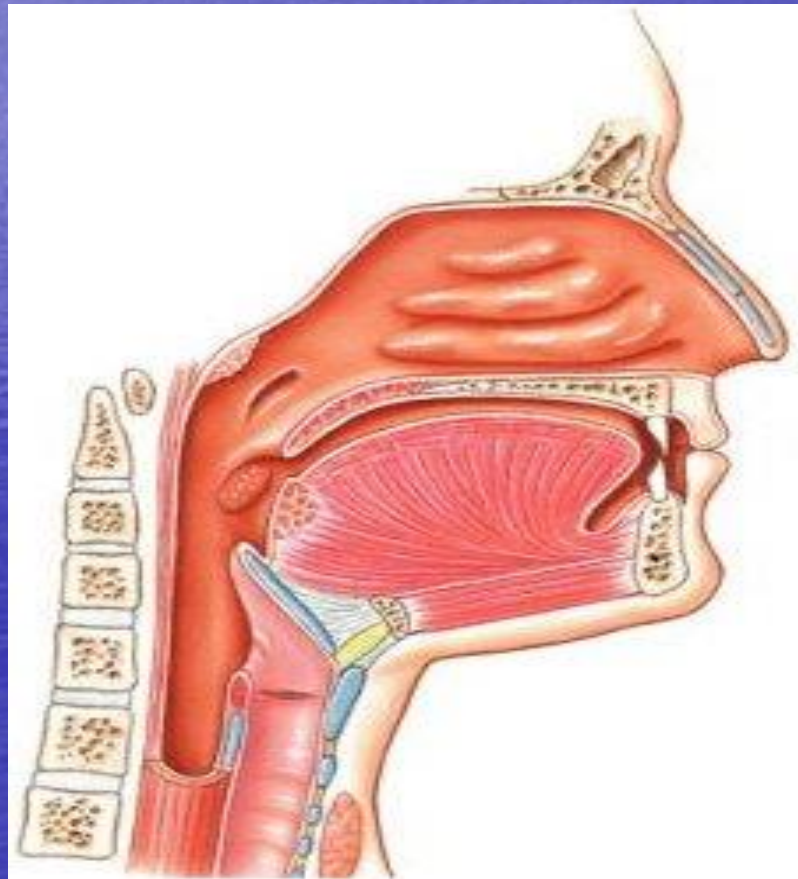


ISOLATION OF BACTERIA FROM THE EAR, NOSE, & THROAT



- Blood agar (BAP) is a common medium used to culture bacteria because
- 1) it is a great enrichment medium for **fastidious** bacteria.
- 2) Hemolysis of blood cells can be very useful as an identification test.
- Blood agar is made with 5% sheep blood.

- Hemolysis is the breakdown of red blood cells: hemolysins are enzymes produced by some bacteria and are released into the medium around the bacterial colony.
- It can be a complete breakdown of the cells, with the release of hemoglobin and a clearing of the red from the surrounding medium around the colony. Or the hemolysis can be a partial breakdown, resulting in a greenish or green-yellow zone around the colony.

- You will find a variety of bacteria in the throat: it is normal. This is true also of the ear and nose, but there is less diversity. In addition to your identification of different hemolytic reactions, you are also going to isolate a *Staphylococcus* species to be used in **the Kirby-Bauer test for antibiotic sensitivity.**

THE TEST OBJECTIVES

- Differentiate among various species from a clinical specimen.
- Isolate a species of Staphylococcus for antibiotic susceptibility testing.
- Identify the 3 hemolytic types on blood agar.

THE PROCEDURE

- **THROAT CULTURE:**

Take a sterile swab and place it in the orifice of your patient. If performing a throat swab, be sure to hold the tongue down with the tongue depressor while going to the **BACK** of the throat with the swab.

THROAT, NOSE, EAR CULTURES:

- Prepare a streak plate However, you will use the swab for the first section of the plate, rolling it around to get as many bacteria off as possible).
- Switch over to your inoculating loop and continue on as you would a regular streak plate.

- **KISS PLATE:** The agar plate is inoculated by kissing it---*gently*, because the agar medium is not very strong. You also want to slightly touch the nose while kissing, so you can see the various populations in different areas.
- Place the plates upside down in the candle jar. When all plates are in the jars, the candle will be lit, and the jars incubated at 37 °C.

INTERPRETATION

- After incubation you will check the plates for:
- **different hemolytic reactions**
 - complete hemolysis = beta hemolysins produce a clear, yellow zone
 - partial hemolysis = alpha hemolysins produce an opaque green/green-yellow zone
 - no hemolysis = gamma , no hemolysins, no zone



gamma



gamma



beta



alpha

- **Different gram reactions**
- Gram stain a couple of different species. In particular, you are looking for a G+ coccus in clusters (potentially *Staph*).
- a *Staph* isolate

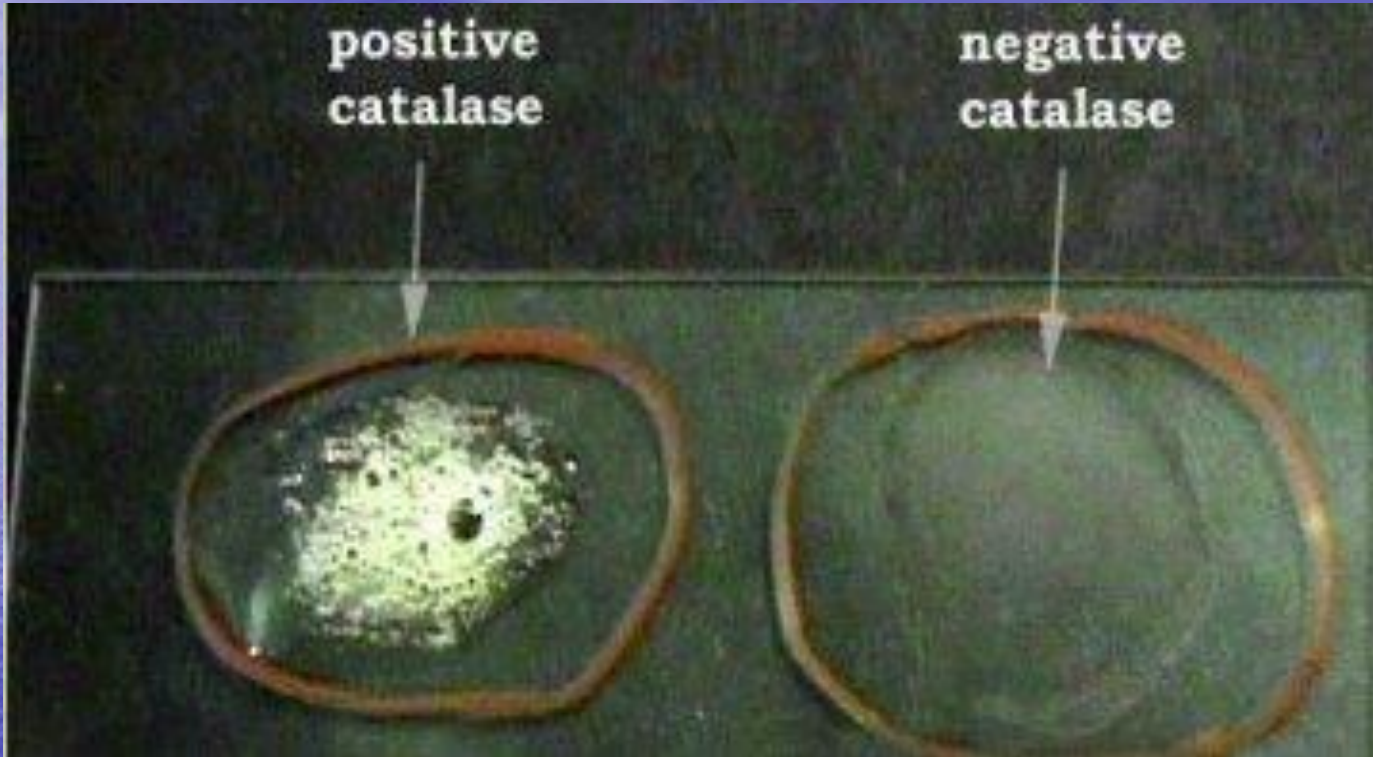
- Find a colony that is white, off white, or cream pigmented on the BAP. Gram stain. You will confirm that it is a ***Staph*** not only with a gram stain, but with a catalase test.
- Once the colony is confirmed as a ***Staph*** species, subculture the colony onto a TSA plate, streak for isolation.
- Incubate at 37 °C and then check for purity of the culture.
- You will run the antibiotic susceptibility test on your own species of ***Staph*** in the next week or after.
- Before the day we are to perform the antibiotic sensitivity test, you need to subculture your ***Staph*** isolate into an TSB broth.

CATALASE TEST

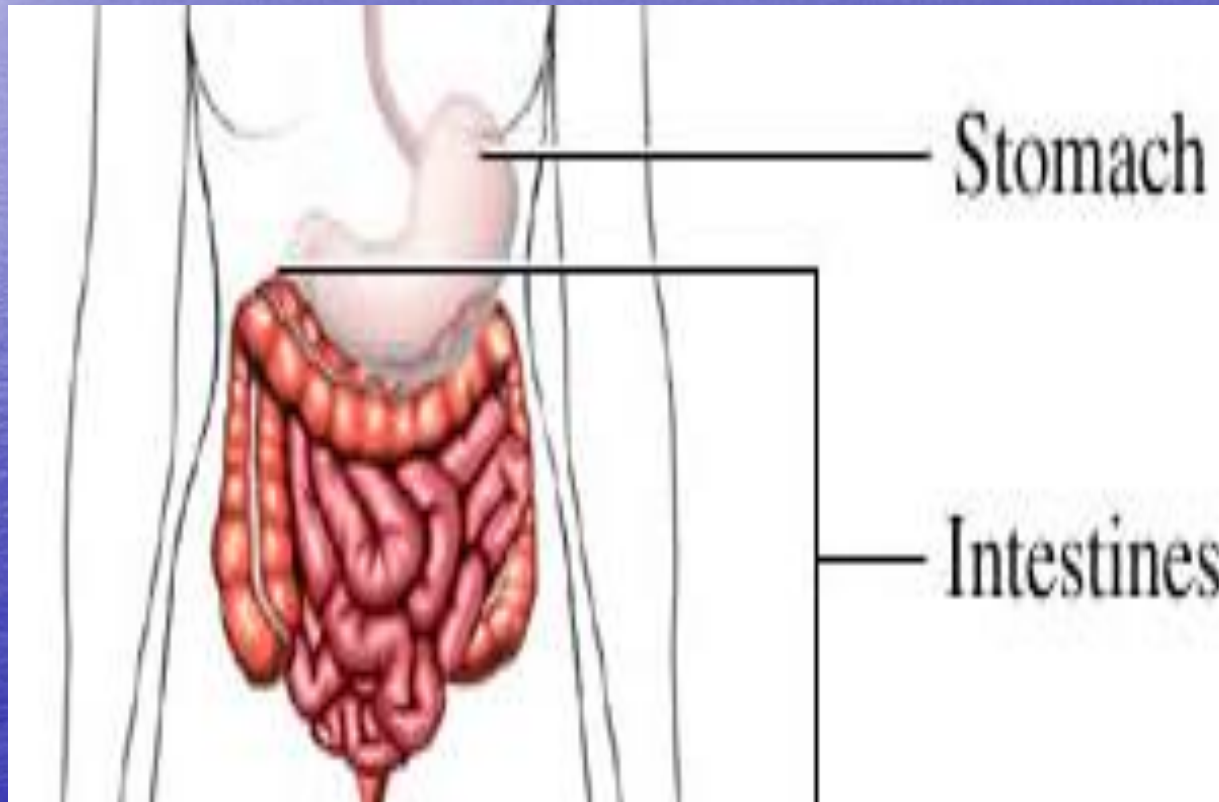
- Place the inoculum from the BAP onto a microscope slide (this is not a smear, no water!).
- Add one drop of H_2O_2 and watch for immediate bubbles (O_2 released from the hydrogen peroxide).

**positive
catalase**

**negative
catalase**



Stool Culture



Test Overview

- A stool culture is done to identify bacteria or viruses that may be causing an infection **using MacConkey Agar**.
- More than 50 different kinds of bacteria normally live in the **intestines**. However, disease can result if large numbers of abnormal organisms (bacteria, viruses, fungi, or parasites) grow in the intestines.
- Certain types of viruses, fungi, or parasites can be identified with a stool culture. A stool culture may be done if you have persistent diarrhea.

- For a stool culture, a stool sample is collected in a clean container and placed under conditions that allow bacteria or other organisms to grow.
- Usually several stool samples are collected over a period of days for accurate test results.

Why It Is Done

- Detect and identify certain types of bacteria, viruses, fungi, or parasites that can cause disease. Symptoms of an intestinal disease may include prolonged diarrhea, bloody diarrhea, an increased amount of gas, nausea, vomiting, loss of appetite, bloating, abdominal pain and cramping, and fever.
- Identify a person who may not have any symptoms of disease but who carries bacteria that can spread to others. This person is called a carrier. A person who is a carrier and who handles food is likely to infect others.

How To Prepare

- No special preparation is required before having this test.
- Do not collect a stool sample if you have bleeding hemorrhoids. Women should not collect a stool sample during their menstrual period; wait until 3 days after your period has stopped.
- If you have recently taken antibiotics, traveled out of your native country, or had a recent test with contrast material, tell your health professional when you receive the stool sample collection container.

How It Is Done

- The stool sample for this test may be collected at home or hospital.



- **STOOL CULTURE BOTTLE**
With spoon 25ml (Disposable)

- Wear gloves before collecting your specimen.
- Pass stool (but not urine) into a dry container. You may be given a container that can be placed under the toilet seat. Either solid or liquid stools can be collected. Avoid mixing toilet paper, water, or soap with the sample.
- Wash your hands well after collecting the sample to avoid spreading an infection.
- Deliver the sealed container as soon as possible to your health professional's office or directly to the lab.
- Your health professional may collect a stool sample by gently inserting a cotton swab into the rectum of the patient if he/ she was unable to pass a stool sample.

Risks

- There are no risks associated with collecting a stool sample.
- It is important to wear gloves before and wash your hands well after collecting the sample so that you do not spread an infection.

Results

- A stool culture is done to identify bacteria, viruses, fungi, or parasites that may be causing an infection. Stool culture test results usually take 2 to 3 days.

- **Normal:** No disease-causing (pathogenic) bacteria, viruses, fungi, or parasites are present or grow in the culture.
- **Abnormal:** Pathogenic bacteria (such as salmonella, shigella, campylobacter, certain types of *Escherichia coli*, or *Yersinia enterocolitica*) grow in the culture. Some of the more common diseases found using a stool culture include food poisoning and pseudomembranous enterocolitis. Fungi or parasites such as *Giardia lamblia* are found.
- If bacteria are found in the culture, sensitivity testing may be done to determine the best antibiotic to kill the bacteria.

Clostridium difficile is a bacterium commonly found in the intestinal tract but which, under the right circumstances such as after or during antibiotics therapy, can be the cause of enterocolitis.



What Affects the Test

Factors that can interfere with your test and the accuracy of the results include:

- Recent use of antibiotics, medication (such as bismuth) to control diarrhea, enemas, or laxatives.
- Recent X-ray tests using a contrast material containing barium.
- A stool sample contaminated with urine or blood.
- Delay in getting the stool sample to the lab for testing.

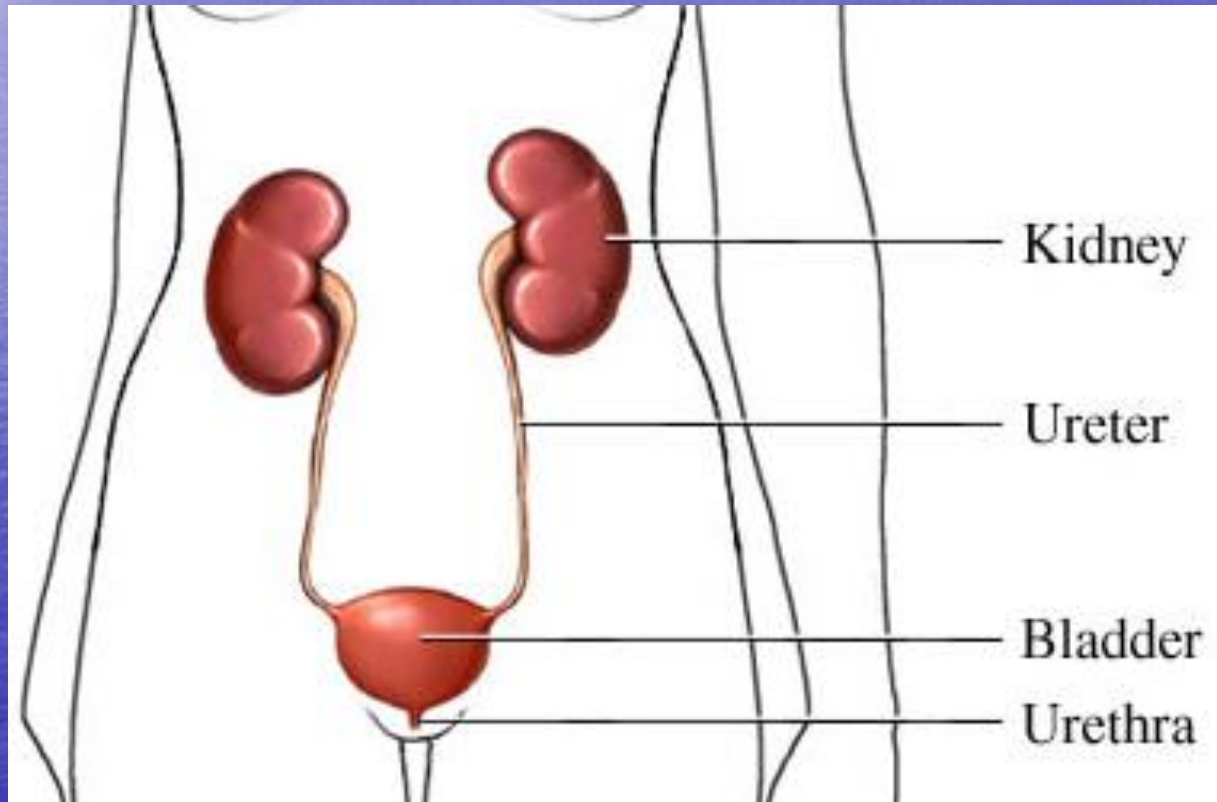
What To Think About

- A stool culture that does not grow any disease-causing (pathogenic) organism may not rule out an infection. Factors such as the amount of stool collected, the type of culture done, and previous use of antibiotics can prevent the growth of an organism in the culture.
- Sensitivity testing helps the health professional choose the best medication to kill the specific types of bacteria or fungi infecting a person.

- A test for parasites may be done using a sample of stool. Parasites are neither bacteria nor viruses and include organisms such as worms (pinworms, roundworms, tapeworms) and the protozoan *Giardia* that causes giardiasis. These parasites commonly infect the intestines.
- The parasites or their eggs can often be seen during an examination of the stool sample under a microscope.
- A stool sample can also be checked for the presence of:

- White blood cells, which may indicate an infection. If many white blood cells are present but no harmful bacteria grow in the stool culture, other diseases (such as inflammatory bowel disease) may be present.
 - Blood, which may indicate sores inside the intestines or stomach.
 - Poisonous substances (toxins) produced by some types of bacteria.

Urine Culture



Test Overview

- A urine culture is a test to detect and identify organisms (usually bacteria) that may be causing a urinary tract infection (UTI). Urine in the bladder normally is sterile—it does not contain any bacteria or other organisms (such as fungi)—but a urine sample can pick up bacteria in the urethra during urination.

- A urine sample is kept under conditions that allow bacteria and other organisms to grow. If few organisms grow, the test is negative.
- If organisms grow in numbers large enough to indicate an infection, the culture is positive. The type of organisms causing the infection are identified with a microscope or by chemical tests.

- If bacteria that can cause infection grow in the culture, another test may be done to determine which antibiotic will be most effective in treating the infection. This is called susceptibility or sensitivity testing.

Why It Is Done

A urine culture may be done to:

- Diagnose a urinary tract infection (UTI).
- Identify the best antibiotic to treat a urinary tract infection. This is called sensitivity testing.
- Determine whether a urinary tract infection has been cured.

How To Prepare

- No special preparation is required before having this test. If you are currently taking or have recently taken antibiotics, tell your health professional.

How It Is Done

- You will be asked to collect a clean-catch midstream urine sample for testing. The first urine of the day is preferred because bacterial levels will be higher.

Clean-catch midstream urine collection

- This collection method prevents contamination of the sample.



URINE CULTURE BOTTLE

- **Wash your hands to make sure they are clean before collecting the urine.**
- **If the collection container has a lid, remove it carefully and set it down with the inner surface up.**
- **Clean the area around your genitals.**
- **After the urine has flowed for several seconds, place the collection container in the stream and collect of this “midstream” urine without interrupting the flow.**
- **Avoid touching the rim of the container to your genital area, and avoid getting toilet paper, hair, feces, or menstrual blood in the urine sample.**
- **Carefully replace the lid on the container. Wash your hands. Return the urine sample to the lab. If you are collecting the urine at home and cannot get it to the lab within an hour, refrigerate the sample. It can be refrigerated for up to 24 hours. Follow the instructions from your lab.**

How It Feels

Collecting a urine sample does not normally cause any discomfort.

Risks

- There are no risks associated with collecting a urine sample.

Results

- A urine culture is a test to detect and identify organisms (usually bacteria) that may be causing a urinary tract infection (UTI). Urine culture results are usually ready in 1 to 3 days. Some organisms take longer to grow in the culture; for this reason, results may not be available for several days.

Urine culture

Normal:

No bacteria or other organisms (such as fungi) grow in the culture. The culture result is negative.

Normal results may vary from lab to lab.

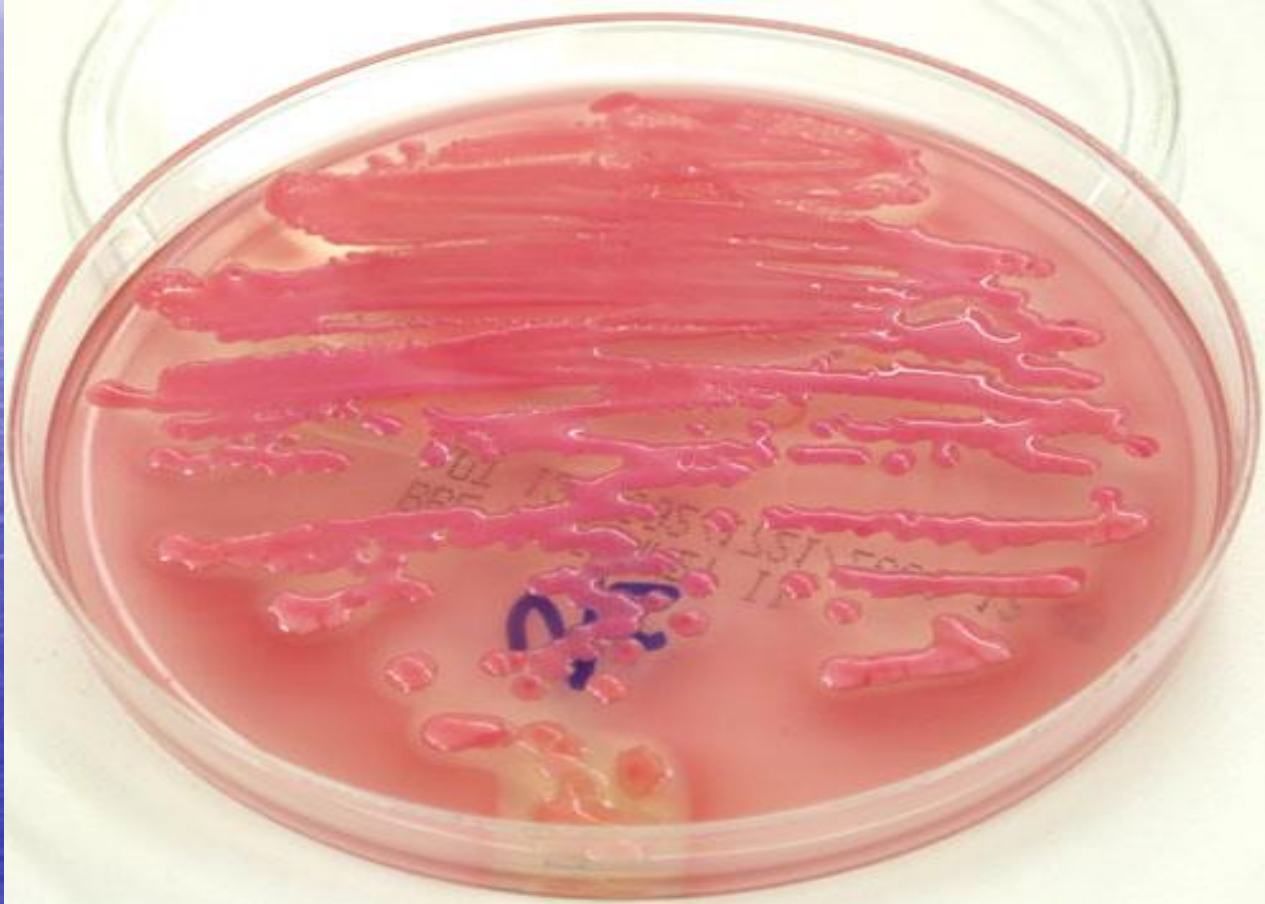
Abnormal:

Organisms (usually bacteria) grow in the culture. The culture result is positive. Urinary tract infections are usually caused by bacteria that normally live in the intestines, such as *E. coli*. The number of bacteria in a given quantity of urine is usually estimated. A count of 100,000 or more bacteria per milliliter (mL) of urine may indicate an infection. A count ranging from 100 to 100,000 could be due either to infection or to contamination of the sample (you may need a repeat urine culture). If the count is 100 or less, infection is unlikely; however, a count of 100 or less may also be seen if you are taking antibiotics for a UTI.

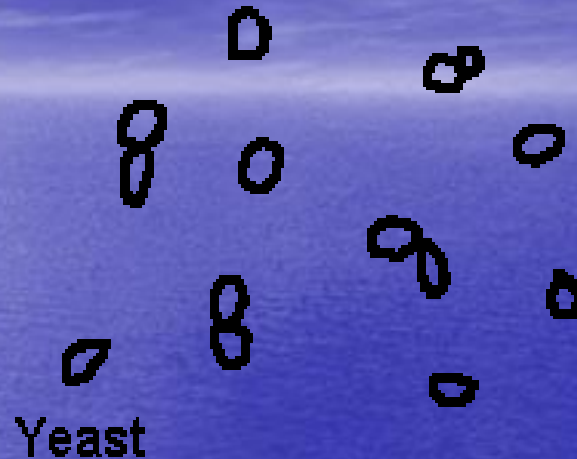
Lactose-fermenting, Gram negative rods.
MacConkey agar plate of *E. coli* from urine ($>10^6$ CFU/mL).
Note pink, non-shiny, lactose+ colonies that are characteristic
for *E. coli*.



Urine culture on MacConkey agar. Lactose+, mucoid colonies identified as *E. coli* by API testing (not to be confused with mucoid nature of *Klebsiella pneumoniae*).



Yeast



- Yeast cells may be contaminants or represent a true yeast infection. They are often difficult to distinguish from red cells and amorphous crystals but are distinguished by their tendency to bud. Most often they are *Candida*, which may colonize bladder, urethra, or vagina.

What Affects the Test

Factors that can interfere with your test and the accuracy of the results include:

- Using diuretics or drinking a large amount of liquid. This may dilute your urine and lower the concentration of bacteria, causing inaccurate test results.
- Recent use of antibiotics or taking high doses of vitamin C.
- Some organisms take several days to grow in the culture.

What To Think About

- A urine culture done in the early stage of a urinary tract infection (UTI) may be less accurate than one that is done after the infection becomes established.
- A urine culture may be done when an abnormal result from a urinalysis (such as an increased number of white blood cells) indicates an infection.
- A urine culture may be repeated after the UTI has been treated to make sure the infection is cured.

- To reduce the chance of contaminating the urine sample with bacteria (other than the bacteria causing the infection), a health professional may collect a urine sample by inserting a urinary catheter through the urethra into the bladder.
- Catheterization is sometimes done to collect urine from a person in the hospital who is very ill or unable to provide a clean-catch sample. This method reduces the risk that the sample will be contaminated but may occasionally cause a UTI.
- People who have a urinary catheter in place for a long time are at high risk of developing a UTI.

- Collecting a urine sample from a small child or baby may be done by using a special plastic bag with tape around its opening (a U bag). The bag is attached around the child's genitals until he or she urinates (usually within an hour). Then the bag is carefully removed.
- To collect a urine sample from a very sick baby, a health professional may insert a needle through the baby's abdomen directly into the bladder.
- To diagnose tuberculosis that has spread to the urinary tract, a special test will be done using all of the first morning urine on three separate days.

- Sensitivity testing helps a health professional choose the best antibiotic for the specific type of bacteria infecting a specific person. Differences in the genetic material (DNA) in some types of bacteria make them resistant to certain antibiotics.
- In such cases, those antibiotics cannot kill all the bacteria. When an effective antibiotic is chosen, its entire course must be completed to prevent the bacteria from developing resistance to the antibiotic. Stopping antibiotic treatment early kills only the most sensitive bacteria, while bacteria that are more resistant can multiply and prolong the infection.

MACROSCOPIC URINALYSIS

- The first part of a urinalysis is direct visual observation. Normal, fresh urine is pale to dark yellow or amber in color and clear. Normal urine volume is 750 to 2000 ml/24hr.
- Turbidity or cloudiness may be caused by excessive cellular material or protein in the urine or may develop from crystallization or precipitation of salts upon standing at room temperature or in the refrigerator. Clearing of the specimen after addition of a small amount of acid indicates that precipitation of salts is the probable cause of turbidity.

- A red or red-brown (abnormal) color could be from a food dye, eating fresh beets, a drug, or the presence of either hemoglobin or myoglobin. If the sample contained many red blood cells, it would be cloudy as well as red.

Examples of appearances of urine

- Three urine samples are shown. The one at the left shows a red, cloudy appearance. The one in the center is red but clear. The one on the right is yellow, but cloudy.

