Microbiological culture

- A **microbiological culture**, or **microbial culture**, is a method of growing a microbial organism to determine what it is, its abundance in the sample being tested, or both. It is one of the primary diagnostic methods of microbiology. A tool is often used to determine the cause of infectious disease by letting the agent multiply (reproduce) in predetermined media in laboratory.

- The most common method of microbiological culture uses Petri dishes with a layer of agar-based growth medium in them to grow bacterial cultures. This is generally done inside of an incubator. Another method is liquid culture, where the bacteria are grown suspended in a liquid nutrient medium. Bottles of liquid culture are often placed in shakers in order to introduce oxygen to the liquid and maintaining the uniformity of the culture. The term culture can also, though infrequently and informally, be used as a synonym for tissue culture, which involves the growth of cells or tissues explanted from a multi-cellular organism.

Types of culture

- Blood culture
- Sputum culture
- Stool culture
- Culture of various fluids such as pleural fluid and peritoneal fluid
- Urine culture

BLOOD CULTURE

- WHAT IS A BLOOD CULTURE

looking for microbes (germs)
that may be causing infection
For your blood

Why is a blood culture performed?

- Detect a bacterial infection, such as meningitis, osteomyelitis, or sepsis, in the bloodstream and identify the type of bacteria causing the infection.
- Detect a fungal infection, such as yeast, in the bloodstream.
- Identify the best antibiotics to kill bacteria. This is called sensitivity testing.
- Evaluate unexplained fever or shock.

Test Overview !

- A bacterial infection in the blood, called bacteremia, is usually serious because the blood can spread the bacteria to any part of the body. A blood infection most commonly occurs with other serious infections (such as those affecting the kidneys, bowel, gallbladder, or heart valves).

- A blood infection may also develop when the immune system is weakened. This can occur in infants and older adults from disease (such as cancer or AIDS) or from medications (such as corticosteroids or chemotherapy).

- To test for infection in the blood, a sample of blood is collected and placed in a container with substances that promote the growth of bacteria or fungus.

- The type of bacteria or fungus that grows is identified by chemical tests and by examining the culture under a microscope.

- To increase the chances of identifying bacteria or fungi in the blood, two or three blood samples from different veins are usually taken. If no bacteria or fungus grow, the blood culture is called negative.

How To Prepare?

- No special preparation is required before having this test.
- Tell your health professional if you have recently taken antibiotics.

How It Is Done?

- Wrap an elastic band around your upper arm to stop the flow of blood. This makes the veins below the band larger so it is easier to put a needle into the vein.
- Clean the needle site with alcohol or iodine.
- Put the needle into the vein. More than one needle stick may be needed.
- Attach a tube to the needle to fill it with blood.
- Remove the band from your arm when enough blood is collected.
- Apply a gauze pad or cotton ball over the needle site as the needle is removed.
- Apply pressure to the site and then a bandage.

- Blood is usually collected from at least two different locations, or it may be collected at two different times a few hours apart.
- In some situations, people may have long-term catheters placed in a major vein because they are receiving chemotherapy or nutrition supplements for weeks or months at a time. For these people, blood will be collected from their catheters for this test.

Method used

- The most important determinant of the sensitivity of blood cultures is the amount of blood inoculated into the blood. Not only does this increase the number of organisms introduced into the bottle (because the density of bacteria in the blood is usually very low, sometimes less than 1 organism per milliliter) but it also increases the nutrient content of the medium (since blood itself is extremely nutrient-rich).
- Some bottles can accomodate up to 20 ml of added blood. True, 20 ml, for two sets of culture bottles, is a lot of blood, but if it permits an accurate diagnosis to be made earlier, may save on total blood drawn subsequently, and for all but young children, is a very small fraction of total blood volume.

Subculturing positive blood culture bottles

- Blood culture bottles (Trypticase Soy Broth, for aerobic culture) were inoculated with bacteria. Note: no blood was added, this is only for laboratory demonstration purposes. In an actual clinical setting, the bottle would be blood red.

		
Capped culture bottle, and capped needle	First cap off, inserted into bottle	Second cap off, ready for plating

- Drops are then inoculated onto Blood agar and MacConkey agar, to streak for single colony isolation.

Blood Agar

MacConkey Agar

How It Feels ?

- You may feel nothing at all from the needle puncture, or you may feel a brief sting or pinch as the needle goes through the skin. Some people feel a stinging pain while the needle is in the vein.

Risks

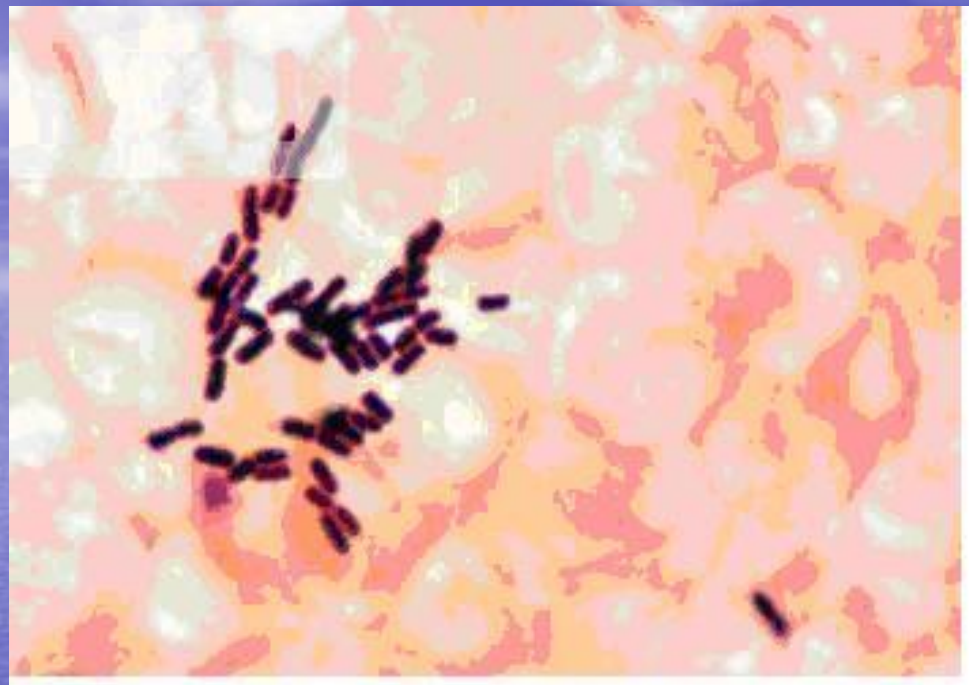
- You may develop a small bruise at the puncture site. You can reduce the risk of bruising by keeping pressure on the site for several minutes after the needle is withdrawn.
- Rarely, the vein may become inflamed after the blood sample is taken. This condition is called phlebitis and is usually treated with a warm compress applied several times daily.
- Continued bleeding can be a problem for people with bleeding disorders. Aspirin, warfarin (Coumadin), and other blood-thinning medications can also make bleeding more likely. If you have bleeding or clotting problems, or if you take blood-thinning medication, tell your health professional before your blood is drawn.

Results

- **Normal:** No bacteria or fungus are found. Normal culture results are called negative.

- **Abnormal:** Bacteria or fungus grow in the culture. Abnormal culture results are called positive.

- If bacteria are found in the culture, another test is usually done to find the antibiotic that is most effective at killing the bacteria.
- This is called sensitivity or susceptibility testing. Sensitivity testing is important to treat a blood infection effectively and prevent the development of bacteria that are resistant to antibiotics.



Escherichia coli **Bacteria from human blood culture. LM X1000.**

What Affects the Test

- Factors that can interfere with your test and the accuracy of the results include:
- Recent antibiotic treatment.
- Contamination of the blood sample by bacteria or fungus on the skin.

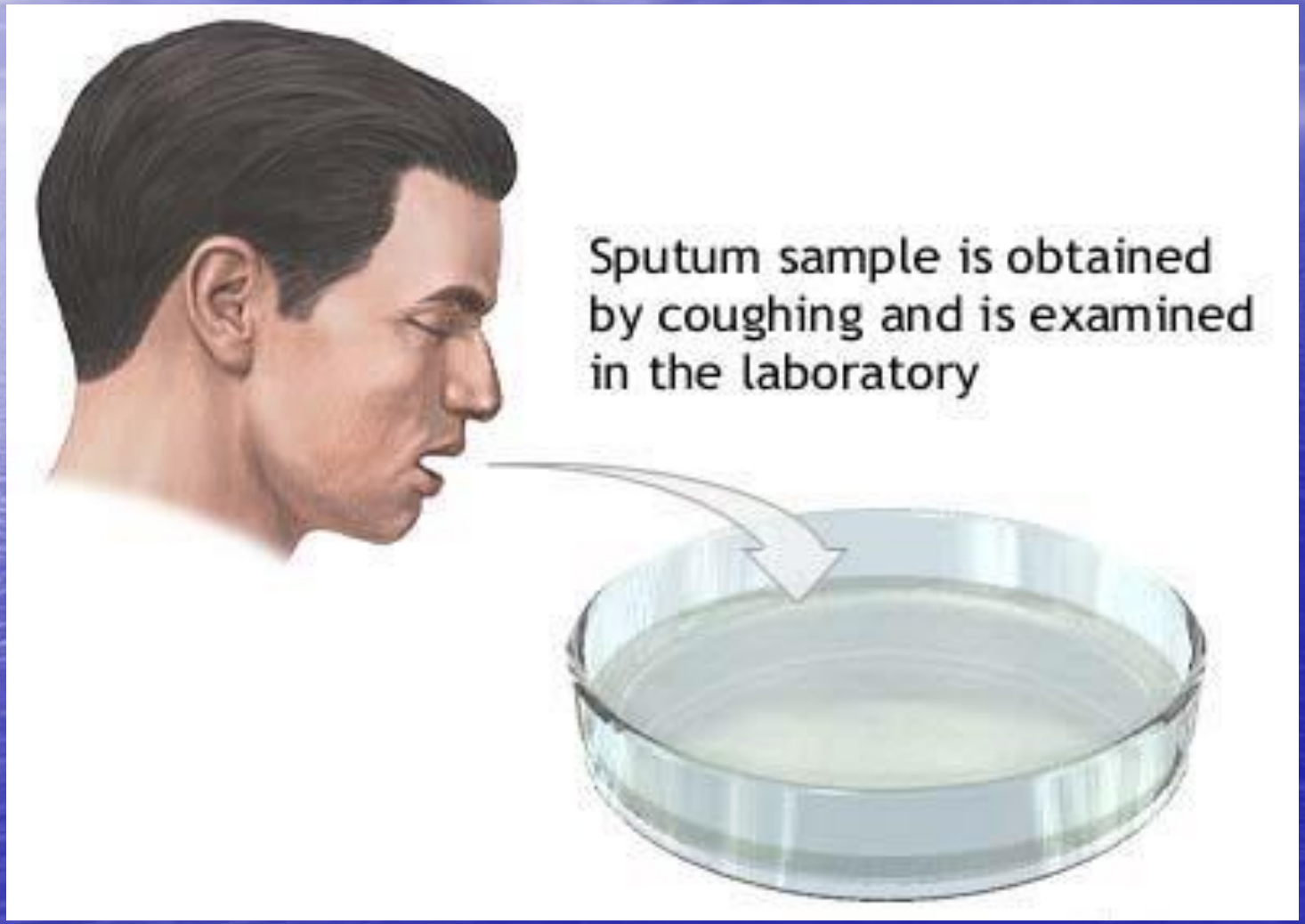
What To Think About ?

- Some types of bacteria can temporarily infect the blood when an infection of the kidneys, throat, lungs, or another part of the body is present. This may not indicate a serious infection of the blood; usually bacteria persist in the blood when a serious blood infection is present.

- About 5% of blood cultures are contaminated with normal skin bacteria (usually a type of staph bacteria). Therefore, it is sometimes difficult to determine whether the bacteria that grow in the culture are actually causing a blood infection or have contaminated the blood sample. When the same bacteria grow in several blood cultures, it is likely that those bacteria are in the blood and are not a contaminant. When staph bacteria grow in the culture in less than 48 hours, it is likely that the staph bacteria are in the blood and are not a contaminant.

- A culture that fails to grow any bacteria does not always rule out a blood infection. Factors such as the amount of blood taken, the timing of the blood sample, the type of culture done, and previous use of antibiotics can affect the growth of bacteria in the culture.

Sputum culture



- Sputum is material coughed up from the lungs and expectorated (spit out) through the mouth.

- A sputum culture is done to find and identify the microorganism causing an infection of the lower respiratory tract such as pneumonia (an infection of the lung). If a microorganism is found, more testing is done to determine which antibiotics will be effective in treating the infection.

Purpose

- A person with a fever and a continuing cough that produces pus-like material and/or blood may have an infection of the lower respiratory tract. Infections of the lungs and bronchial tubes are caused by several types of microorganisms, including bacteria, fungi (molds and yeast), and viruses.

- A chest x ray provides visual evidence of an infection; a culture can grow the microorganism causing the infection. The microorganism is grown in the laboratory so it can be identified, and tested for its response to medications, such as antifungal and antibiotics.

Description

- Based on the clinical condition of the patient, the physician determines what group of microorganism is likely to be causing the infection, and then orders one or more specific types of cultures: bacterial, viral, or fungal (for yeast and molds).

- For all culture types, the sputum must be collected into a sterile container. The sputum specimen must be collected carefully, so that bacteria that normally live in the mouth and saliva don't contaminate the sputum and complicate the process of identifying the cause of the infectious agent. Once in the laboratory, each culture type is handled differently.

Bacterial culture

- A portion of the sputum is smeared on a microscope slide for a Gram stain. Another portion is spread over the surface of several different types of culture plates, and placed in an incubator at body temperature for one to two days.

- A Gram stain is done by staining the slide with purple and red stains, then examining it under a microscope. Gram staining checks that the specimen does not contain saliva or material from the mouth. If many epithelial (skin) cells and few white blood cells are seen, the specimen is not pure sputum and is not adequate for culture.

- Depending on laboratory policy, the specimen may be rejected and a new specimen requested. If many white blood cells and bacteria of one type are seen, this is an early confirmation of infection.

- The color of stain picked up by the bacteria (purple or red), their shape (such as round or rectangular), and their size provide valuable clues as to their identity and helps the physician predict what antibiotics might work best before the entire test is completed. Bacteria that stain purple are called gram-positive; those that stain red are called gram-negative.

- During incubation, bacteria present in the sputum sample multiply and will appear on the plates as visible colonies. The bacteria are identified by the appearance of their colonies, by the results of biochemical tests, and through a Gram stain of part of a colony.

- A sensitivity test, also called antibiotic susceptibility test, is also done. The bacteria are tested against different antibiotics to determine which will treat the infection by killing the bacteria.

- The initial result of the Gram stain is available the same day, or in less than an hour if requested by the physician. An early report, known as a preliminary report, is usually available after one day.

- This report will tell if any bacteria have been found yet, and if so, their Gram stain appearance--for example, a gram-negative rod, or a gram-positive cocci. The final report, usually available in one to three days, includes complete identification and an estimate of the quantity of the bacteria and a list of the antibiotics to which they are sensitive.

Fungal culture

- To look for mold or yeast, a fungal culture is done. The sputum sample is spread on special culture plates that will encourage the growth of mold and yeast. Different biochemical tests and stains are used to identify molds and yeast. Cultures for fungi may take several weeks.

Viral culture

- Viruses are a common cause of pneumonia. For a viral culture, sputum is mixed with commercially-prepared animal cells in a test tube. Characteristic changes to the cells caused by the growing virus help identify the virus. The time to complete a viral culture varies with the type of virus. It may take from several days to several weeks.

Special procedures

- Tuberculosis is caused by a slow-growing bacterium called *Mycobacterium tuberculosis*. Because it does not easily grow using routine culture methods, special procedures are used to grow and identify this bacterium. When a sputum sample for tuberculosis first comes into the laboratory, a small portion of the sputum is smeared on a microscope slide and stained with a special stain, called an acid-fast stain.

- The stained sputum is examined under a microscope for tuberculosis organisms, which pick-up the stain, making them visible. This smear is a rapid screen for the organism, and allows the physician to receive a preliminary report within 24 hours.

- To culture for tuberculosis, portions of the sputum are spread on and placed into special culture plates and tubes of broth that promote the growth of the organism. Growth in broth is faster than growth on culture plates.

- Instruments are available that can detect growth in broth, speeding the process even further. Growth and identification may take two to four weeks.

- Other microorganisms that cause various types of lower respiratory tract infections also require special culture procedures to grow and identify.

- *Mycoplasma pneumonia* causes a mild to moderate form of pneumonia, commonly called walking pneumonia; *Bordetella pertussis* causes whooping cough; *Chlamydia pneumoniae*.

Preparation

- The specimen for culture should be collected before antibiotics are begun. Antibiotics in the person's system may prevent microorganisms present in the sputum from growing in culture.

- The best time to collect a sputum sample is early in the morning, before having anything to eat or drink. The patient should first rinse his or her mouth with water to decrease mouth bacteria and dilute saliva. Through a deep cough, the patient must cough up sputum from within the chest.

- Taking deep breaths and lowering the head helps bring up the sputum. Sputum must not be held in the mouth but immediately spat into a sterile container. For tuberculosis, the physician may want the patient to collect sputum samples on three consecutive mornings.

- A sputum sample is obtained by coughing deeply and expelling the material that comes from the lungs into a sterile cup. The sample is taken to a laboratory and placed in a medium under conditions that allow the organisms to grow.

- A positive culture may identify disease-producing organisms that may help diagnose bronchitis, tuberculosis, a lung abscess, or pneumonia.

- If tuberculosis is suspected, collection of sputum should be carried out in an isolation room, with all attending healthcare workers wearing masks.
- In addition to special precautions in collecting sputum when tuberculosis is suspected, workers in hospital laboratories must take extra care to inactivate unstained smear preparations that may contain *M. tuberculosis*.

Normal results

- Sputum from a healthy person would have no growth on culture. A mixture of microorganisms, however, normally found in a person's mouth and saliva often contaminates the culture. If these microorganisms grow in the culture, they may be reported as normal flora contamination.

Abnormal results

- The presence of bacteria and white blood cells on the Gram stain and the isolation of a microorganism from culture, other than normal flora contamination, is evidence of a lower respiratory tract infection.
- Microorganisms commonly isolated from sputum include:
- *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Legionella pneumophila*, *Mycoplasma pneumonia*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bordetella pertussis*, and *Escherichia coli*.