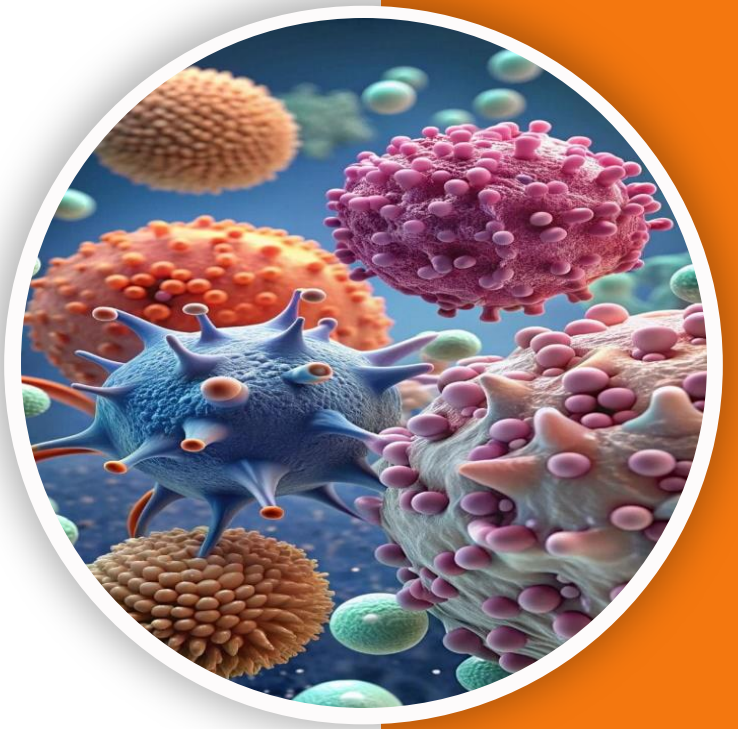
بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Microbiology | Lecture 7

Bacterial Taxonomy pt.2



Written by : Lujain alqadi
NST

Reviewed by : Hebah Salah

Lecture 5 - Part-2

Bacterial taxonomy, Classification, and laboratory diagnosis

Vitek system

Urine culture technique

Blood culture

Vitek system

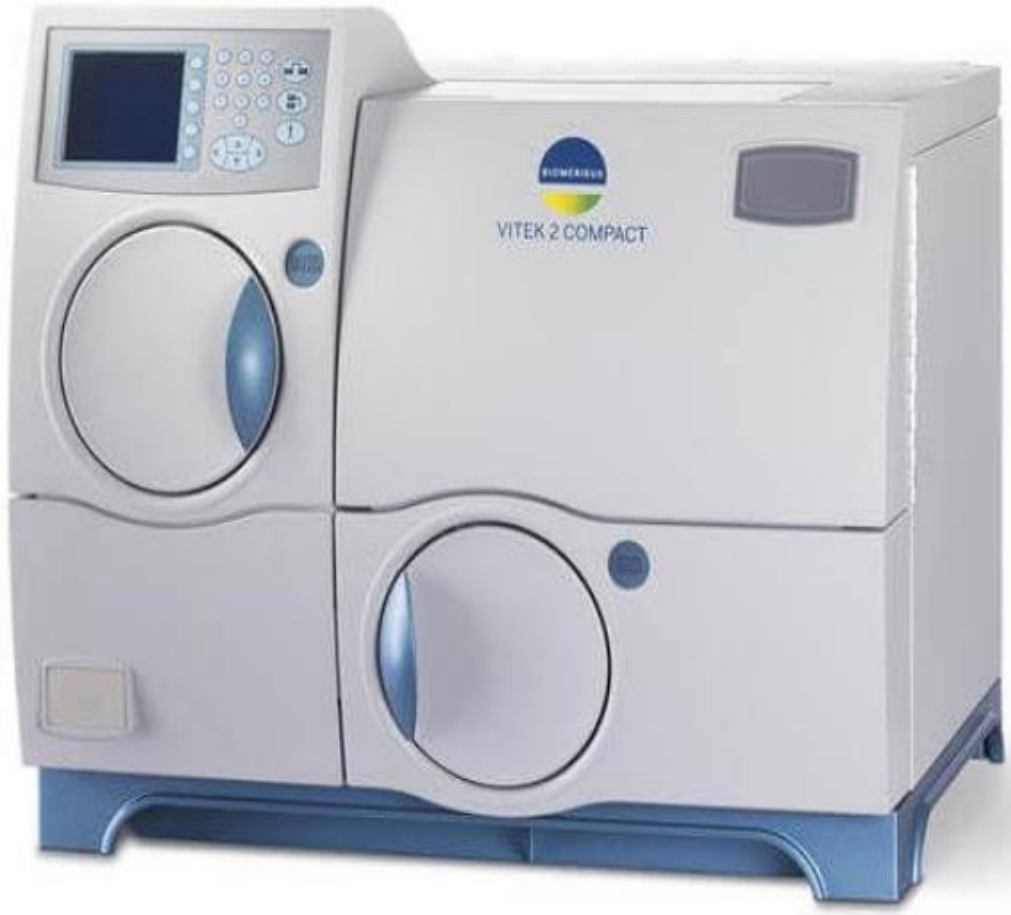
Vitek system

An automated instrument

Main uses:

1) Identification
   It can identify bacteria and fungi
2) Antibiogram
   It can perform antimicrobial susceptibility testing, which tells us the proper antibiotic to give the patient.
3) Antifungals

It can also identify the appropriate antifungal the patient may use.

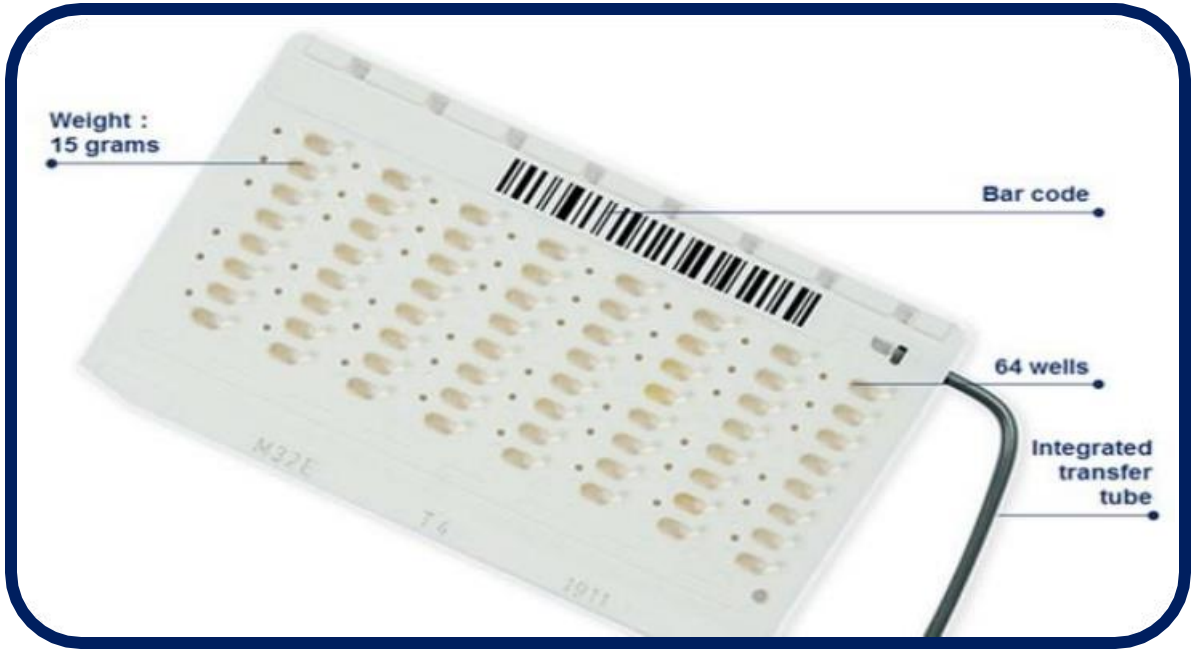


Vitek system

Vitek system

** Two cards

1) Identification card (ID card)
   contains:
   *47 biochemical tests.
   It helps us in the identification process to determine the type of bacteria or fungi.
   *Specific Card for GN (Gram -)
   *Specific Card for GP(Gram +)
   *Specific Card for Yeast



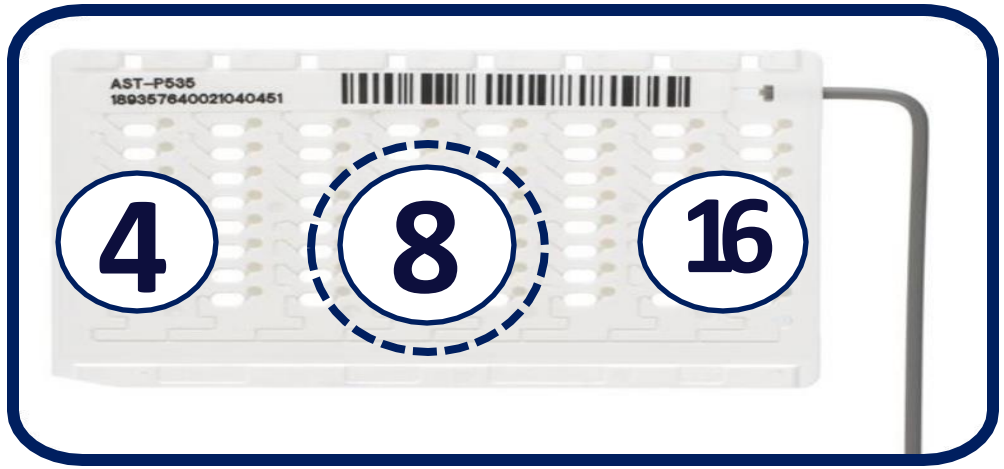
بالمختبر يا دوب نعمل
10 biochemical tests
فالجهاز جدًا ممتاز بخليني أعمل
47 biochemical tests

Vitek system

It provides us with all the antibiotics that the patient can use and identifies the most effective ones.

2) Antimicrobial susceptibility test card (AST card)

22 antibiotics



MIC :

It can determine the minimum inhibitory concentration (MIC) of the antibiotic.

Ex:For patients with kidney problems who have a certain infection, and when I want to avoid giving them a strong antibiotic, I check the MIC of the antibiotic to choose the lowest effective dose.

These wells each contain different dilutions of the antibiotic for example, 4, 8, and 16.
We look at the turbidity (the cloudiness)in each well to see if bacteria are still growing.
In the well with 4, the microorganisms **are still growing; so the antibiotic isn't strong enough there.**
>>At 8, about 80% of the bacteria are killed.
>>At 16 ,all the microorganisms **were killed.**
#So the MIC (the minimum inhibitory concentration)is 8.

- Two patients might have the same type of bacteria, but that doesn't mean they should get the same antibiotic. Each patient's body is different — one might have kidney problems, another might be allergic to a certain drug, or have a weak immune system. That's why, even after identifying the bacteria, we still need to perform AST. It helps us choose the most appropriate antibiotic based on the patient's condition, not just the bacterial species.That's why we don't rely on identification (ID) alone — diagnosis and treatment depend not only on the organism's name, but also on how the bacteria and the patient respond together .

Steps of work

1

Organism isolation (Pure)

We cultured a sample and obtained colonies. These colonies must be pure and not contaminated, to identify the causative agent responsible for the disease.

Steps of work

2

Bacterial suspension (2 tubes)

The sample contains bacteria and liquid help us to identify the type of bacteria and how to eliminate it

ID

Help us to identify the type of bacteria

AST

It helps us to determine which antibiotic can kill this bacterium

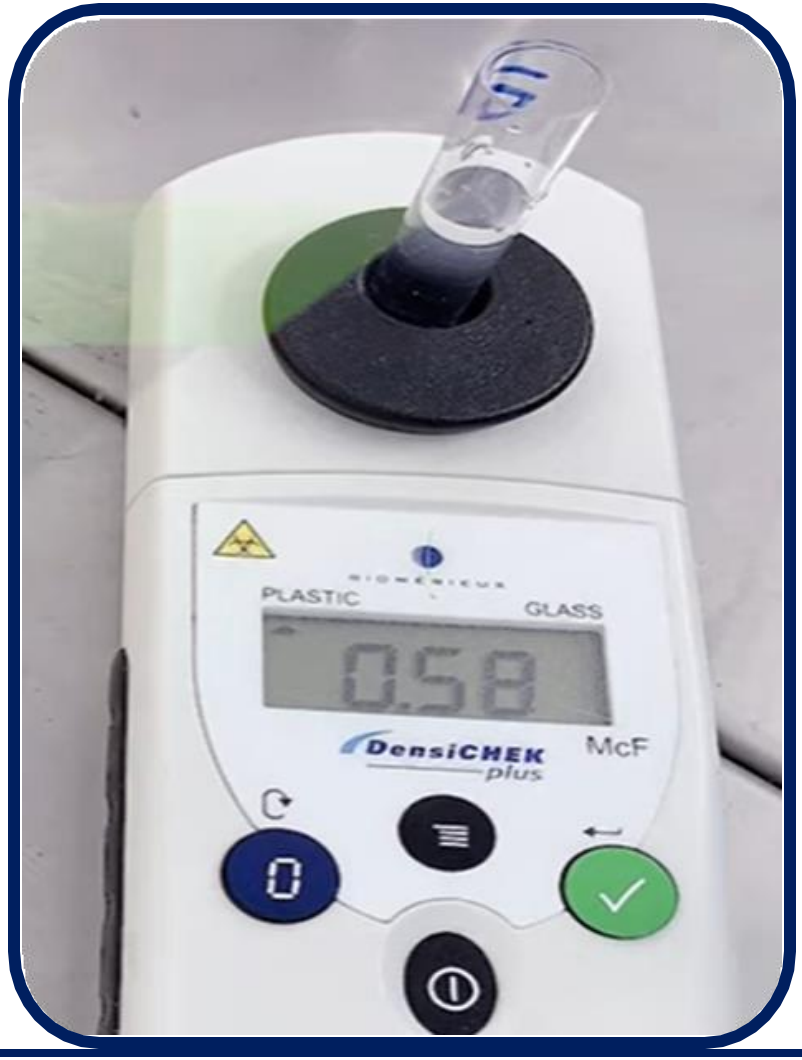
We have two test tubes, and in each one we place some bacterial colonies into nutrient broth to prepare a bacterial suspension. This suspension causes turbidity, and the level of turbidity must be standardized.Why standardized? Because turbidity reflects the bacterial concentration. If the suspension is too turbid, meaning there are too many microorganisms, the antibiotic may appear less effective than it actually is – leading to a **false negative result**. On the other hand, if the turbidity is too low and there are very few microorganisms, the antibiotic may appear more effective than it really is – **resulting in a false positive**. Therefore, the bacterial concentration in the suspension must be standardized to ensure accurate antimicrobial susceptibility testing

>> that, we need to have another small device that measures the turbidity, which is a turbidometer

Steps of work

3

Measure turbidity (0.5 -0.63)



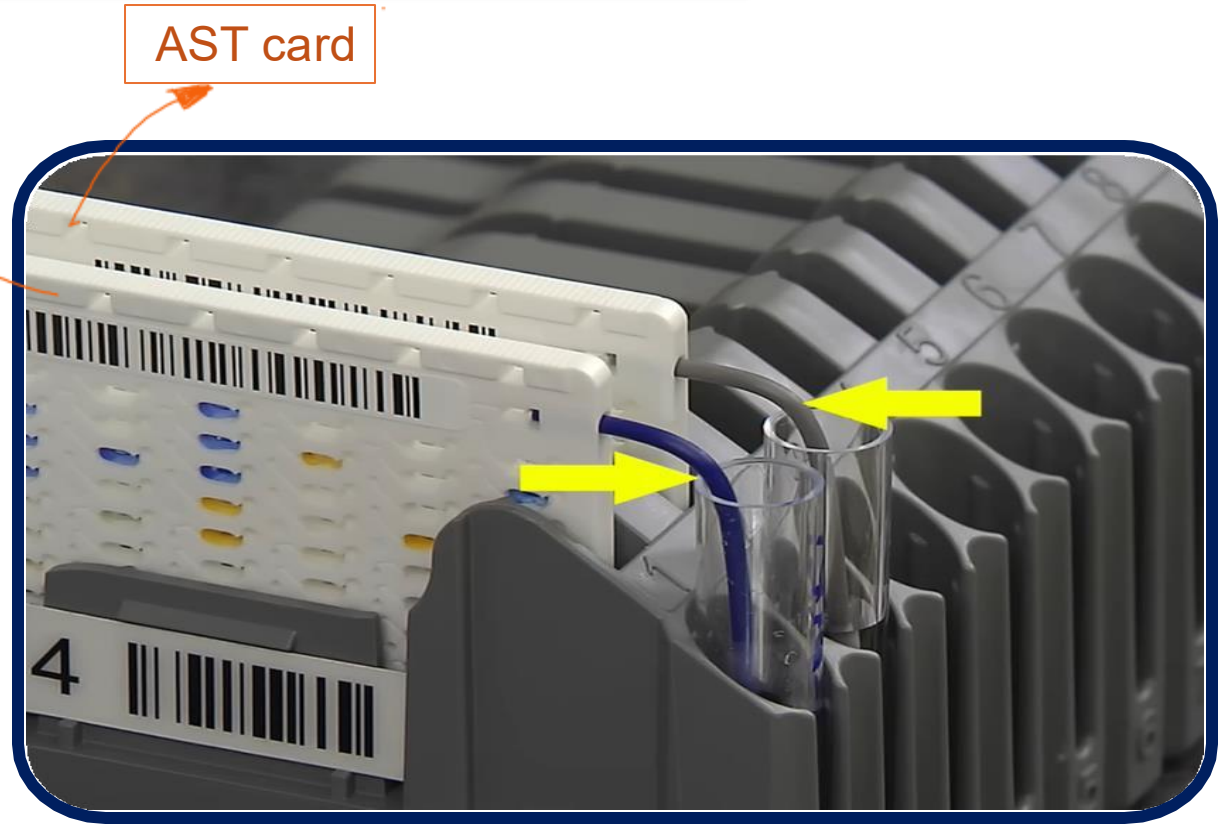
Turbidimeter

Steps of work

4

Insert cards in bacterial Suspension tubes

We bring a rack or cassette, and in it, we first place the ID card (for bacterial identification), then the AST card (for antibiotic susceptibility testing). There is also a capillary tube, where we place the bacterial suspension that we have prepared according to the standardized turbidity.



Steps of work

5

Into the filling room (Transfer the bacterial suspension into the wells of the ID & AST cards)

Steps of work

6

Transfer the cassette into the loading room

(Diagnostic) 5-10hrs

Steps of work

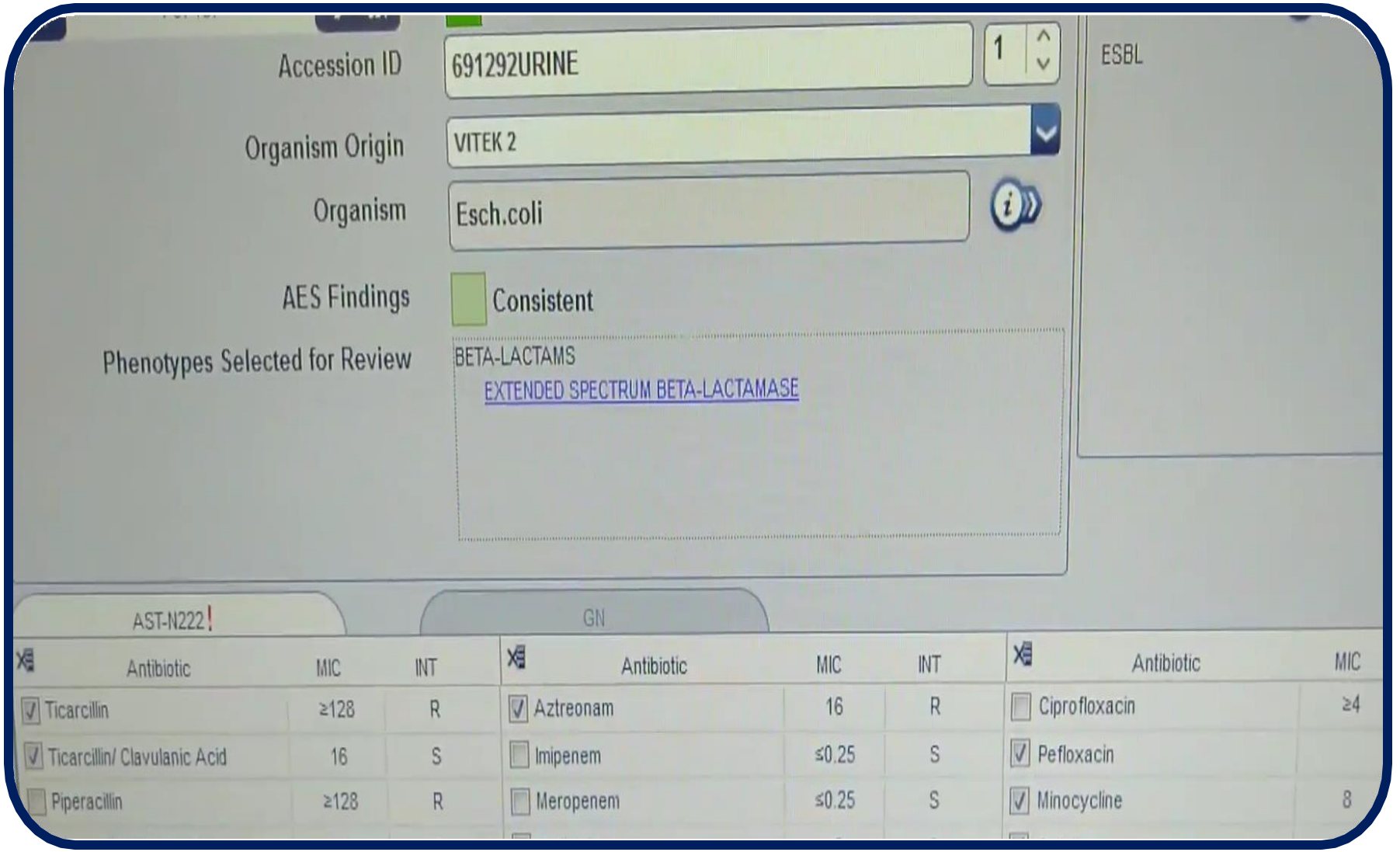
7

NEXT SLIDE

Colorimetric (Barcode)

- This card contains a barcode. When the barcode is scanned, the device reads all the biochemical tests, metabolic reactions, and color changes, then compares them to the stored data. The system has a large database that includes most of the microorganisms likely to appear in clinical samples, along with their associated biochemical test results, color changes, and metabolic activities.When the device analyzes the results and finds a 90% or higher match with the stored data, it identifies the bacterial species and determines the most appropriate antibiotic, using a colorimetric detection method.

Steps of work



Urine culture technique

Purpose

Specimen

Method

Interpretation

Purpose

To diagnose Urinary tract infection

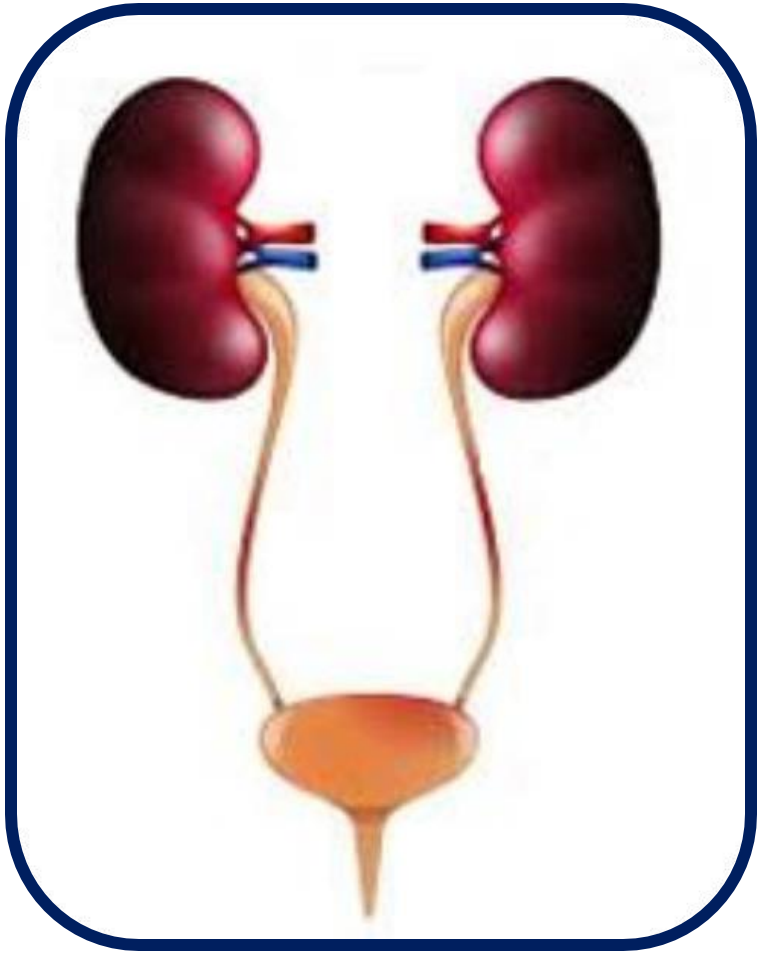
(UTI) -> Urinary Tract Infection.

(Bacteriuria)

They have bacteria in their urine. The area of bladder and above it is supposed to be sterile means it doesn't have any microorganisms ,so if microorganisms are there there's something wrong

Pyelonephritis

Occur when the bacteriuria found in the kidney



Cystitis

Occur when the pathogen found in the bladder

Purpose

UTI

Bacteriuria

Dysuria → The patient has a burning sensation during urination

Frequency → Patient goes to the bathroom frequently

Purpose

UTI

Its not any bacteriuria it should be significant bacteriuria

Bacteriuria

Significant

means patient must have

≥105 CFU/ml (100,000 CFU/ml)

Colony forming unit (CFU)

Bacterial count

In urine culture we interested of counting coloni

Purpose

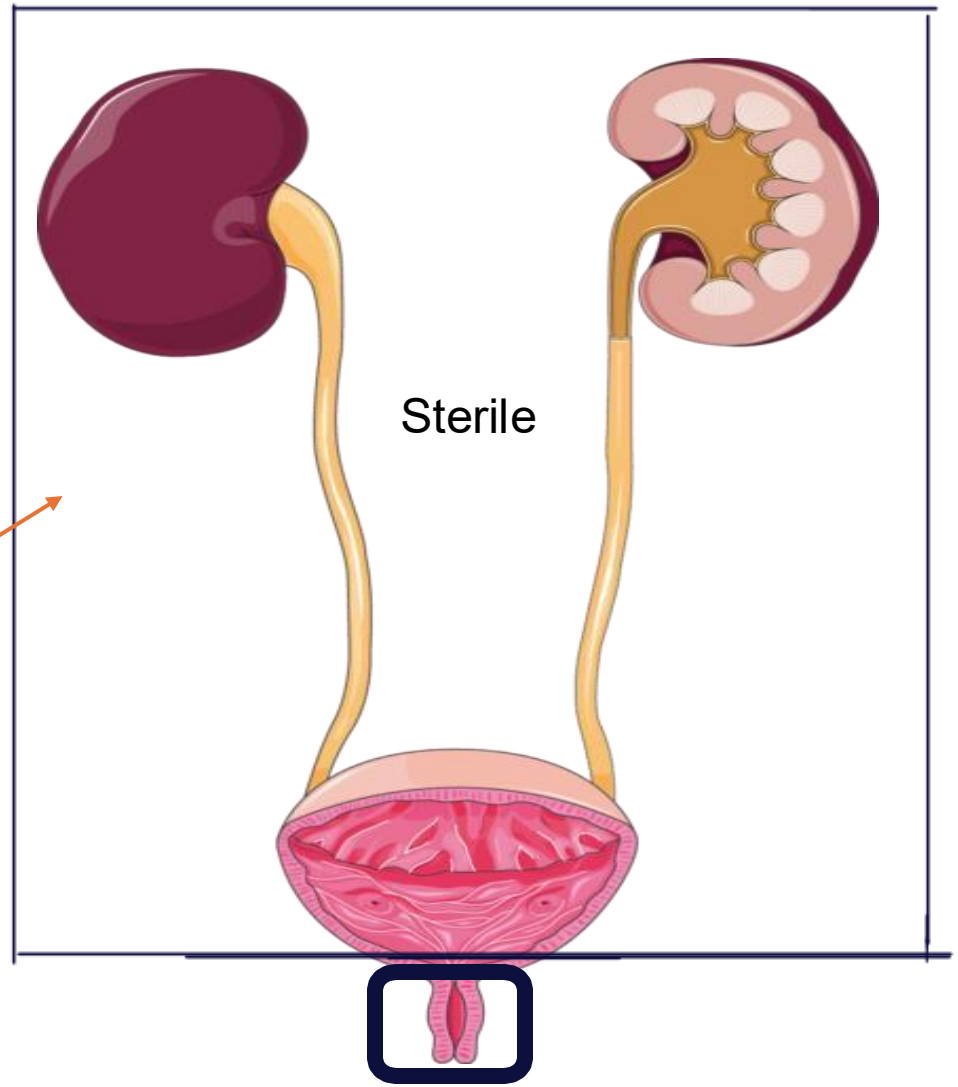
Bacterial count

Bacteriuria

≥10^5 CFU/ml

Significant

This area – the bladder and above – is supposed to be sterile, without any microorganisms. The problem often starts in the urethra, where contamination with bacteria can occur, allowing them to enter and cause infection



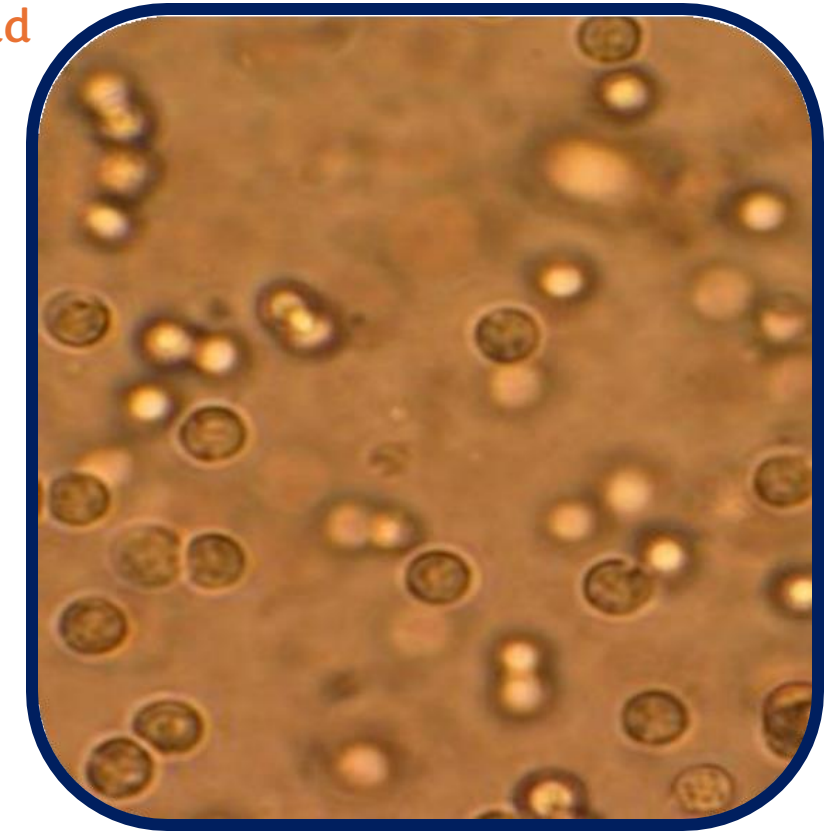
Purpose

Pyuria

We found cell in urine

(Pus in urine > 10 cells/hPF).

High power field



Significant Bacteriuria

Pyuria is commonly associated with significant bacteriuria, but there are some exceptions. Pyuria can be present without infection, and bacteriuria may occur without a significant number of white blood cells.

Specimen

1

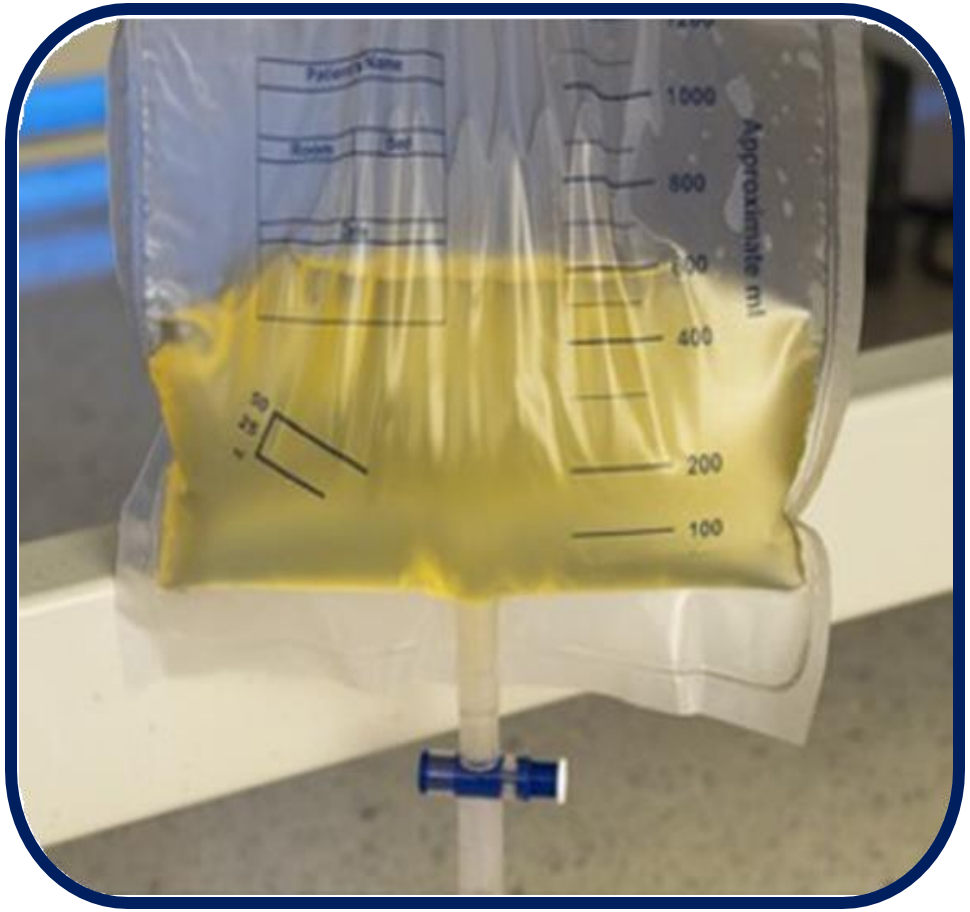
Mid stream urine

Specimen

2

Catheterization

Urinary catheterization is the insertion of a thin tube (catheter) into the bladder through the urethra to drain urine or to monitor urine output.

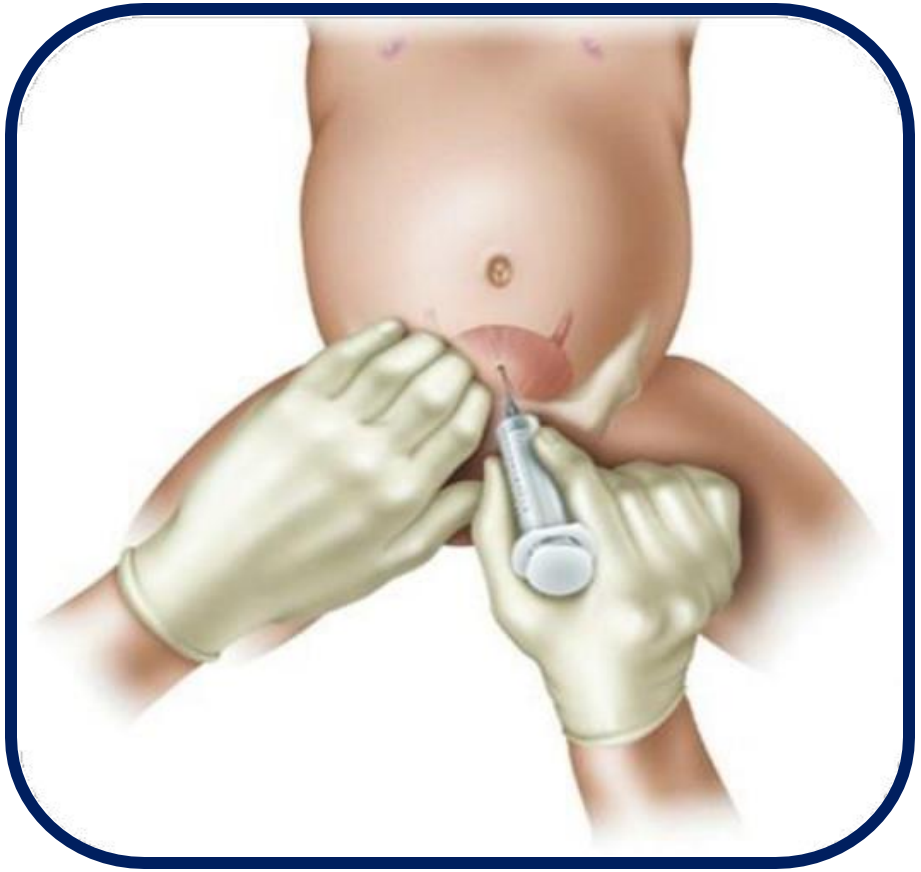


Specimen

3

Suprapubic aspiration

Urinary catheterization in children is done when they cannot urinate, to accurately measure urine output



how to collect Mid stream urine

Stop antibiotics (for 3 days)

is done to avoid false-negative results, because antibiotics reduce or eliminate bacteria in the urine and can make the test appear normal even when infection is still present.

how to collect Mid stream urine

بعدين بحكيله كيف يجهز العينة :

1 Wash and dry your hands.

2 Clean genital area

3 Remove the lid on the **container** (Sterile)

how to collect Mid stream urine

4) Pass a small amount of urine into the toilet. (at morning)

5)**Mid stream urine**

6)Pass the reimaging urine into the toilet.

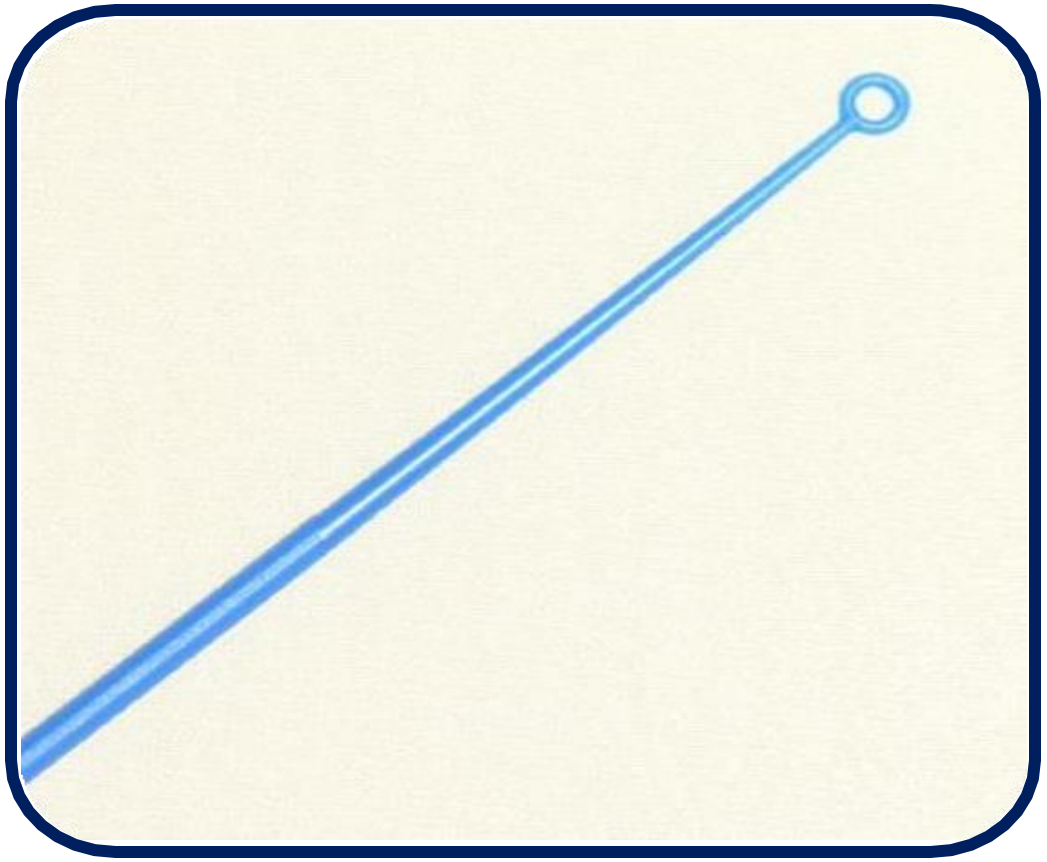
Mid stream urine

➢Mid-stream urine is collected to avoid contamination from the urethra and external genital area that appears in the first part of urination, so the middle portion provides a cleaner and more accurate sample that reflects the true bladder urine for reliable analysis and culture.

Method

1

- Mix urine (uncentrifuged) & by Calibrated loop



- 1µL (0.001ml)

10µL (0.01ml)

mix the urine sample then use a calibrated loop to take a **precise volume** of urine for inoculation onto 2 cultures medium

The two loops shown represent different calibrated volumes:

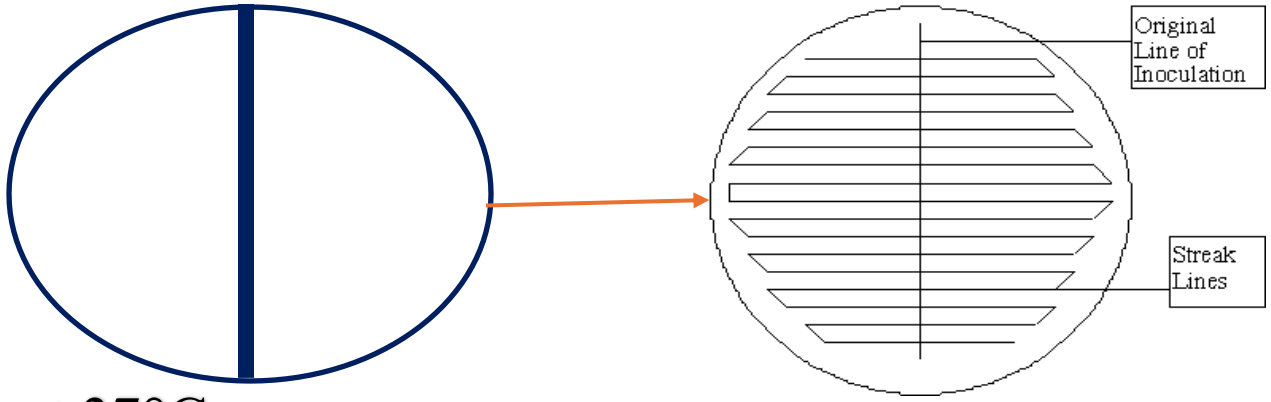
1 µL (0.001 mL) ينصح نتعامل معاه

10 µL (0.01 mL)

Using a known volume allows accurate calculation of the bacterial count per milliliter after incubation.

Method

2



Inoculation on

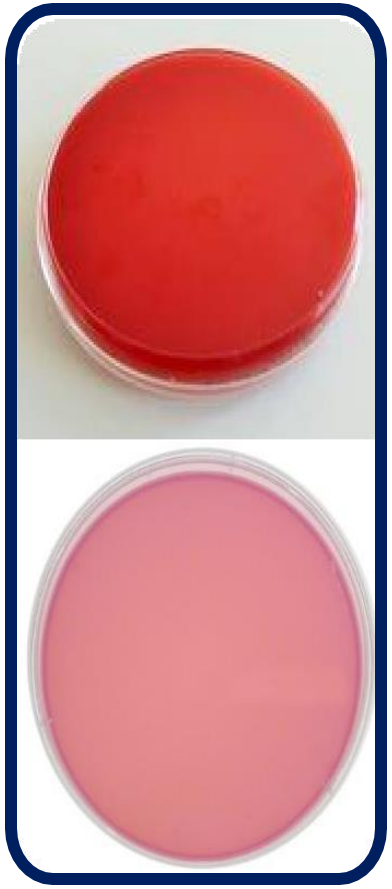
By streaking & incubate at 37°C

For 24hrs.

Urine culture is different from other cultures:

1)Put urine on the agar plate with a loop.
2)Make a central line.
3)Zigzag from the central line to spread the bacteria.
4)Incubate 37°C for 24hrs

Blood agar



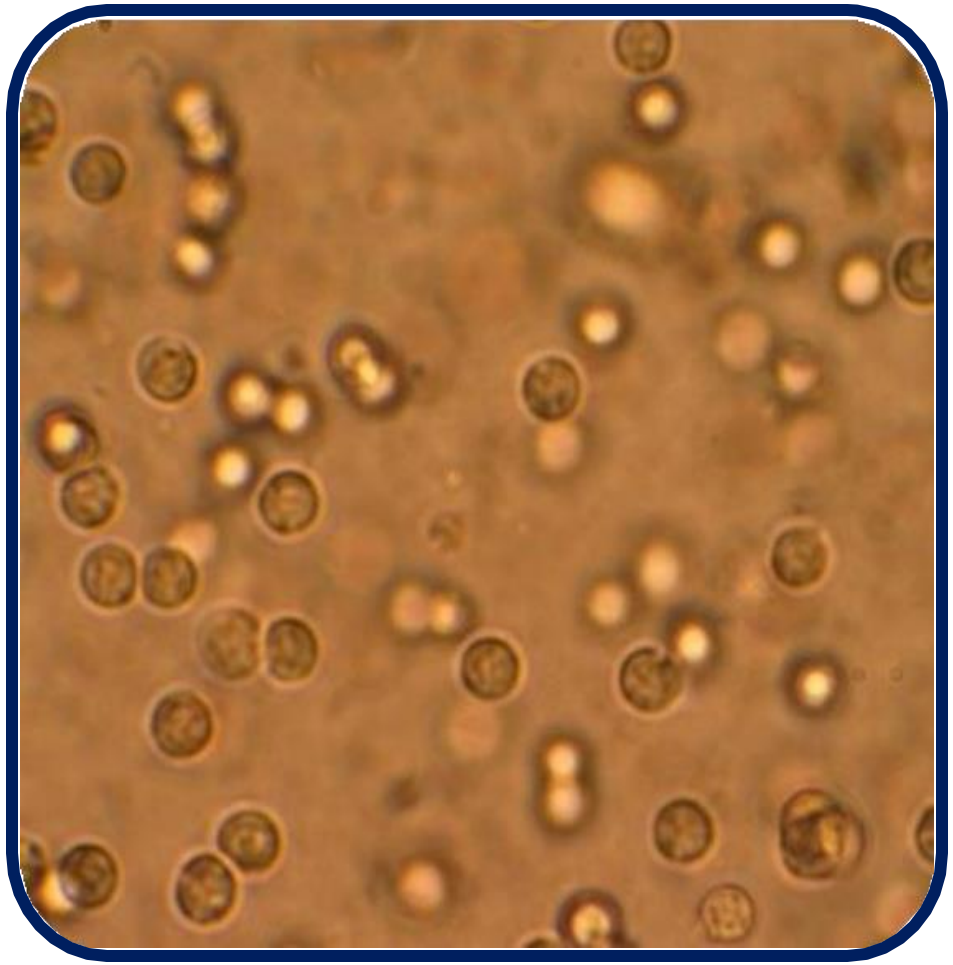
MacConkey

Method

3

Examine centrifuged urine ($\geq$ 10 cells/hPF)

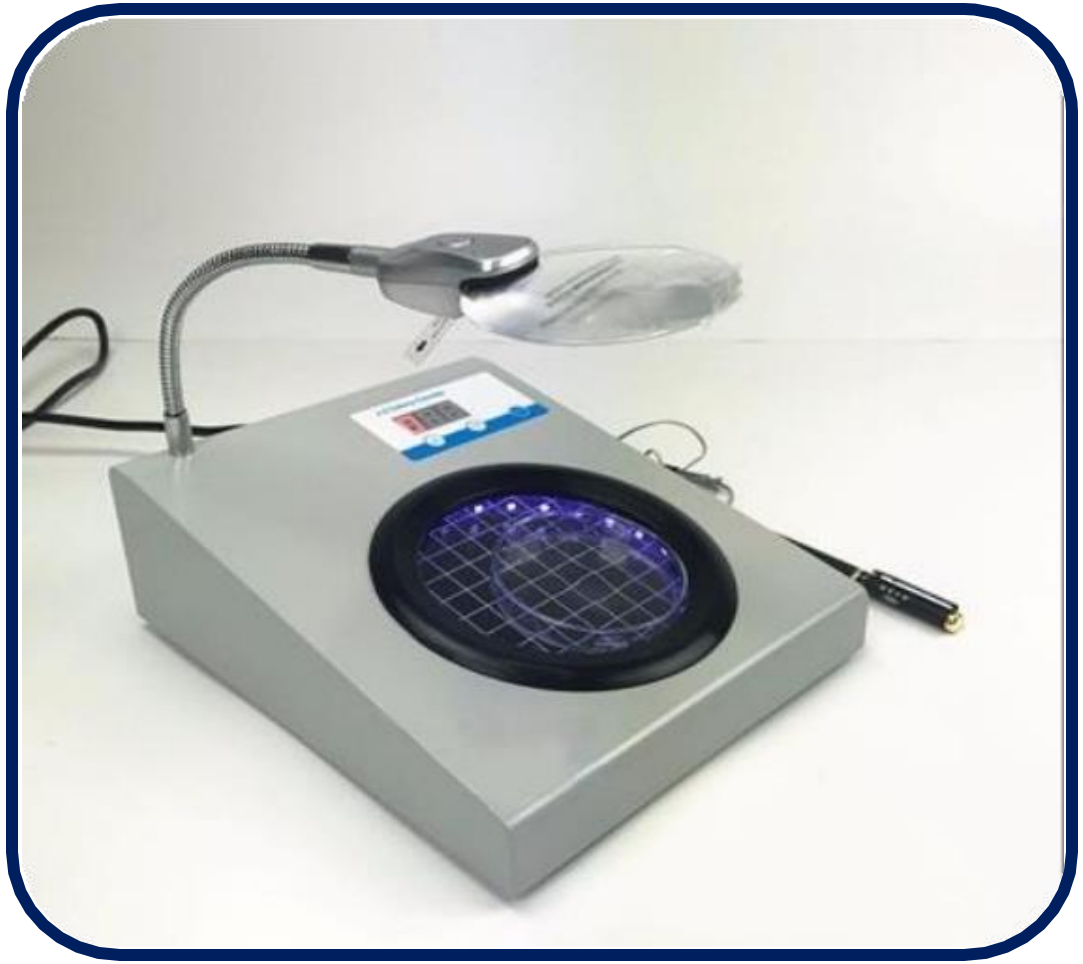
Pyuria



Method

4

Count the growth colonies



the main methods of urine culture

1) Preparing the sample

2) Choosing the calibrated loop

3) Inoculating the agar plate : عملية نقل العينة

Dip the loop into the urine sample.

Make a straight line (center line) then zigzag pattern.

4) Incubation:

The plates are incubated at 37 °C for 24 hours until colonies become visible.

5) Counting and calculation

Method

4

10µL(0.01ml)

No. of colonies**X100**= 105 CFU/ml

1µL (0.001ml)

No. of colonies**X1000**= 105 CFU/ml

Multiply the count by dilution factor

You plated 10 µL (0.01 ml) of a urine sample on a blood agar plate and after incubation you counted 35 colonies.

Calculate the CFU/ml of the original sample.

Solution:

Since 10 µL = 0.01 ml, we multiply by 100 to convert to 1 ml.

Why do we multiply the colony count by 100 or 1000 when calculating CFU/ml?

1) You do not plate the full 1 ml on the agar plate.
You only plate a small portion (either 10 µL or 1 µL).

2) After plating the small volume and counting the colonies,
you must go back and estimate how many colonies there would have been if you had plated the full 1 ml.

3) To convert back to 1 ml:
- If you plated 10 µL = 0.01 ml, you multiply by 100
- If you plated 1 µL = 0.001 ml, you multiply by 1000

In summary:
Take the number of colonies you counted on the plate and multiply it by the dilution factor (100 or 1000).

تخيّل إنك شربت نقطة واحدة من عصير برتقال مركز، وبعدها حاولت تتخيل طعمه لو شربت الكوب كامل.
نفس الشيء لما بنعد بكتيريا من جزء صغير من العينة، بنضرب العدد عشان نعرف كم كان رح يكون لو أخذنا المليلتر كامل

Method

0.01ml (10µL)

No. of coloniesX100= 105 CFU/ml

No. of colonies= 10

10X100= 1000 CFU/ml

10^3

Not significant

Counting and calculation
Example: using a 1 µL loop
Suppose 100 colonies appear on the plate
Calculation:
100 colonies × 1000 = 10^5 CFU/mL
We multiply by 1000 because 1 µL = 1/1000 mL.

Urine culture: Interpretation

≥**10^5** CFU/ml

Significant bacteriuria

Identification

UTI

Interpretation

≥10^4 CFU/ml

6X1000= 6000 CFU/ml

Significant bacteriuria

Identification

S. aureus

10^4 or 10^3 CFU/ml + Staph. aureus = significant

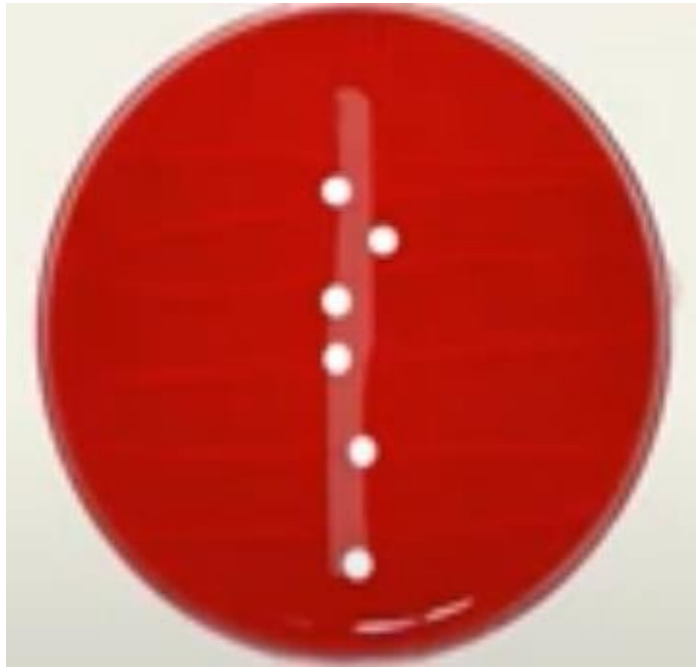
Interpretation

≥10^3 CFU/ml

6X1000= 6000 CFU/ml

Significant bacteriuria

Identification



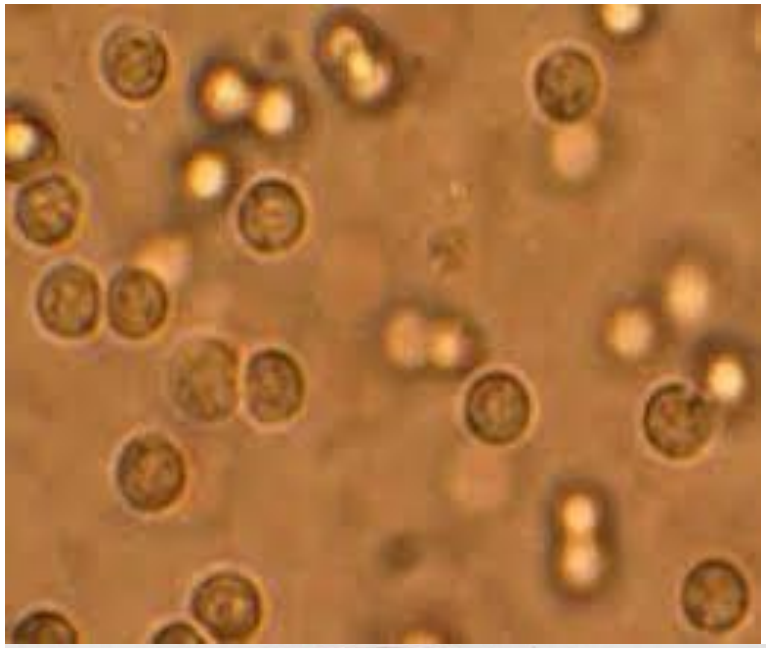
S. aureus

Interpretation

Sterile pyuria

Pus without any bacterial growth in ordinary media

No microoorganisms present why ?



Sterile pyuria

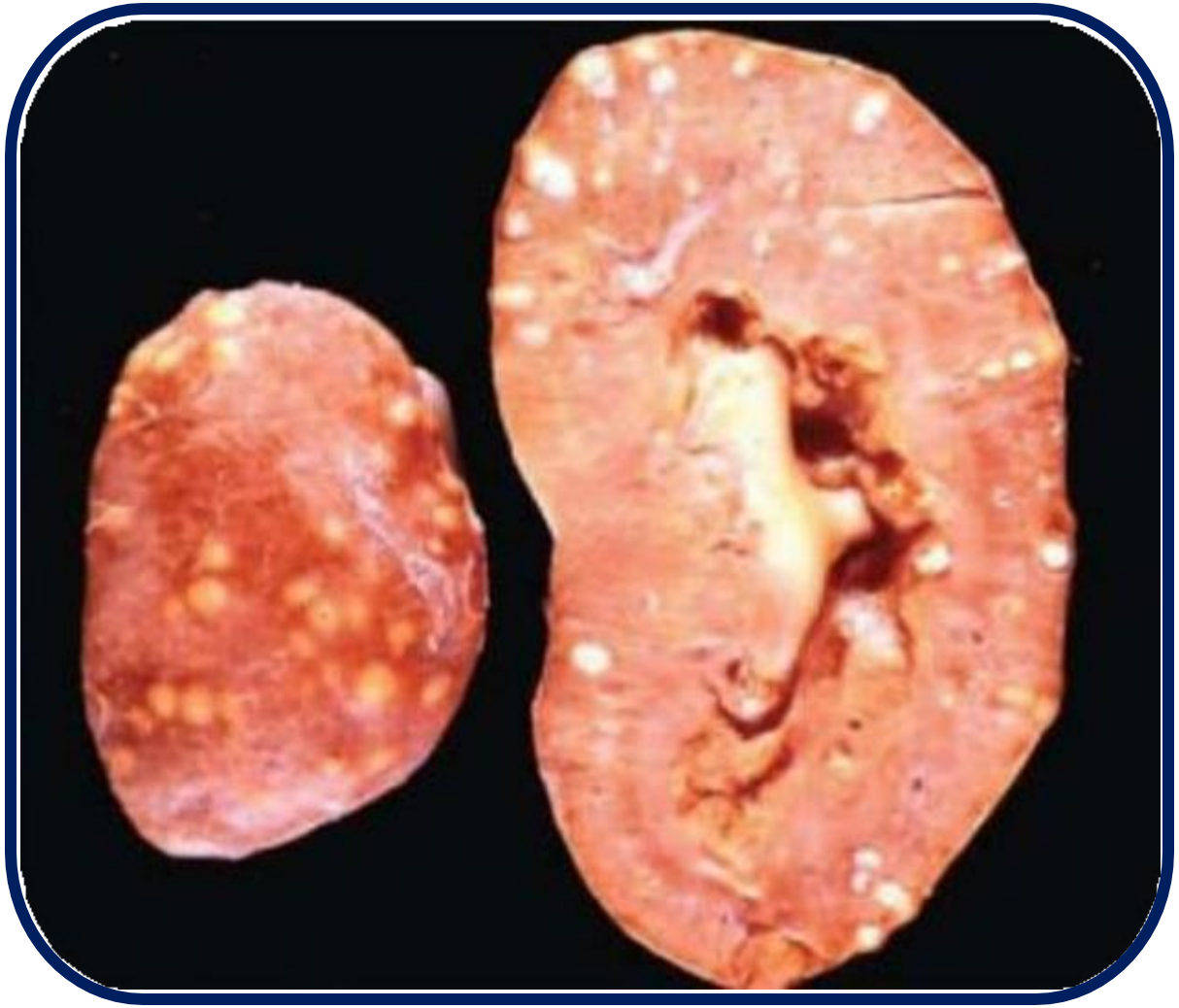
1

Taking antibiotics

The antibiotic kills the bacteria before the urine is cultured,
so the culture appears negative even though infection was there.

Sterile pyuria

2



Renal tuberculosis

It’s responsible for making cells

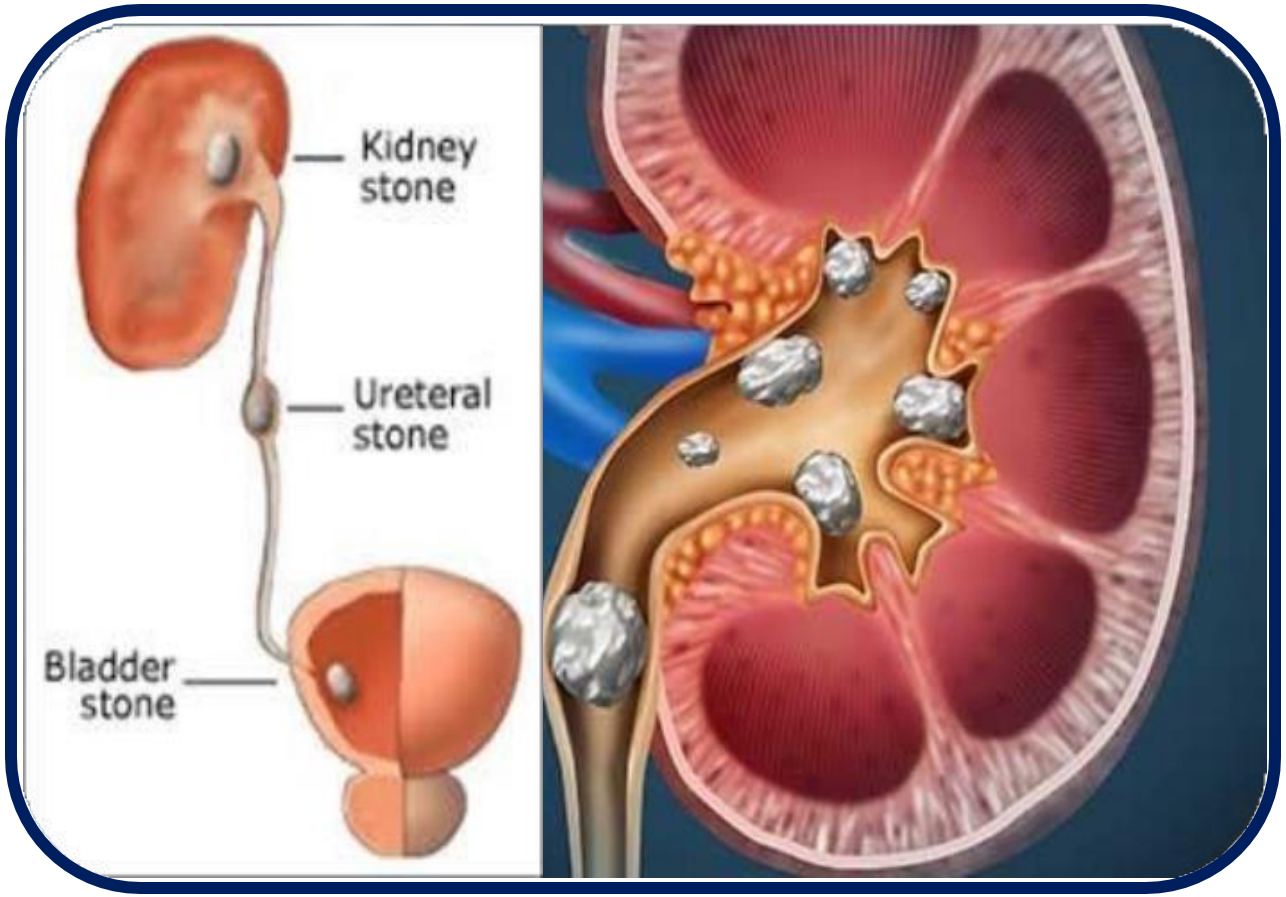
EXTRA INFO:The infection causes inflammation in the kidney. This inflammation attracts white blood cells (WBCs) to fight the infection.
The WBCs then appear in the urine → pyuria

Sterile pyuria

3

Renal stones

Stones irritate the urinary tract and cause inflammation with WBCs, but not necessarily bacterial infection.



Sterile pyuria

4

Organism not grow on ordinary media

need a certain medium to grow

Mycoplasma

L-form bacteria Anaerobic infection

Interpretation

10^3

(No UTI)

Although there is pyuria

1) Prostatitis

inflammation of the prostate (in males)

2) Vaginitis

the vagina (in females)

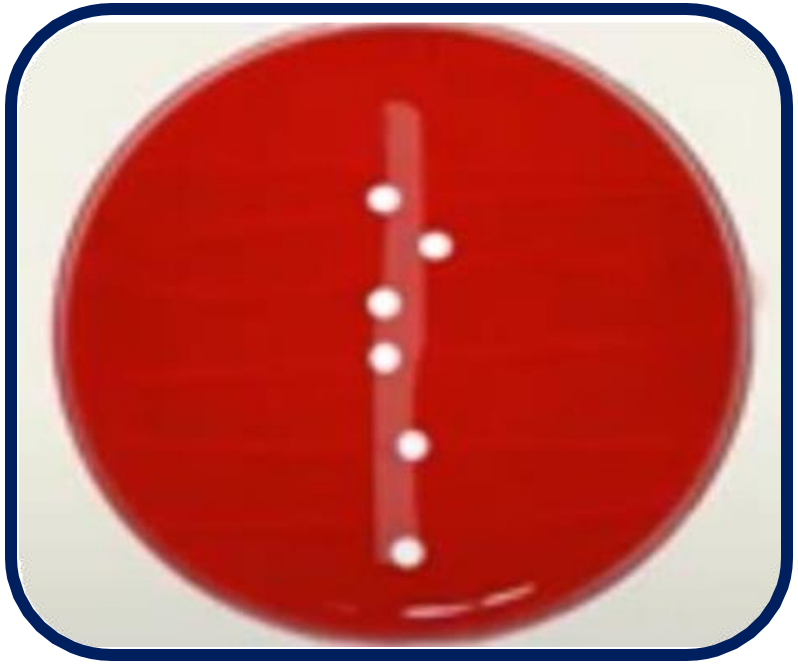
3) Cervicitis

the cervix (in females)

4) Malignancy

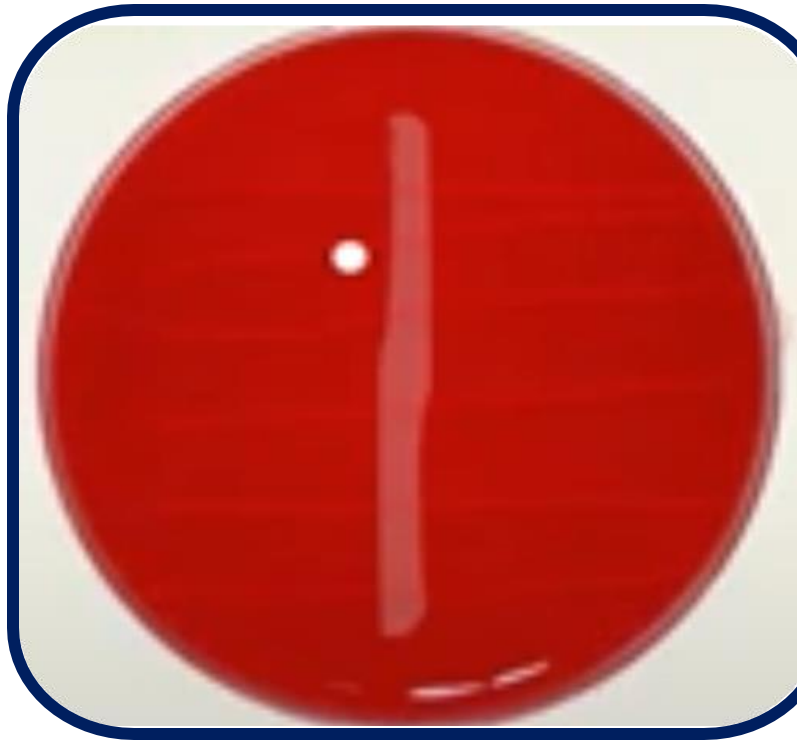
5) Renal calculi

kidney stones



Interpretation

Suprapubic aspiration



10^3

Because it's directly taken from the bladder → meaning there's no contamination.

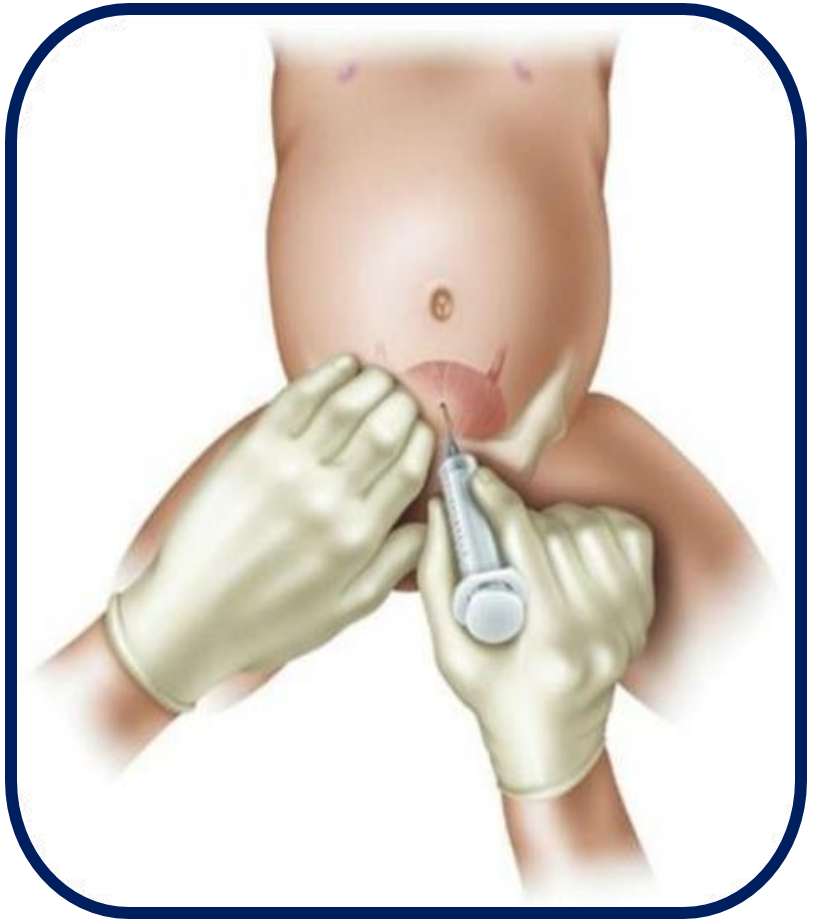
Any growth even one colony is significant bacteriuria

Interpretation

Suprapubic aspiration

1X1000= 1000 CFU/ml

10^3



Any growth is significant bacteriuria

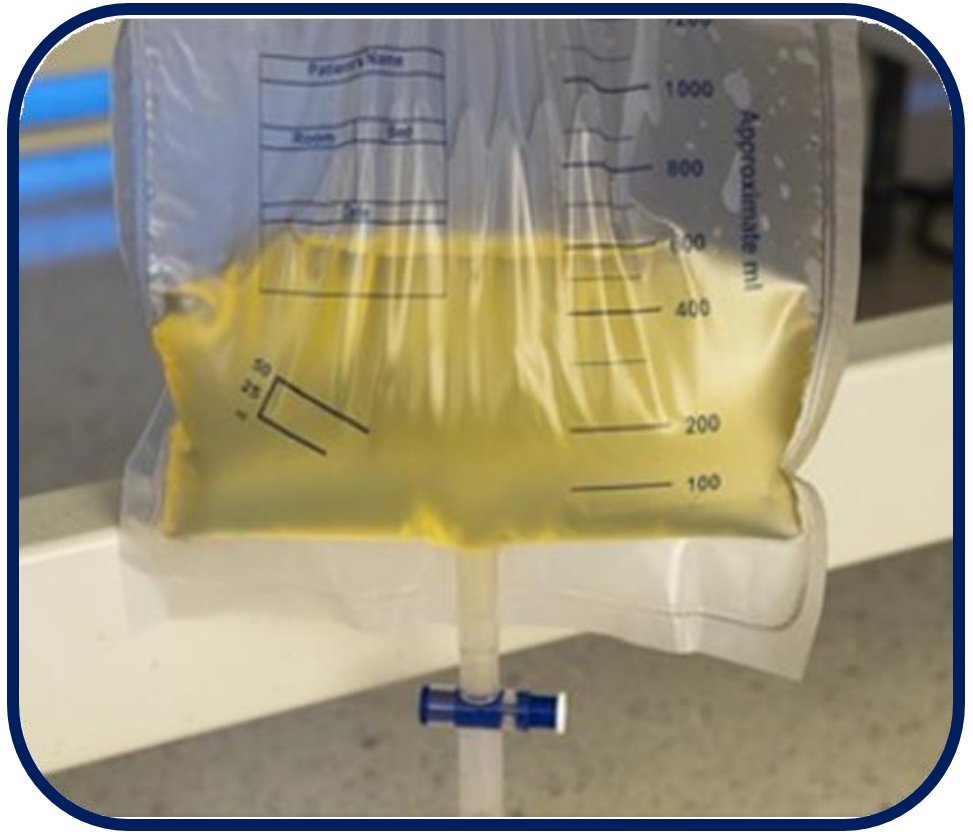
Interpretation

Catheterization

10^3

Any growth is

significant bacteriuria

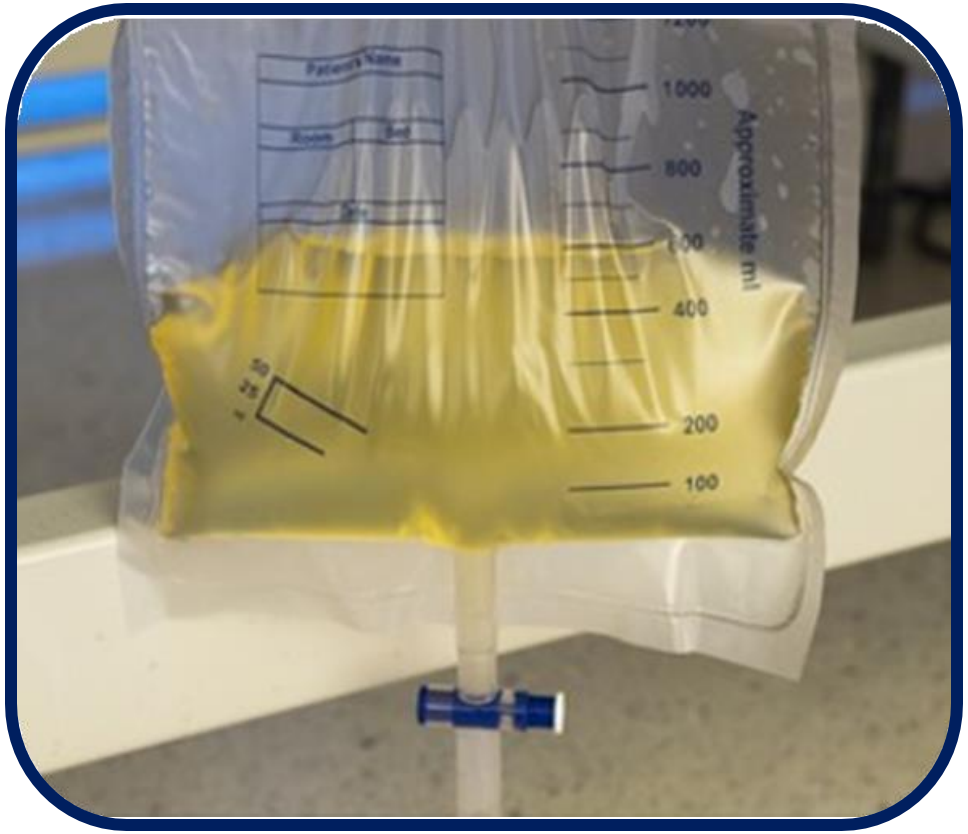


In the case of urine collected by catheterization,

any amount of bacterial growth even very small is considered true bacteriuria

because the sample is taken directly from the bladder area

and there is no contamination from the urethra, vagina, or skin.



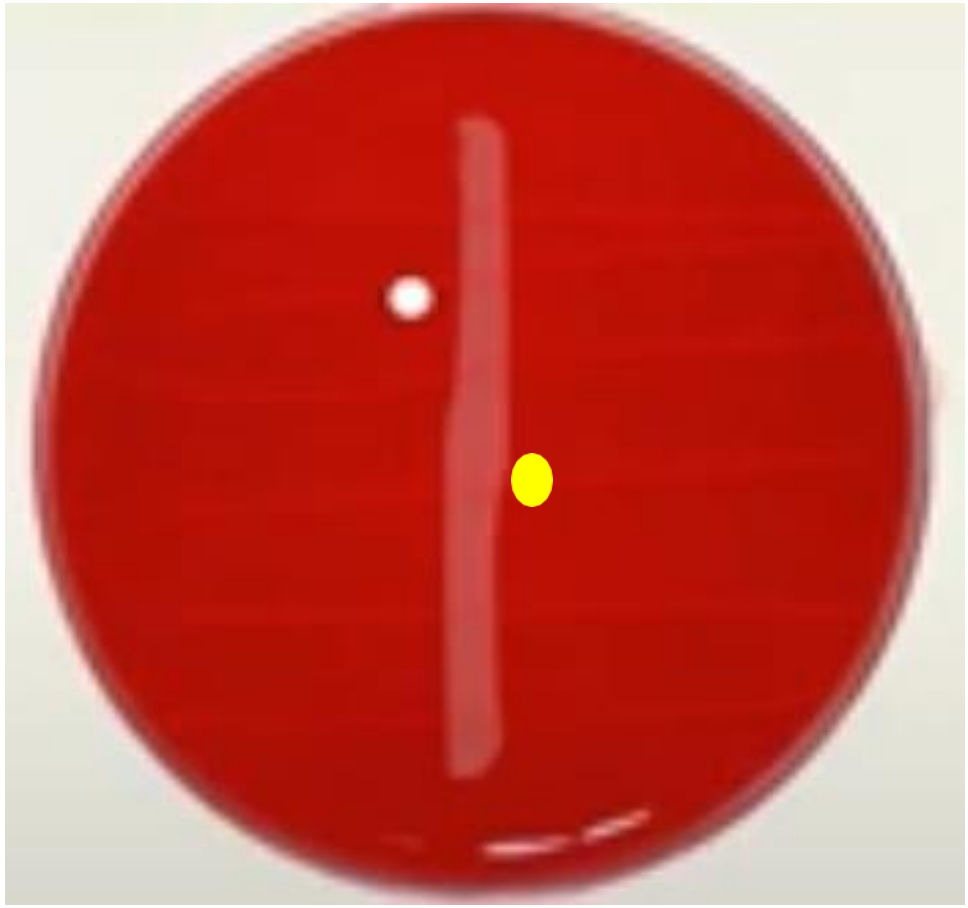
Interpretation

Catheterization

1X1000= 1000 CFU/ml

10^3

Any growth is significant bacteriuria



Urine culture Interpretation

Two pathogen

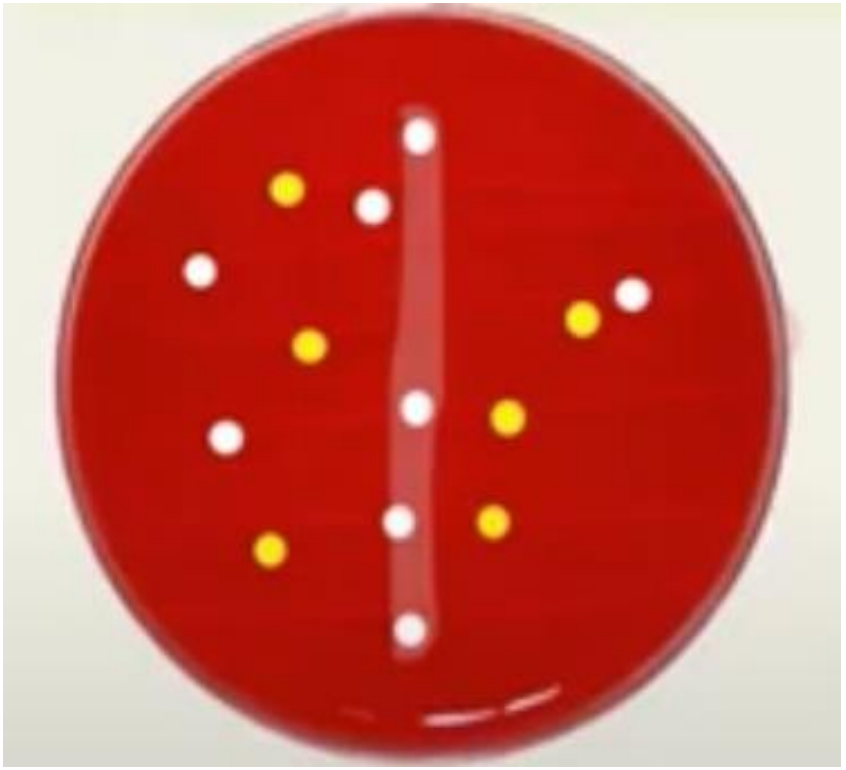
Interpretation

Count 1 ≧ 10^3

8X1000= 8000 CFU/ml

Count 2 ≧ 103

6X1000= 6000 CFU/ml



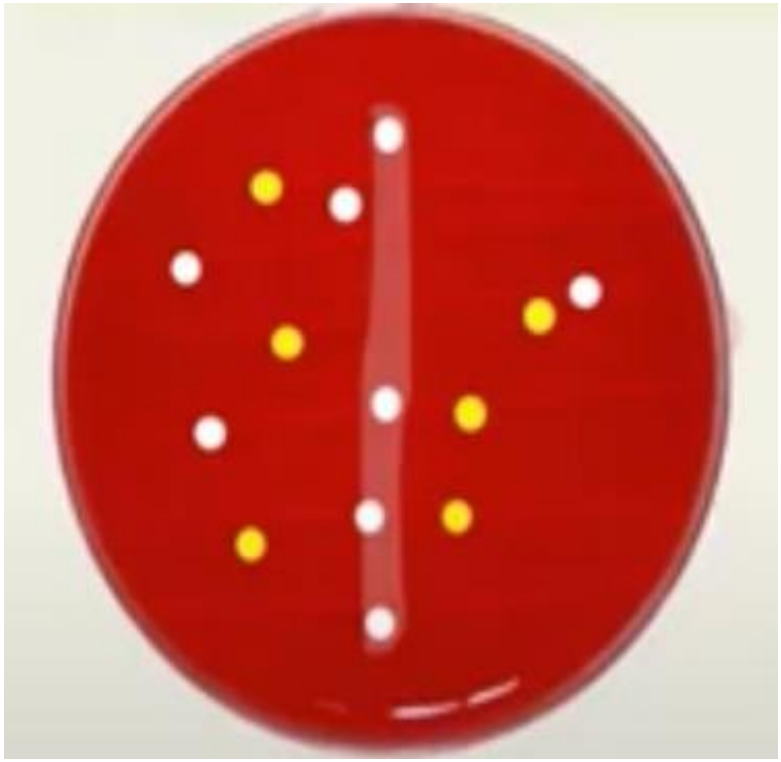
No significant growth

Interpretation

Count 1 ≥ 104

- 13X1000= 13000 CFU/ml
- 6X1000= 6000 CFU/ml

Continue with higher & ignore the other



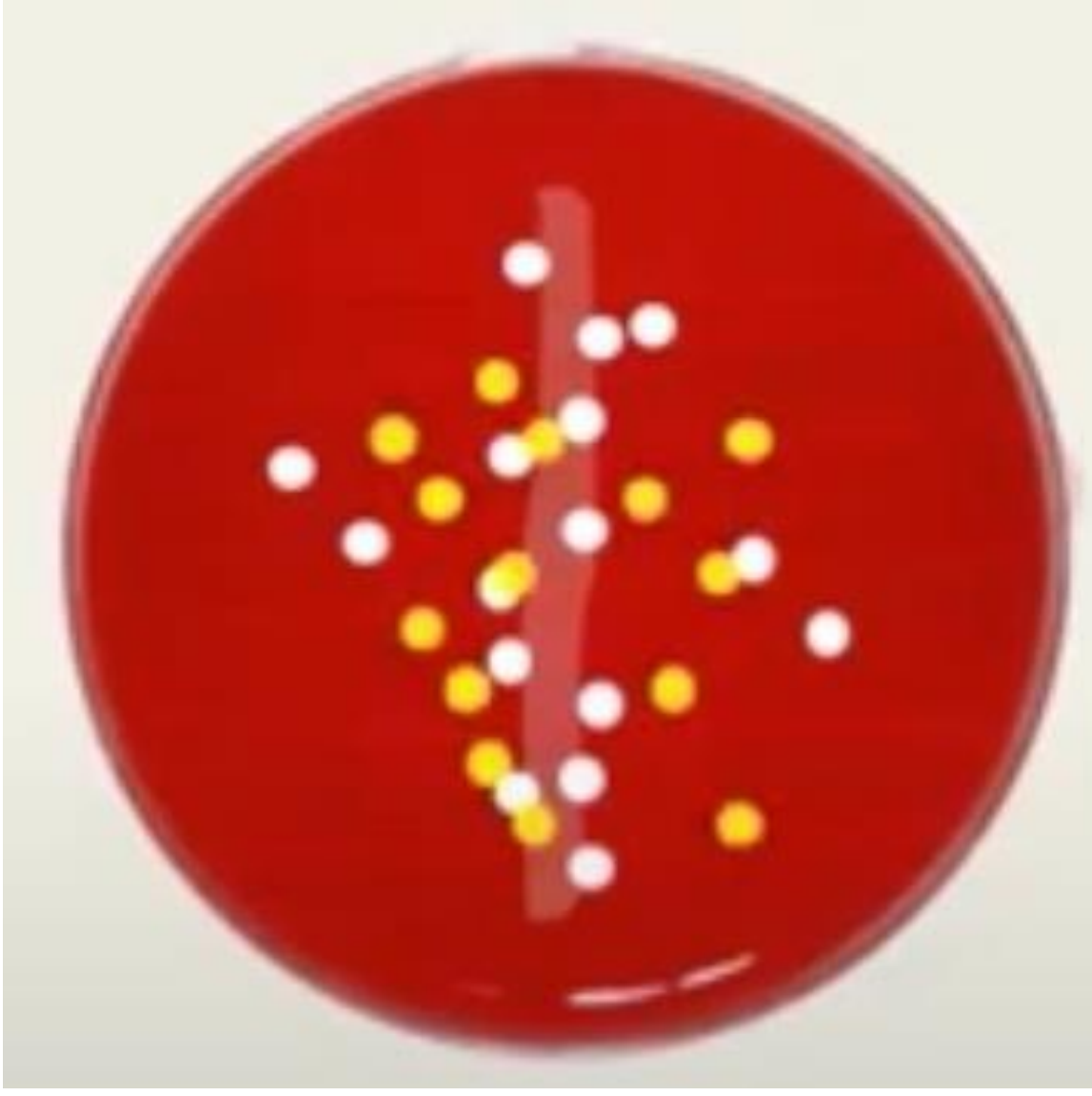
Count 1 ≥ 104

16X1000= 16000 CFU/ml

Count 2 ≥ 104

14X1000= 14000 CFU/ml

Identification for both

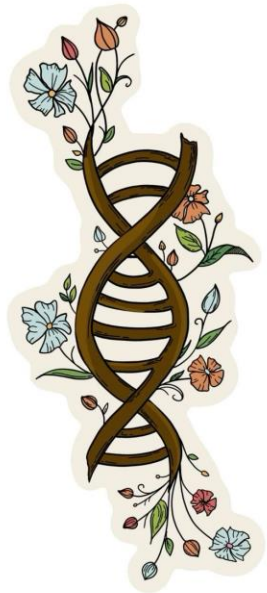


Blood culture

Purpose

Specimen

Method



Purpose

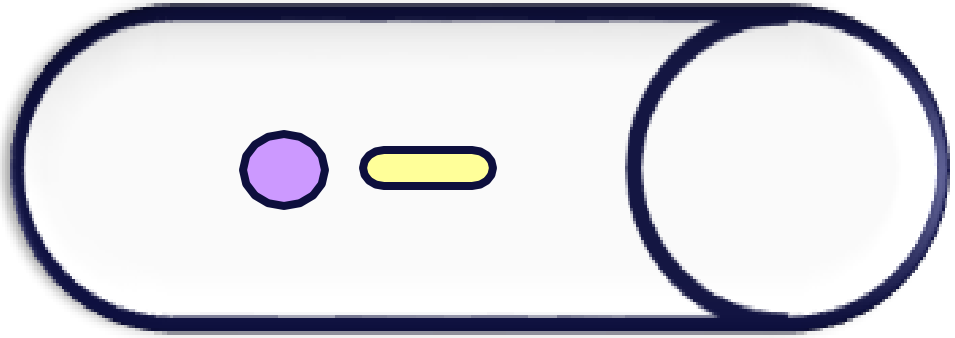
Bacteremic infections can cause diseases like:

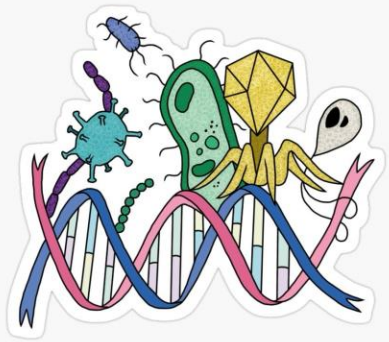
Typhoid fever

Endocarditis Puerperal sepsis

Brucellosis

To diagnose a bacteremic infection, we need to determine whether there is a pathogen present in the blood – in other words, whether the patient has bacteremia or not.





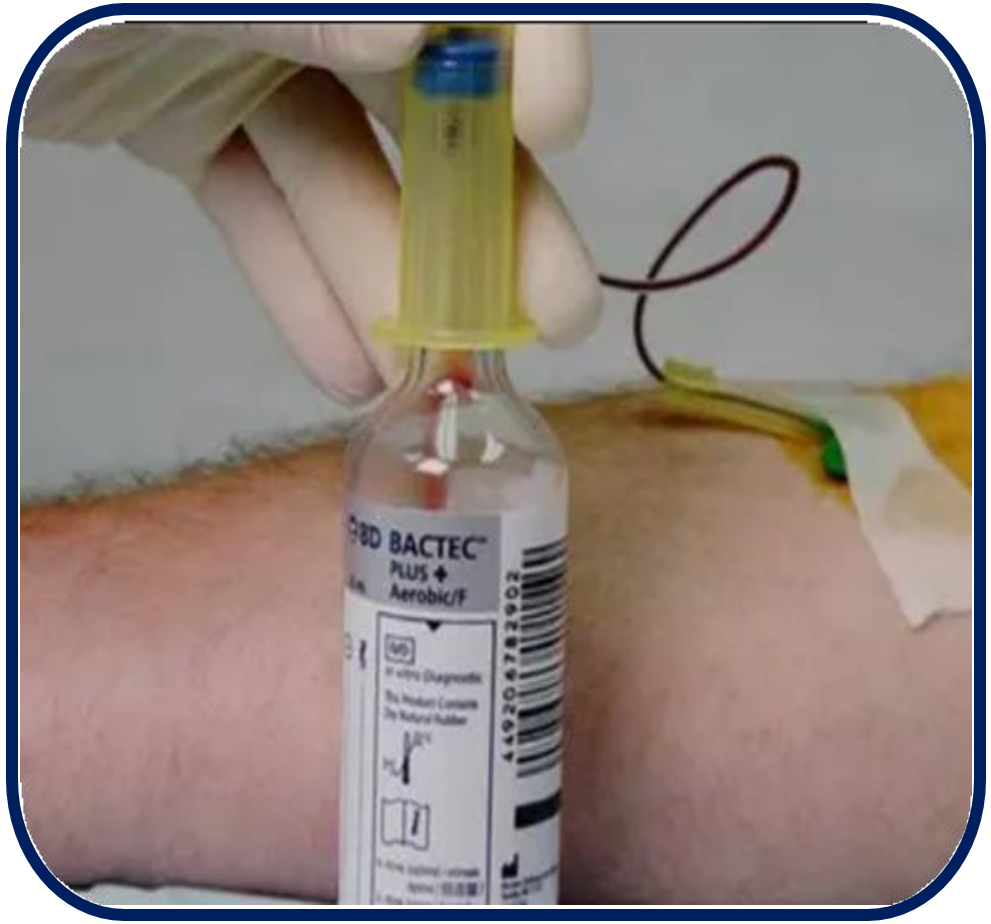
Specimen

Bactec tube have nutrient broth

1

3ml blood to 30 ml broth

For child



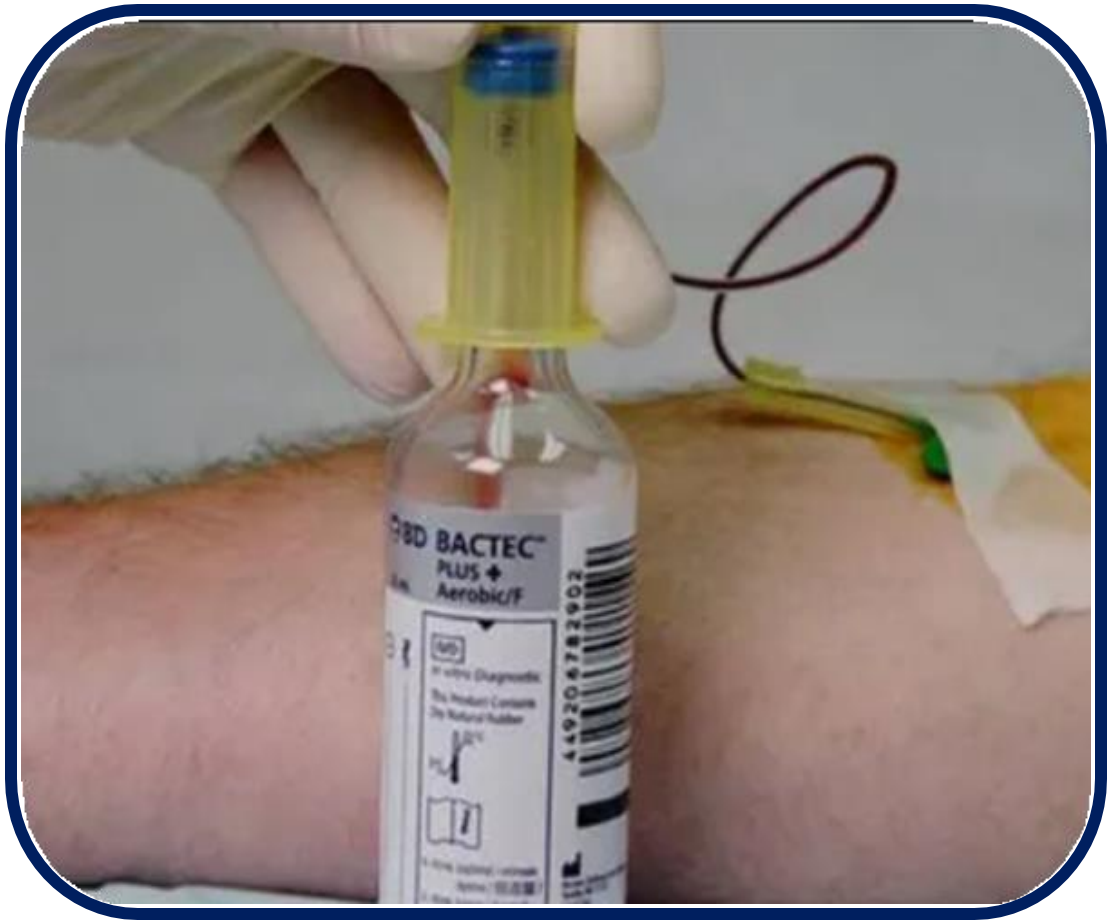
Specimen

1

10 ml blood to

30 ml broth for Adult

(aerobic)



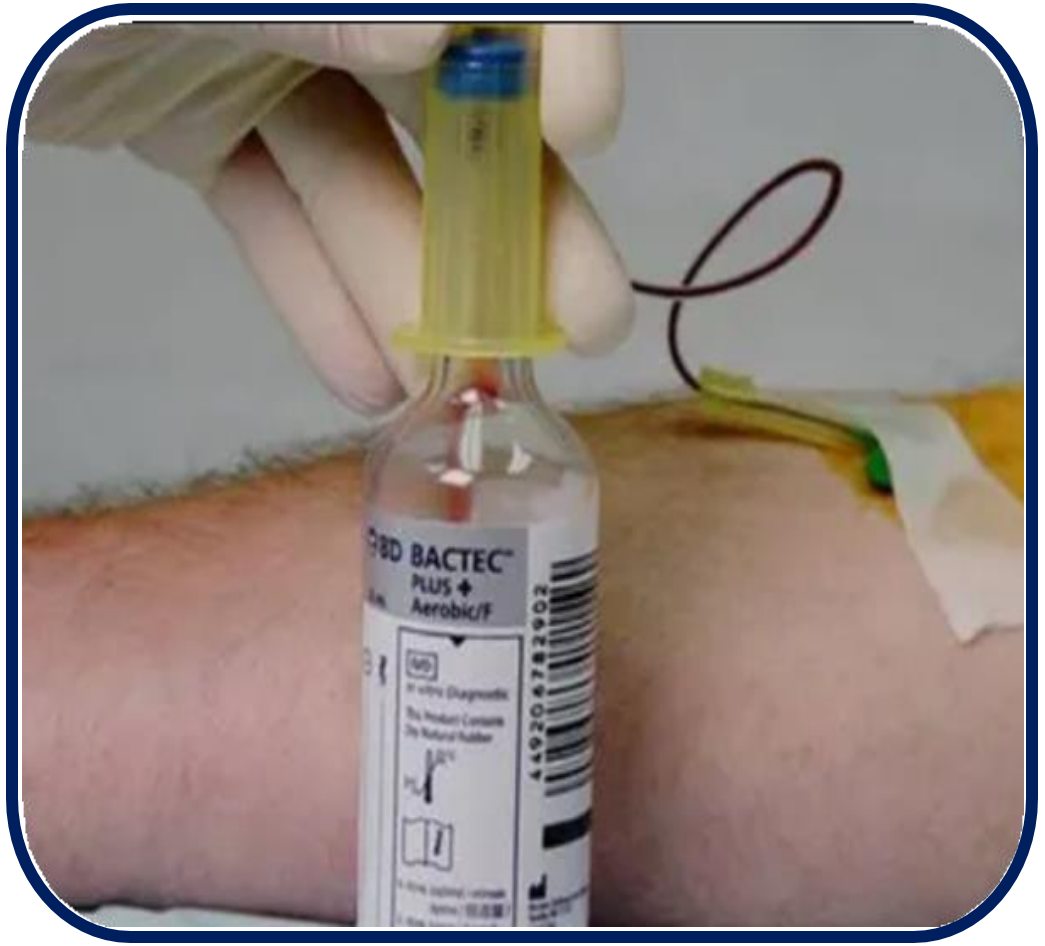
Specimen

1

10 ml blood to

40 ml broth for Adult

(anerobic)



Specimen

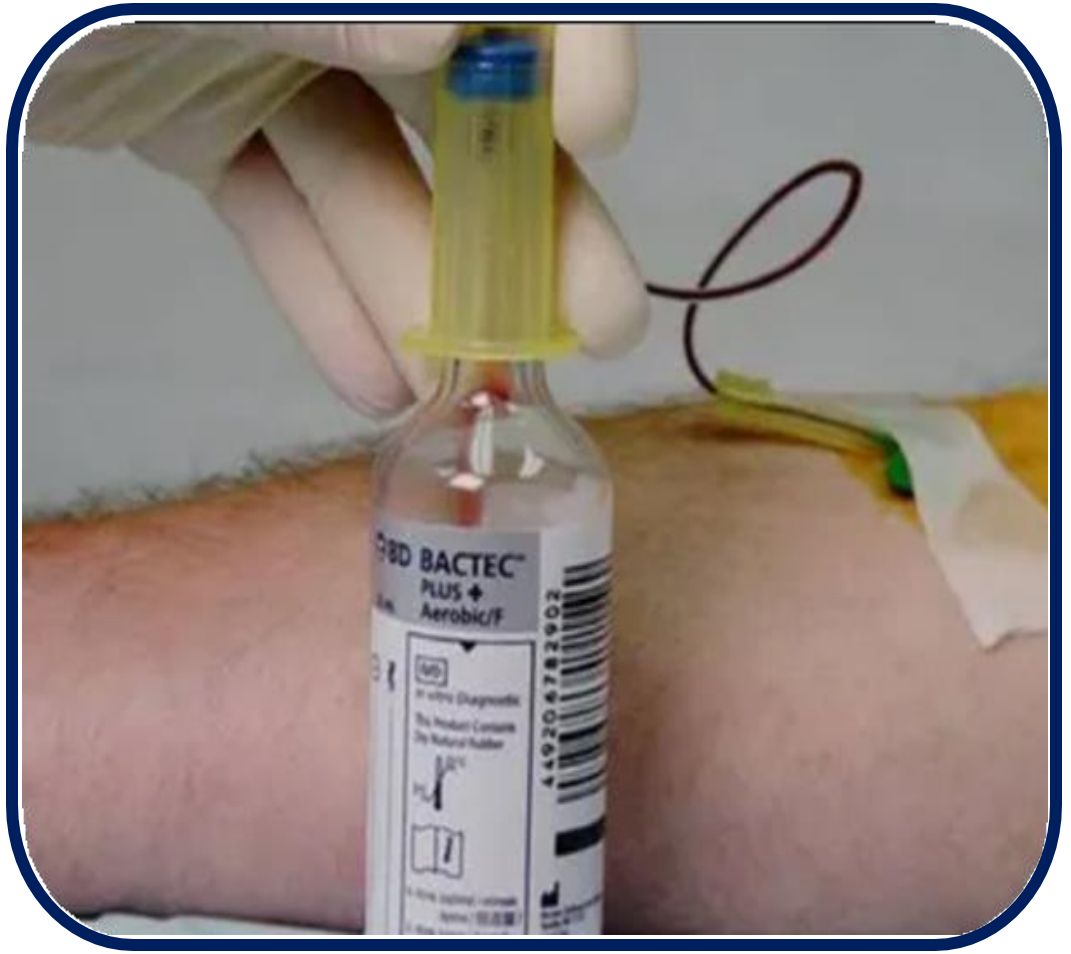
10 ml blood & 30 ml broth

The purpose of this broth :

Dilutes antibacterial

Provides good nutrient

(organism present in small number)



- We perform a culture for three main reasons:First, for detection – if bacteria grow, it indicates a bacterial infection; if not, then there is likely no bacterial infection.Second, for identification (ID) – to determine the causative agent responsible for the disease.And third, for antimicrobial susceptibility testing (AST) – to know which antibiotic is most effective against the isolated organism

Method

Incubation 5 to

21 days

In cases of brucellosis, the culture may require up to 21 days, as Brucella bacteria are slow-growing and may not be detected in the early days of incubation.

Method

Organism present In bactec bottle

⇩

Consume nutrients

⇩

CO2 released

⇩

CO2 reacts with sensor

⇩

Light appears

في bottle تشير الى ان بهاد ال microorganisms

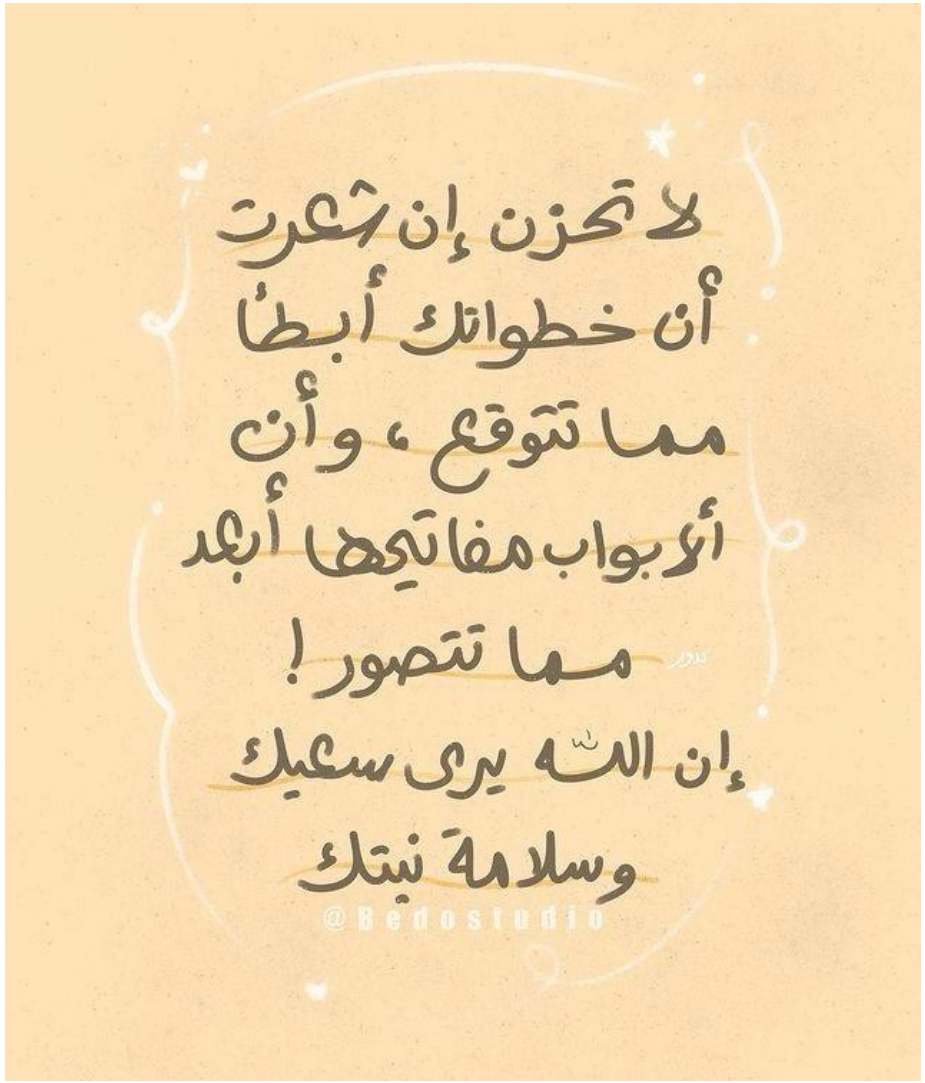
Method

Sub culture & incubate at 37°C for 24h .

Identification

Susceptibility test

رسالة من الفريق العلمي:



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			