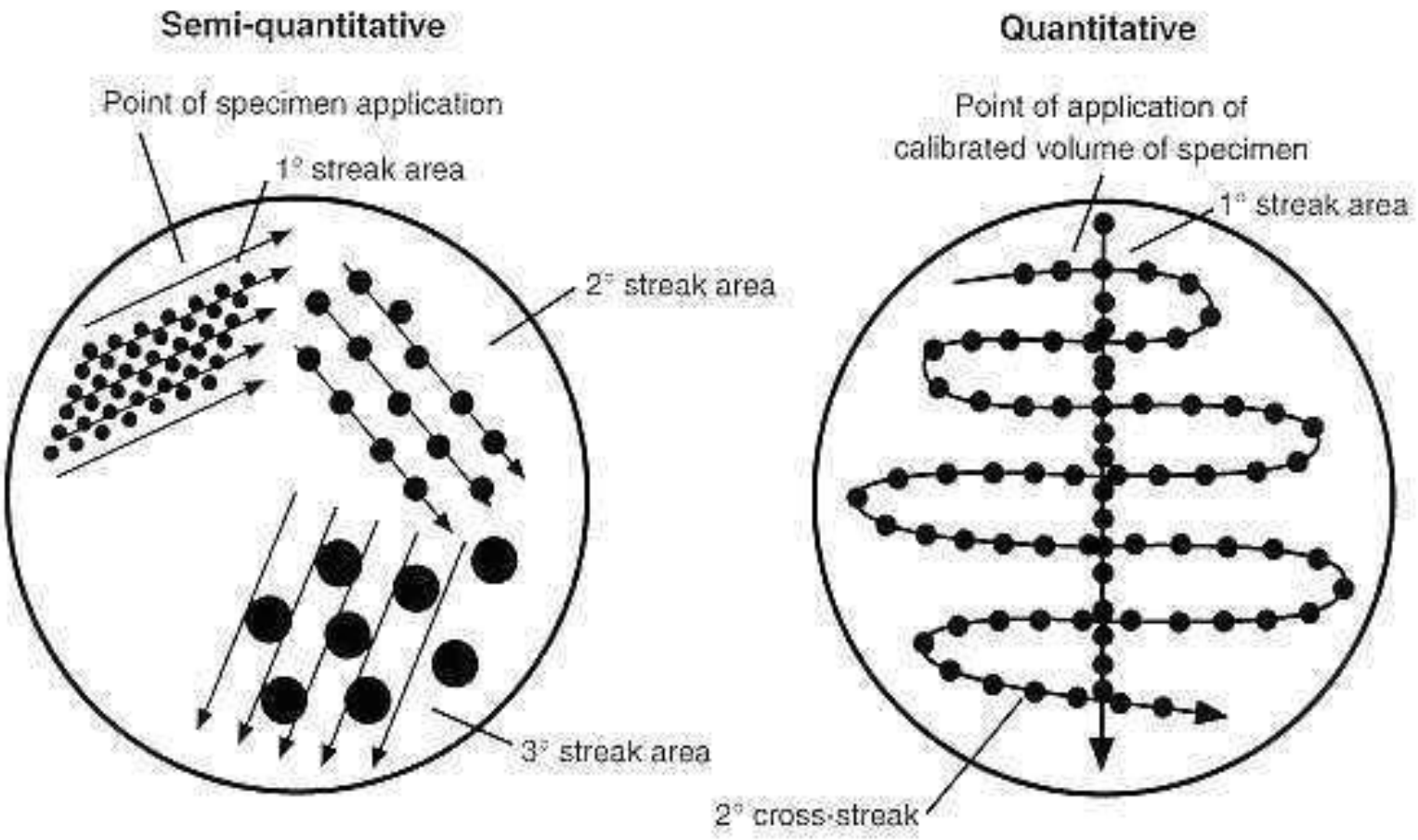
- But
- Infection of the bladder (cystitis) or kidney is usually accompanied by bacteriuria of about > 10^4 CFU/ml.
  - For this reason
- Quantitative cultures of urine must always be performed. For most other specimens a semiquantitative streak method over the agar surface is sufficient.

Quantitative and semiquantitative cultures

- For quantitative cultures, a specific volume of specimen is spread over the agar surface and the number of colonies per milliliter is estimated.
- For semiquantitative cultures, an unquantitated amount of specimen is applied to the agar and diluted by being streaked out from the inoculation site with a sterile bacteriologic loop.

- The amount of growth on the agar is then reported semiquantitatively as many, moderate, or few (or 3+, 2+, or 1+), depending on how far out from the inoculum site colonies appear.
- An organism that grows in all streaked areas would be reported as 3+.

Culture Plate Methods



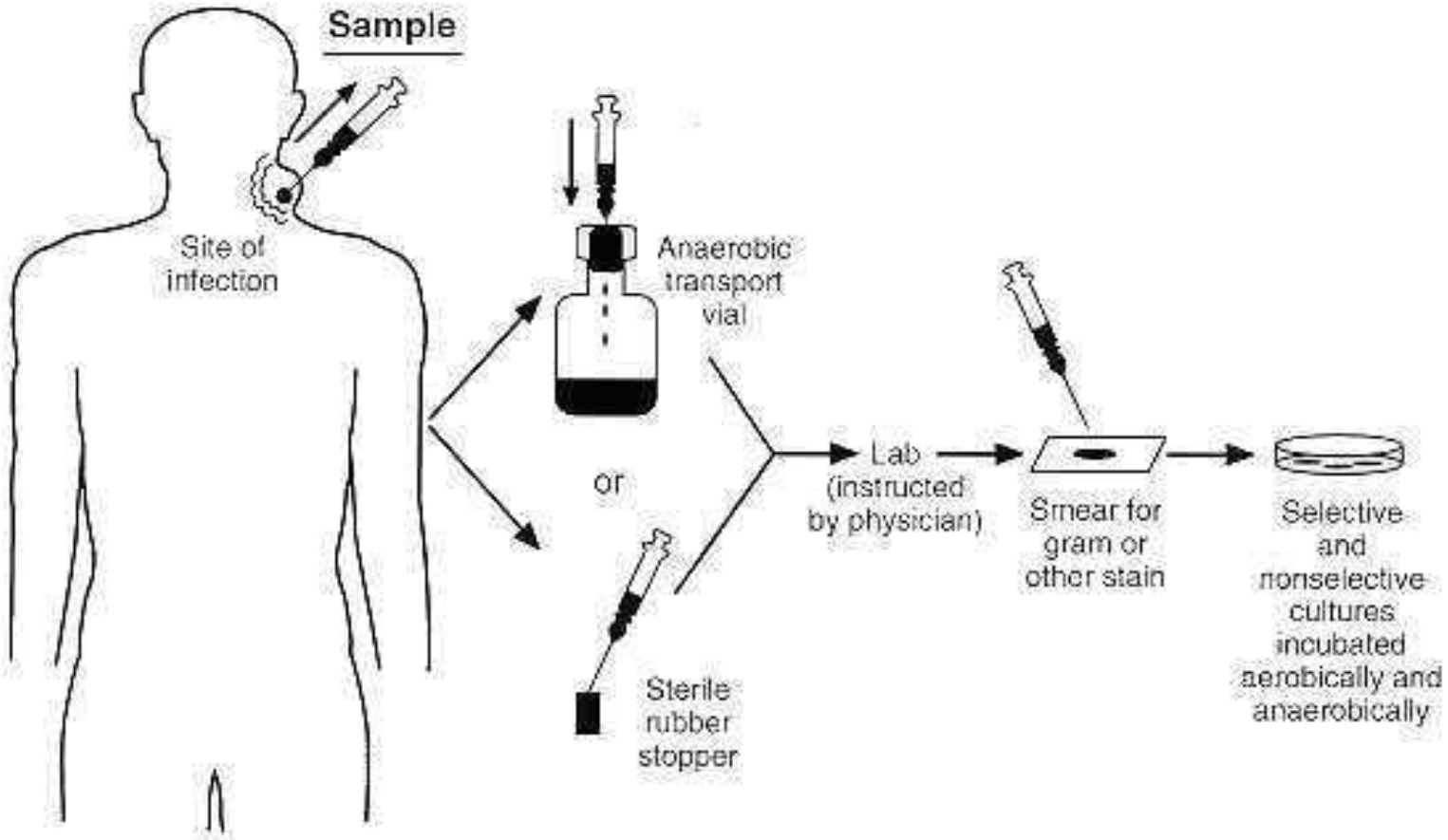
- Chlamydiae and viruses are cultured in cell culture systems, but virus isolation occasionally requires inoculation into animals, such as suckling mice, rabbits, guinea pigs.

- Rickettsiae may be isolated with some difficulty and at some hazard to laboratory workers in animals or embryonated eggs. For this reason, rickettsial infection is usually diagnosed serologically.

- Some viruses, such as the hepatitis viruses, cannot be isolated in cell culture systems, so that diagnosis of hepatitis virus infection is based on the detection of hepatitis virus antigens or antibodies.

- Cultures are generally incubated at 35 to 37°C in an atmosphere consisting of air, air supplemented with carbon dioxide (3 to 10 percent), reduced oxygen (microaerophilic conditions), or no oxygen (anaerobic conditions), depending upon requirements of the microorganism.

- Since clinical specimens from bacterial infections often contain aerobic, facultative anaerobic, and anaerobic bacteria, such specimens are usually inoculated into a variety of general purpose, differential, and selective media, which are then incubated under aerobic and anaerobic conditions.


Sample
Site of infection
Anaerobic transport vial
or
Sterile rubber stopper
Lab (instructed by physician)
Smear for gram or other stain
Selective and nonselective cultures incubated aerobically and anaerobically

Finally

- The duration of incubation of cultures also varies with the growth characteristics of the microorganism. Most aerobic and anaerobic bacteria will grow overnight, whereas some mycobacteria require as many as 6 to 8 weeks.

Microbial Identification

- Microbial growth in cultures is demonstrated by the appearance of turbidity, gas formation, or discrete colonies in broth; colonies on agar; cytopathic effects or inclusions in cell cultures; or detection of genus- or species-specific antigens or nucleotide sequences in the specimen, culture medium, or cell culture system.

How To Identify an organism

- Identification of bacteria (including mycobacteria) is based on growth characteristics (such as the time required for growth to appear or the atmosphere in which growth occurs), colony and microscopic morphology, and biochemical, physiologic, and, in some instances, antigenic or nucleotide sequence characteristics.

General

- The selection and number of tests for bacterial identification depend upon the category of bacteria present (aerobic versus anaerobic, Gram-positive versus Gram-negative, cocci versus bacilli) and the expertise of the microbiologist examining the culture.

Cocci & Bacilli

- Gram-positive cocci that grow in air with or without added CO_2 may be identified by a relatively small number of tests.

- The identification of most Gram-negative bacilli is far more complex and often requires panels of 20 tests for determining biochemical and physiologic characteristics.

Fungi & Viruses

- The identification of filamentous fungi is based almost entirely on growth characteristics and colony and microscopic morphology.

- Identification of viruses is usually based on characteristic cytopathic effects in different cell cultures or on the detection of virus- or species-specific antigens or nucleotide sequences.

Interpretation of Culture Results

- Some microorganisms, such as *Shigella dysenteriae*, *Mycobacterium tuberculosis*, *Coccidioides immitis*, and influenza virus, are always considered clinically significant.

- Others that ordinarily are harmless components of the indigenous flora of the skin and mucous membranes or that are common in the environment may or may not be clinically significant, depending on the specimen source from which they are isolated.

For example

- Coagulase-negative staphylococci are normal inhabitants of the skin, gastrointestinal tract, vagina, urethra, and the upper respiratory tract (i.e., of the nares, oral cavity, and pharynx).

- Therefore, their isolation from superficial ulcers, wounds, and sputum cannot usually be interpreted as clinically significant. They do, however, commonly cause infections associated with intravascular devices and implanted prosthetic materials.

For Example

- Role of coagulase-negative staphylococci in causing infection can usually be surmised only when the microorganism is isolated in large numbers from the surface of an intravascular device, from each of several sites surrounding an implanted prosthetic device, or, in the case of prosthetic valve endocarditis, from several separately collected blood samples.

Another example

- *Aspergillus fumigatus,* is widely distributed in nature, the hospital environment, and upper respiratory tract of healthy people but may cause fatal pulmonary infections in leukemia patients or in those who have undergone bone marrow transplantation.

- The isolation of *A. fumigatus* from respiratory secretions is a nonspecific finding, and a definitive diagnosis of invasive aspergillosis requires histologic evidence of tissue invasion.

Summary

- Physicians must also consider that the composition of microbial species on the skin and mucous membranes may be altered by disease, administration of antibiotics, endotracheal or gastric intubation, and the hospital environment. For example, potentially pathogenic bacteria can often be cultured from the pharynx of seriously ill, debilitated patients in the intensive care unit, but may not cause infection.

Serodiagnosis

- Infection may be diagnosed by an antibody response to the infecting microorganism. This approach is especially useful when the suspected microbial agent either cannot be isolated in culture.

Example

- The diagnosis of hepatitis virus infections can be made only serologically, since neither can be isolated in any known cell culture system. Although human immunodeficiency virus type 1 (HIV-1) can be isolated in cell cultures, the technique is demanding and requires special containment facilities. HIV-1 infection is usually diagnosed by detection of antibodies to the virus.

The disadvantage of serology as a diagnostic tool

- There is usually a lag between the onset of infection and the development of antibodies to the infecting microorganism. Although IgM antibodies may appear relatively rapidly, it is usually necessary to obtain acute- and convalescent-phase serum samples to look for a rising titer of IgG antibodies to the suspected pathogen.

- In some instances the presence of a high antibody titer when the patient is initially seen is diagnostic; often, however, the high titer may reflect a past infection, and the current infection may have an entirely different cause.

- Another limitation on the use of serology as a diagnostic tool is that immunosuppressed patients may be unable to mount an antibody response.

Antimicrobial Susceptibility

- The responsibility of the microbiology laboratory includes not only microbial detection and isolation but also the determination of microbial susceptibility to antimicrobial agents.

How To Made

Disk diffusion method

- Antimicrobial susceptibility tests are performed by either disk diffusion or a dilution method.

- a standardized suspension of a particular microorganism is inoculated onto an agar surface to which paper disks containing various antimicrobial agents are applied. Following overnight incubation, any zone diameters of inhibition about the disks are measured and the results are reported as indicating susceptibility or resistance of the microorganism to each antimicrobial agent tested.

Minimum Inhibitory Concentration

- An alternative method is to dilute each antimicrobial agent in broth to provide a range of concentrations and to inoculate each tube.
- Each well containing the antimicrobial agent in broth with a standardized suspension of the microorganism to be tested. The lowest concentration of antimicrobial agent that inhibits the growth of the microorganism is the minimal inhibitory concentration (MIC).

- The MIC and the zone diameter of inhibition are inversely correlated.

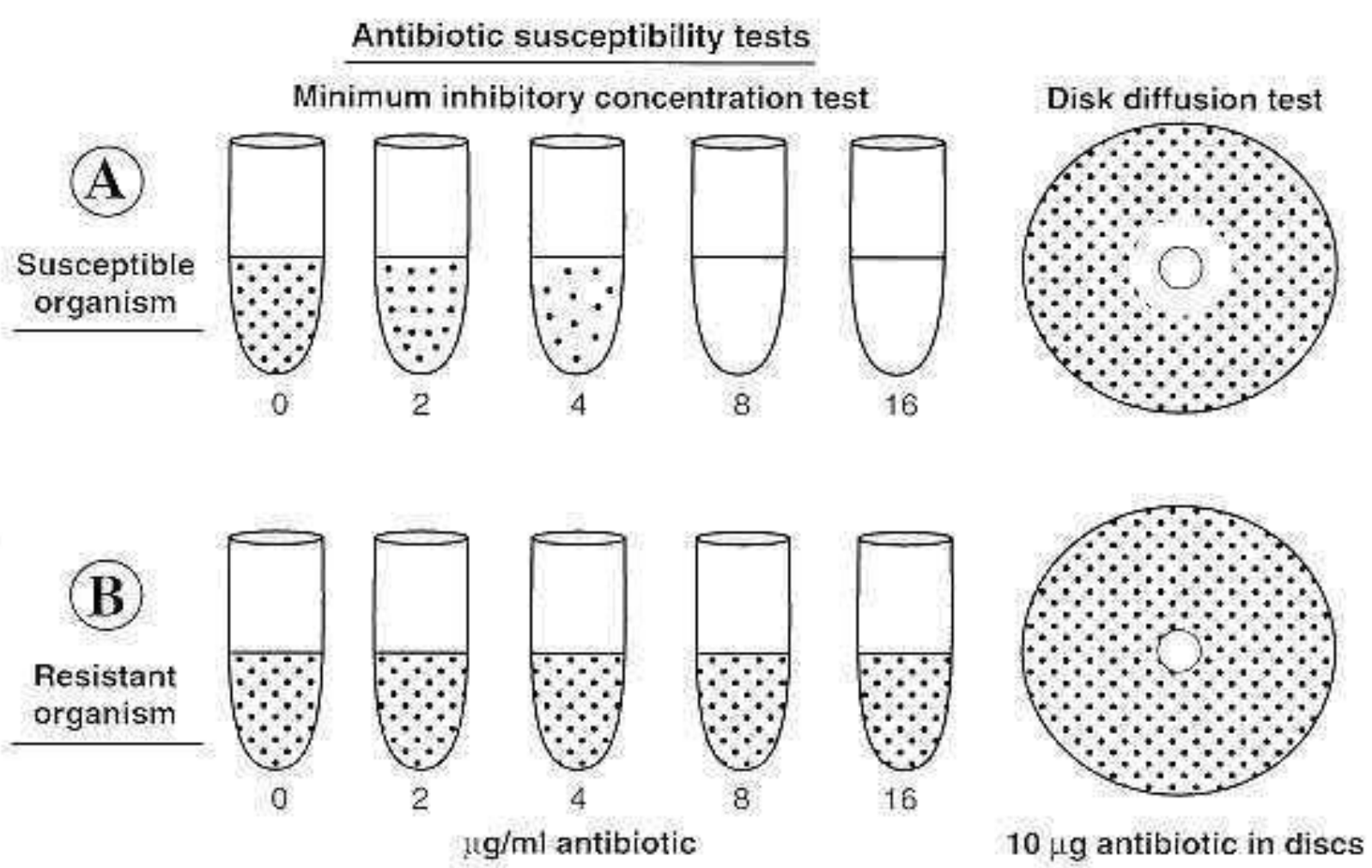
- In other words, the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. Conversely, the more resistant the microorganism, the higher the MIC and the smaller the zone of inhibition.

The term **susceptible**

- Means that the microorganism is inhibited by a concentration of antimicrobial agent that can be attained in blood with the normally recommended dose of the antimicrobial agent and implies that an infection caused by this microorganism may be appropriately treated with the antimicrobial agent.

The term **resistant**

- Indicates that the microorganism is resistant to concentrations of the antimicrobial agent that can be attained with normal doses and implies that an infection caused by this microorganism could not be successfully treated with this antimicrobial agent.


Antibiotic susceptibility tests
Minimum inhibitory concentration test
Disk diffusion test
A
Susceptible organism
0
2
4
8
16
B
Resistant organism
0
2
4
8
16
µg/ml antibiotic
10 µg antibiotic in discs