MICROBIOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 5

Bacterial taxonomy, Classification, and laboratory diagnosis (Pt.2)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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A lecture
is 99+ slides
long…

…but it’s
Dr. Alaa’s
slides.

اللهم لا سهل إلا ما جعلته سهلا وانت تجعل الحزن إذا شئت سهلا

بسم الله نبدأ :)

Lecture 5 - Part-2

Bacterial Taxonomy, Classification, and Laboratory Diagnoses

Vitek system

Urine culture technique

Blood culture

Vitek system

More advanced than API

Vitek system

An automated

Identification

Can identify almost every microorganism.

Antibiogram

Determines the susceptibility of bacteria to various antibodies, which is helpful in determining which treatment is effective for bacterial infections.

Antifungals

Identifying and determining the susceptibility of fungal pathogens to various antifungal agents, to also decide the best approach to effective treatment.

Vitek system

Vitek system

** Two cards

To know the type of the bacteria or fungi (causative agents)

1) Identification card (ID card):

47 biochemical tests

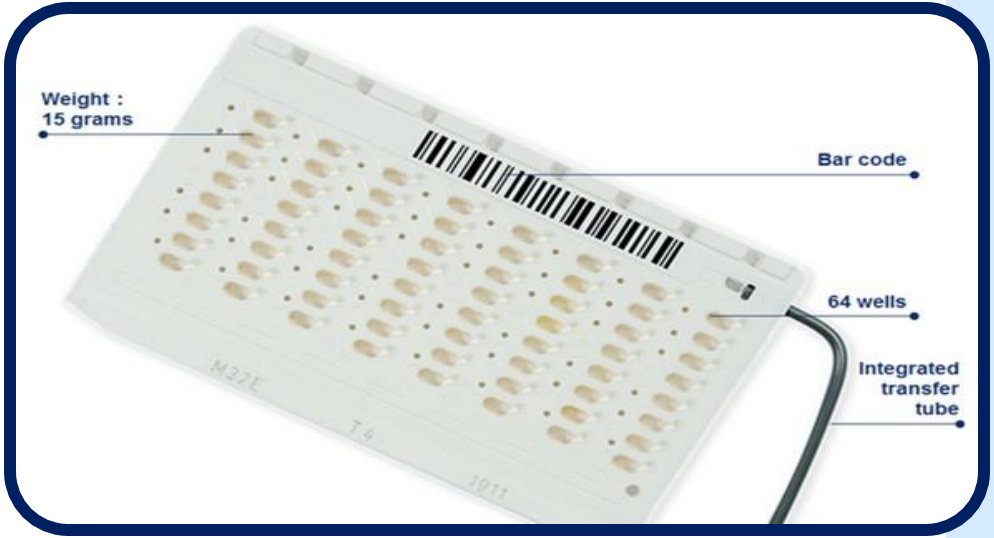
In a routine lab, we can barely get 10 biochemical tests

Specific Card for GN

Gram + & -

Specific Card for GP

Specific Card for Yeast



Vitek system

2) Antimicrobial susceptibility test card

(AST card)

22 antibiotics

Gives us all the antibiotics that we can use effectively for the patient

MIC **M**inimum **I**nhibitory **C**oncentration of antibiotic

Some patients suffer from certain infection, but also have kidney problems, for example. In this case, we don't want to give them a strong antibiotic . So, we look for the MIC of this antibody, which will help the patient while inflicting the least amount of damage. It allows for personalized treatment for the patient by choosing the most effective drug concentration that is compatible with the patient's kidney function and overall health.

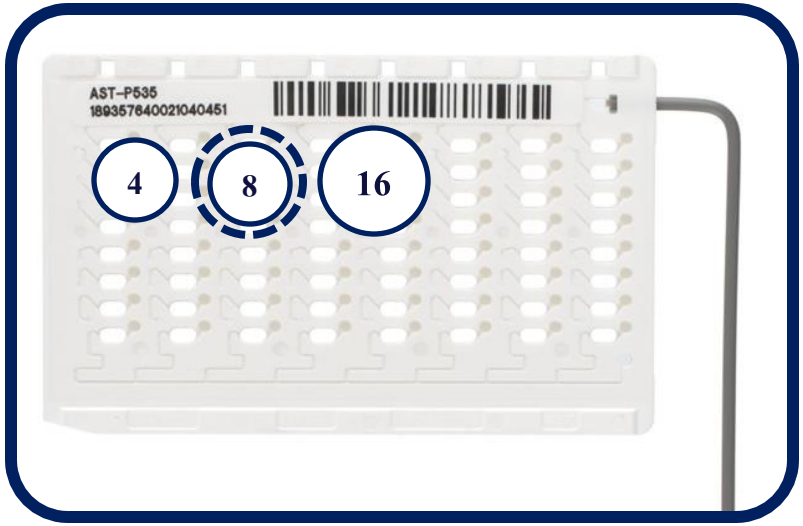
Continued... (: الله بعين

Vitek system

2) Antimicrobial susceptibility test card (AST card)

There are 64 wells present. Every well has a different antibiotic concentration. The first well has an antibiotic concentration of 4 µg/mL . The second has 8 µg/mL. The third has 16 µg/mL, and so on.

First, the 4 µg/mL is observed. The bacteria is still alive and active at this antibiotic concentration.



Then, we observe the 8 µg/mL well. It's apparent that this antibiotic concentration was 80% effective.

However, when we observe the 16 µg/mL well, we can see this antibiotic concentration was 100% effective against this bacteria. Thus, the **MIC** is determined to be **8** µg/mL of this antibiotic.

MIC = the lowest concentration (in µg/mL) of an antibiotic that inhibits the growth of a given strain of bacteria.
(not necessarily 100% inhibition)

Steps of work

1

Organism isolation

(Pure)

These appearing colonies must be pure and uncontaminated, because we are trying to determine the causative agent responsible for this disease.

Then, I take these colonies and produce **two bacterial suspensions.**

Steps of work

2

Bacterial suspension

(2 tubes)

This suspension forms turbidity, which refers to the cloudiness caused by the bacteria's presence. More turbidity = more bacterial growth. This turbidity is a result of the inoculation, or the addition of the colonies to a growth medium (growth broth), which promotes the bacteria's growth. This turbidity should follow a certain standard. Otherwise, if the bacterial growth is exceptionally high, for example, the antibiotics are not going to present their effectiveness fully, which may lead us to think that this antibody is ineffective against this bacteria, when it actually is → false negative. On the other hand, if the bacterial growth was very low, the antibody will present exaggerated effectiveness against this bacteria, when its effectiveness is less → false positive.

These tubes are colonies + growth broth. The first suspension is used for identifying the bacteria. The second suspension is used for the AST.

ID AST

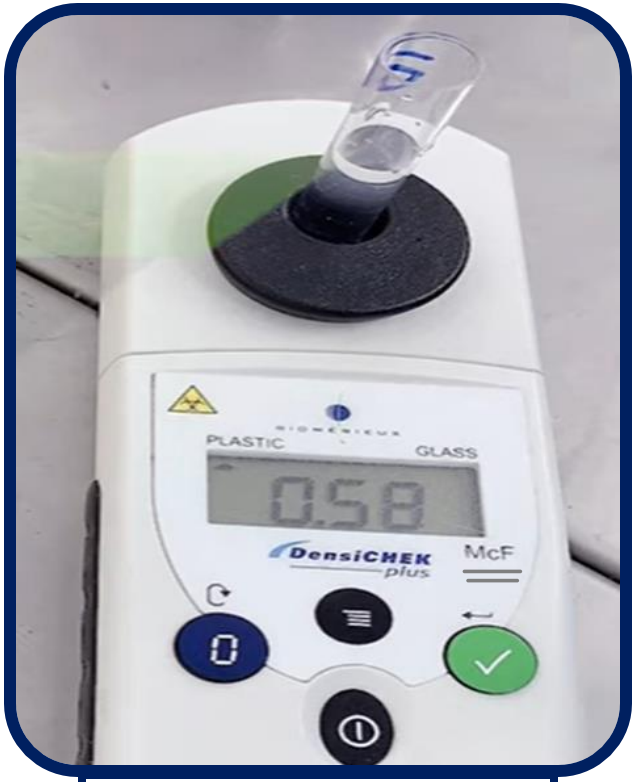
Steps of work

3

The turbidity standard (McFarland Standards)

Measure turbidity

(0.5 -0.63)



Turbidimeter

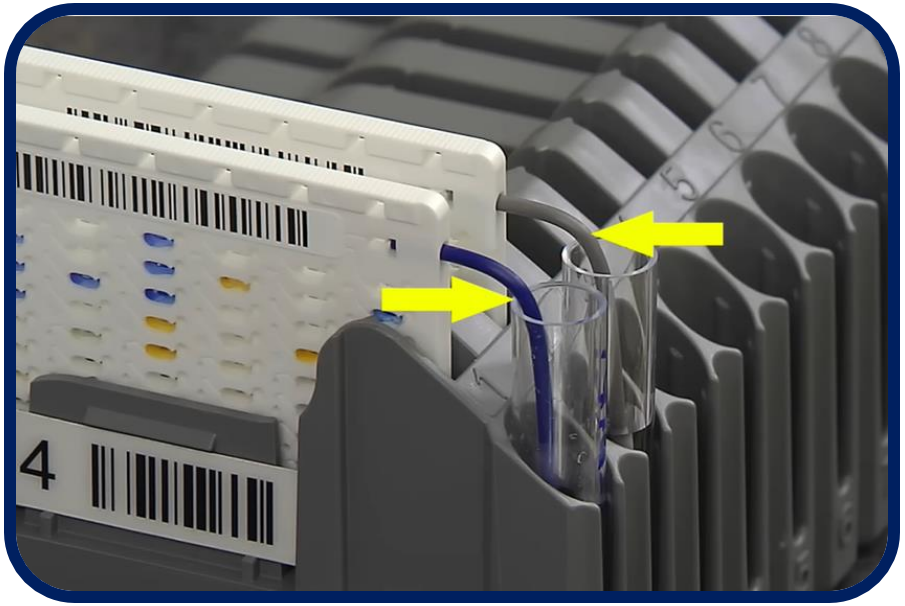
Steps of work

4

ID & AST

Insert cards in bacterial Suspension tubes

This is called a rack or a cassette



We connect each card to one suspension tube

Steps of work

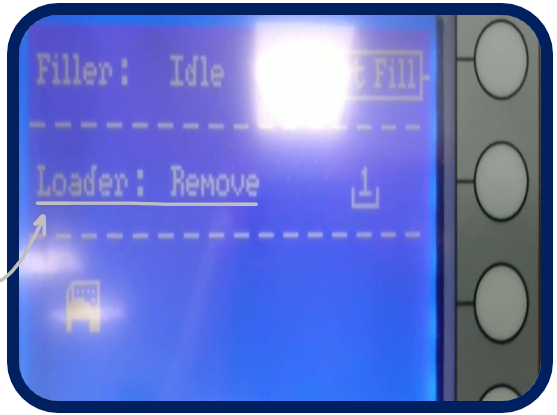
5

Into the filling room

Transfers the bacterial suspension into the wells

Two patients can have the same pathogen (IDs), and still have different AST results because ASTs are personalized and differ from one patient to another (different resistance) .

Each one of these small dashes represent a well



This message lets us know that it has completed the transfer.

Steps of work

6

Transfer the cassette into the loading room

(Diagnostic) 5-10hrs

Compared to API's 24 hours

Steps of work

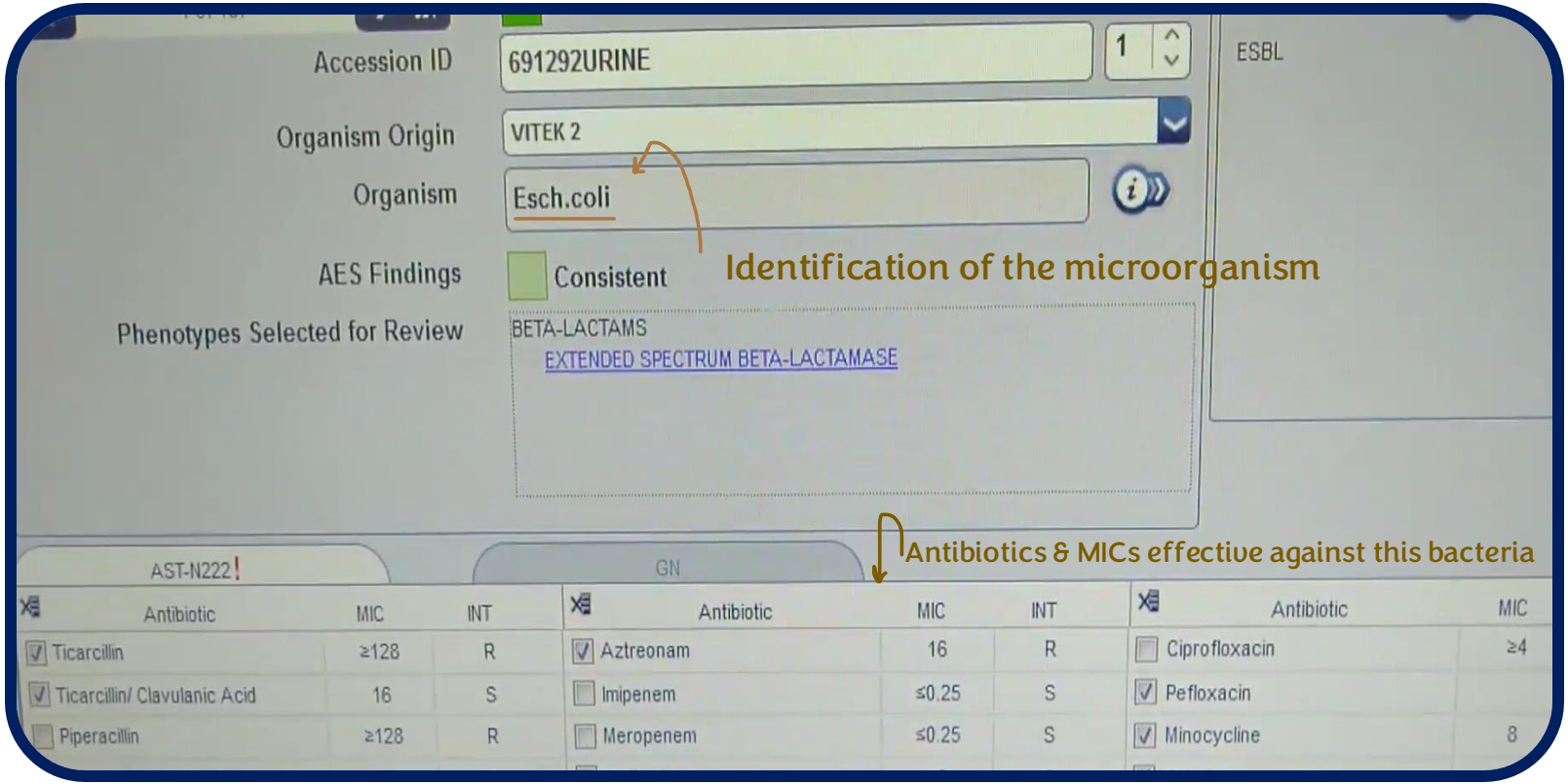
7

Colorimetric

(Barcode)

This barcode reads biochemical tests, color changes, metabolism, etc. and then compares this data to its stored database of almost every microorganisms. If it detects at least a 90% similarity between the scanned and stored data, it identifies the microorganism and the most effective antibiotic treatment.

Steps of work



Urine culture technique

Purpose

Specimen

Method

Interpretation

Purpose

To diagnose

Urinary tract infection

(UTI)

UTI signifies the presence of bacteriuria

(Bacteriuria)

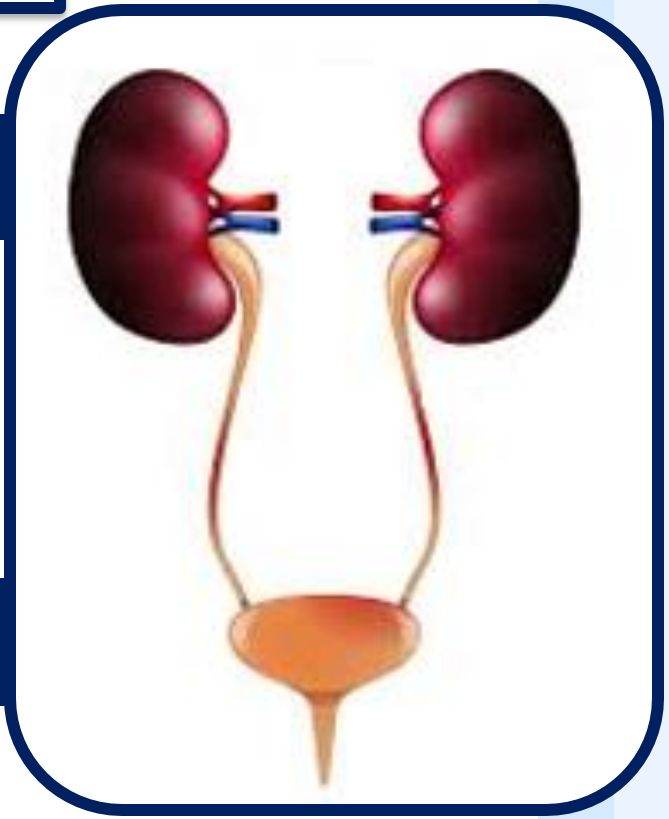
The urinary tract (more specifically, the bladder and organs above) in healthy individuals is sterile and should contain no microorganisms. Microorganisms present in the urinary tract are called bacteriuria.

Pyelonephritis

Bacteriuria found in the kidneys

Cystitis

Bacteriuria found in the bladder



Purpose

UTI

Bacteriuria

Symptoms of bacteriuria

Dysuria

Burning sensation present during urination

Frequency

Lots of bathroom trips

Purpose

UTI

Bacteriuria

Significant

A significant amount of bacteriuria must be present for UTI conformation

Significant amount:

$\geq 10^5$ CFU/ml

(100,000 CFU/ml)

Colony forming unit

(CFU)

When we culture bacteria, we make sure it divides 20-30 times to produce a lot of colonies. If it produces ~100,000 colonies, we can label it as significant bacteriuria.

Bacterial count

Purpose

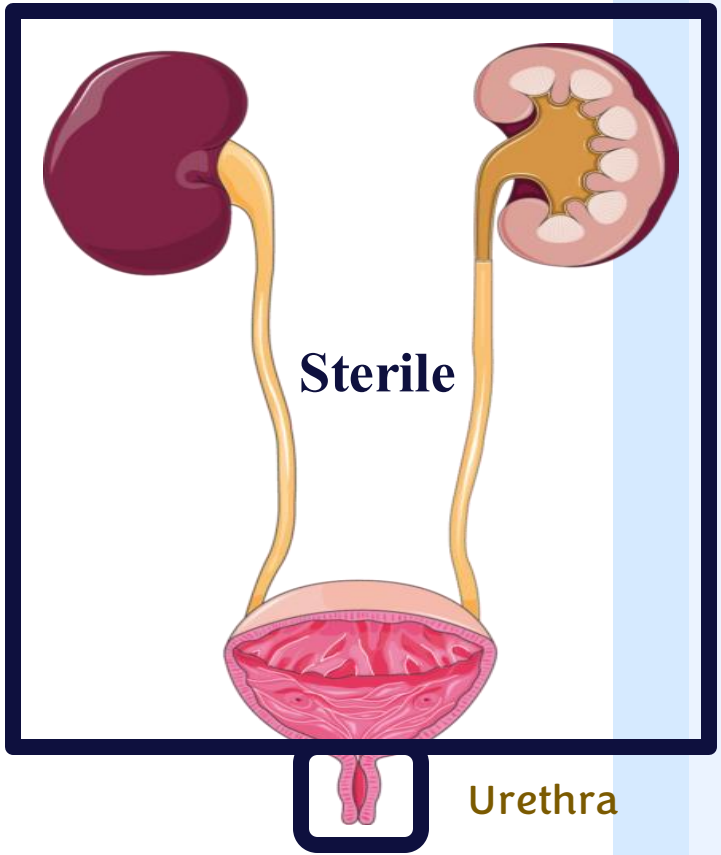
Bacterial count

Bacteriuria

$\leq 10^5$ CFU/ml

Significant

The urethra is the site of contamination, serving as entry point for bacteria. The urinary tract contains normal flora, but if harmful microorganisms interact with it, it can lead to the formation of bacteriuria. If the bacteria travel up to the bladder it causes cystitis, and if it reaches the kidneys, it can lead to pyelonephritis.



Purpose

Pyuria

Finding cells in urine

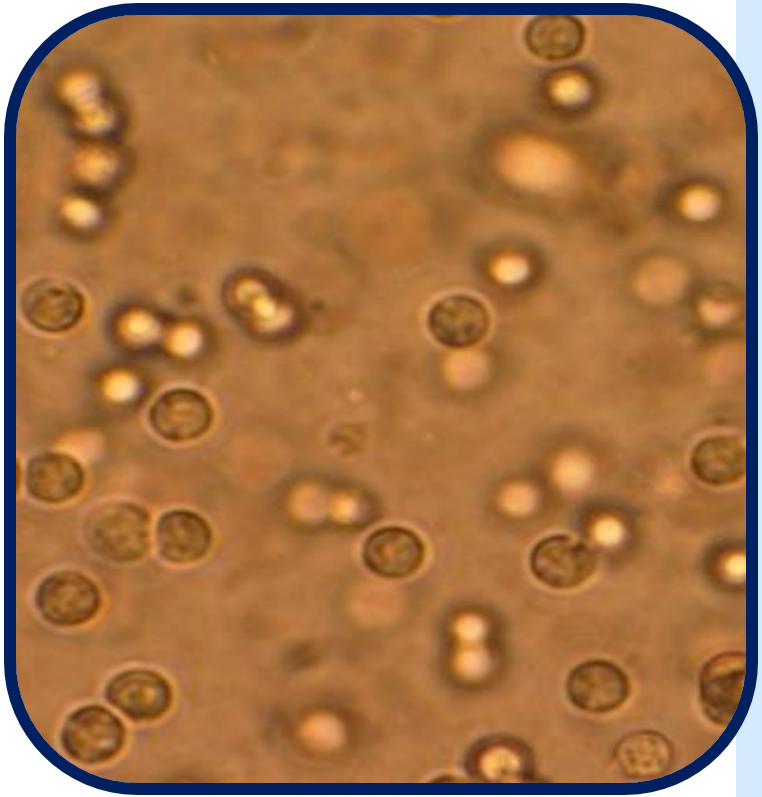
(Pus in urine > 10 cells/HPF).

High power field

Pus signifies an inflammatory response to infection. It consists of WBCs, dead tissues, and bacteria. This pus is thick and discolored (white, yellow, pink, or green). The counted cells in pyuria are WBCs (leukocytes)

Significant Bacteriuria

Bacteriuria and pyuria are often found together.



Specimen

Taken from patients

1

Mid stream urine

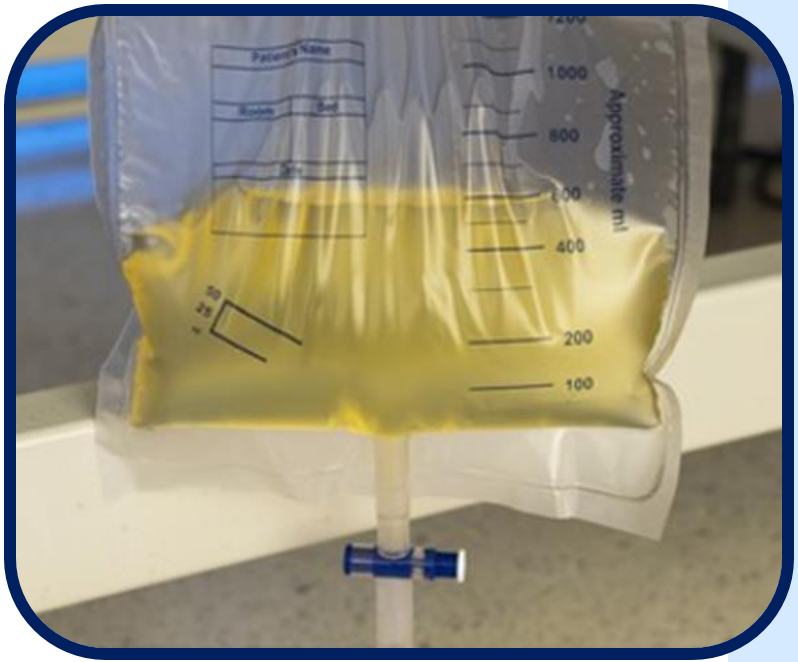
Specimen

2

Catheterization

Urine sample collected by a catheter

A **catheter** is a thin, flexible tube inserted into the body to allow the passage of fluids. (قسطرة)



Specimen

3

Suprapubic aspiration

The specimen is taken **directly** from the **bladder**, especially for **infants** since they cannot control themselves to provide the required sample.

How to collect Mid stream urine

" Instructions "

If they take antibiotics =

Stop antibiotics (for 3 days)

Antibiotics kill the bacteria = no culture obtained

How to collect Mid stream urine

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) → because it's concentrated

5 **Mid stream urine**

Passing the first and last amounts of urine
Only the mid urine is needed

6 **Pass the reimaging urine into the toilet.**

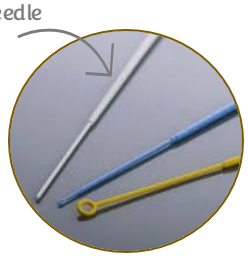
→ **Mid stream urine**

Ready to be taken to the lab as soon as possible

Method

Culturing Process:

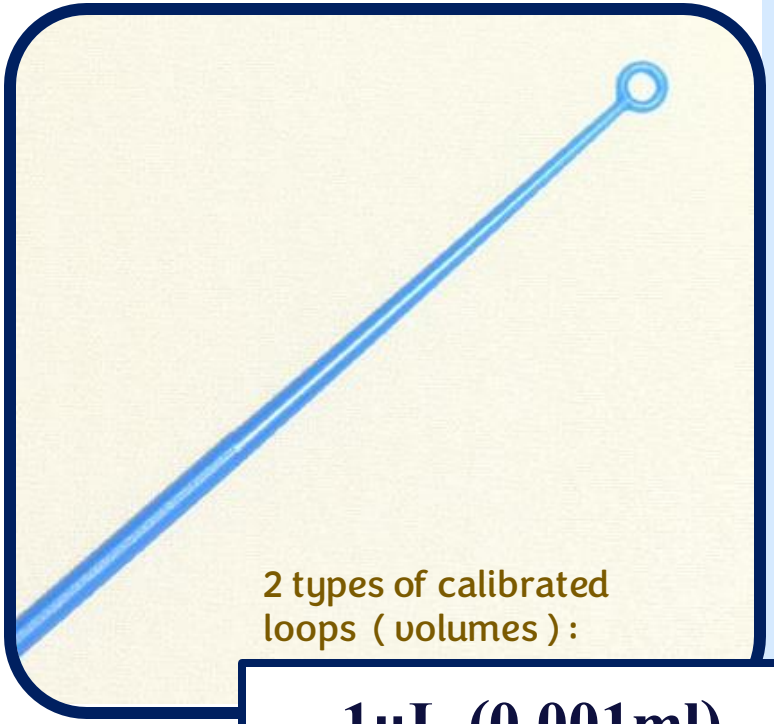
Usually we use an inoculating needle (pointed)
But in **urine** culture = **calibrated loop**



1

Mix urine (uncentrifuged) & by Calibrated loop

It can determine the **volume** taken from the sample to the culture plates ; which is necessary for **bacterial counting**



2 types of calibrated loops (volumes) :

Recommend

- **1µL (0.001ml)**
- **10µL (0.01ml)**

Method

2

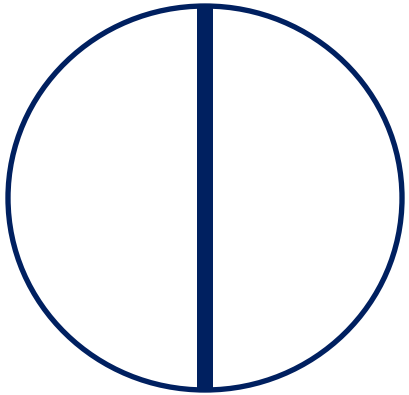
Inoculation on

By streaking & incubate at 37°C

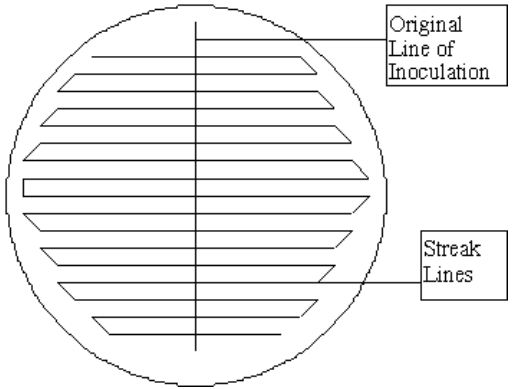
For 24hrs.

Urine culture is different from other cultures due to bacterial counting :

1.Draw a central Line
(original line)

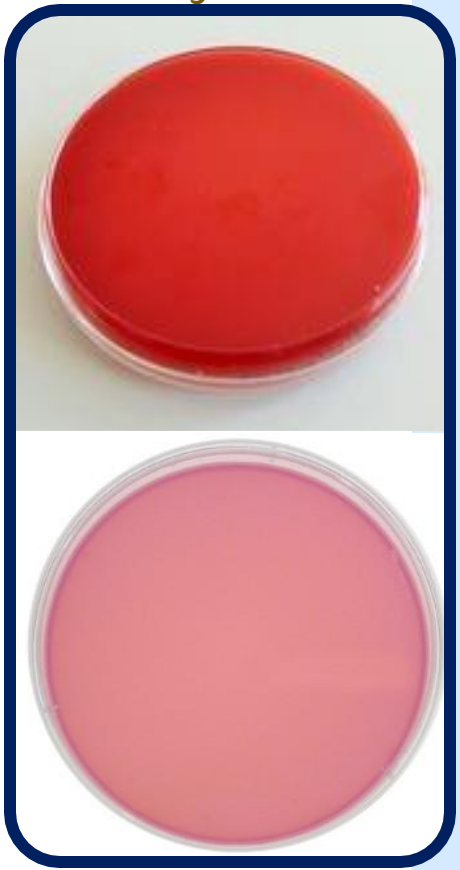


2. Zigzag
(streaking line)



Two types of media :

1.Blood agar



2.MacConkey Agar

Method

After culturing on the two Petri dishes

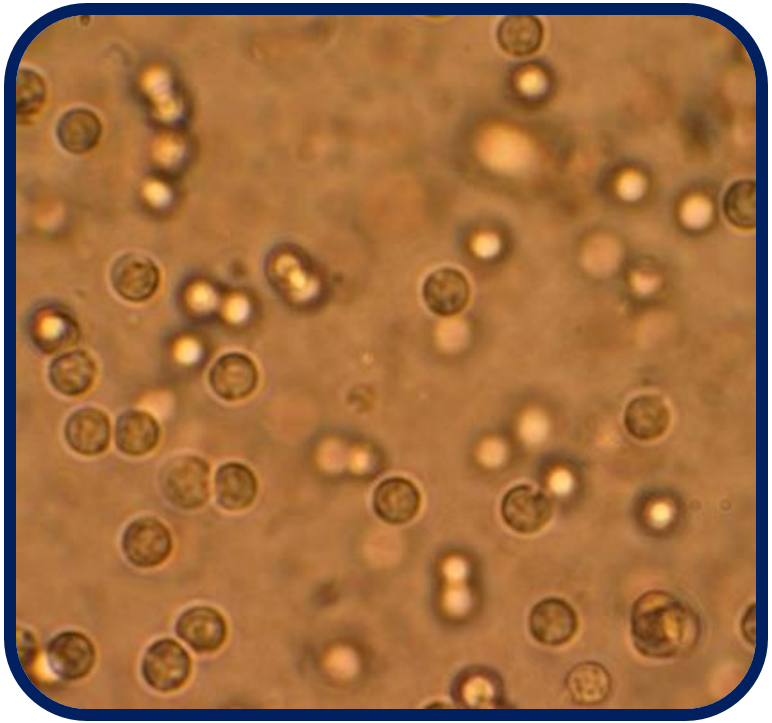
3

Examine

centrifuged urine

(≥ 10 cells/HPF)

Pyuria



centrifugation -> Examine under the microscope -> counting cells -> more than 10 cells = pyuria

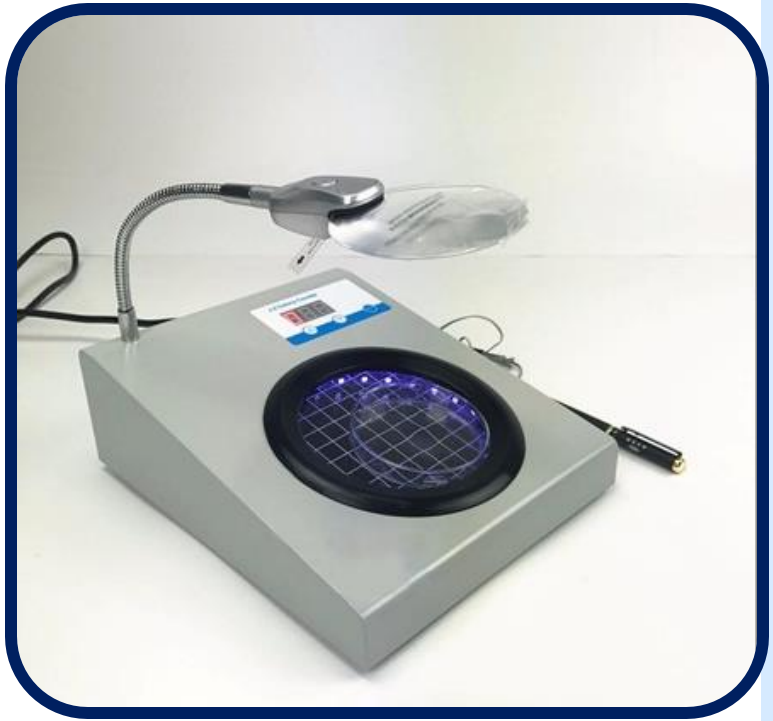
(of the same sample)

Method

Next day...

4

Count the growth colonies



Method

Calibrated loop = 10µL (0.01ml)

↓

No. of colonies X 100 = 10^5 CFU/ml

Calibrated loop = 1µL (0.001ml)

↓

No. of colonies X 1000 = 10^5 CFU/ml

4

Multiply the count by dilution factor

Obviously UTI due to the presence of many colonies

Method

0.01ml (10µL)	No. of coloniesX100= 10^5 CFU/ml

No. of colonies= 10

10X100= 1000 CFU/ml

10^3

Not significant
Less 10^5

Further explanation

$$\frac{\text{CFU}}{\text{mL}}$$

Volume

colony-forming units = no. Of colonies

Example:

No. Of colonies \ 0.01 = CFU x 100

Dilution factor

10 x 100 = 10^3

Urine culture: Interpretation

Analysis of results

$\geq 10^5$ CFU/ml

Significant bacteriuria

Identification

+ Antimicrobial susceptibility test (AST)

UTI

Interpretation

EXCEPTIONs :

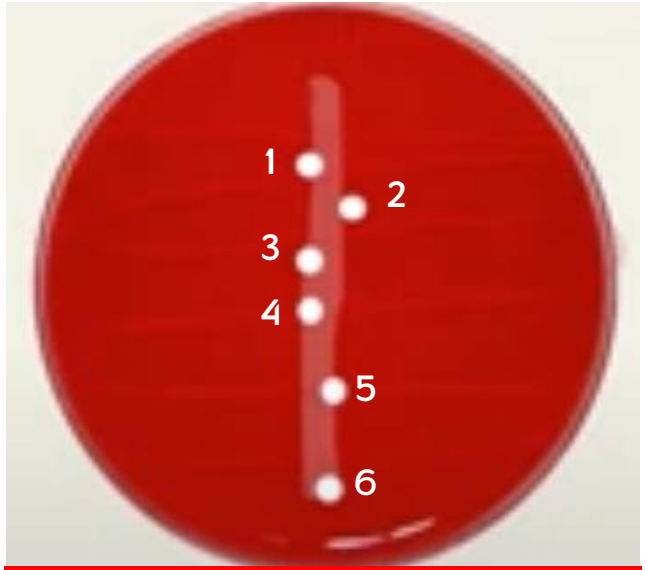
10^4 or 10^3 CFU/ml + Staph. Aureus = significant

$\geq 10^4$ CFU/ml

6 X 1000= 60000 CFU/ml =10^4

Significant bacteriuria

Identification



S. aureus

Check extra info slide 45

Interpretation

$\geq 10^3$ CFU/ml

6X1000= 6000 CFU/ml

Significant bacteriuria

Identification

10^3 CFU/ml + Staph. Aureus = significant

S. aureus

Interpretation

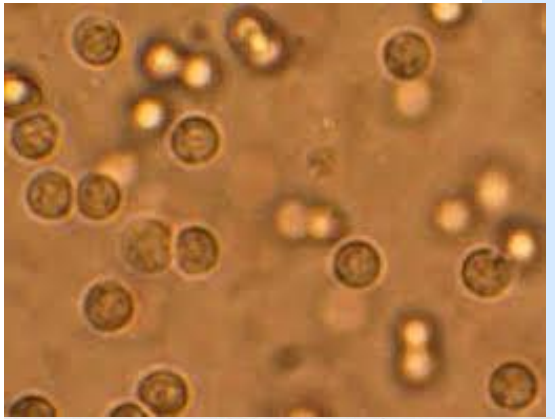
Sterile pyuria

Pus without any bacterial growth in ordinary media

No microorganisms present

Why ?

Next slides ...



Sterile pyuria

1

Taking antibiotics

= Microorganism’s will be eliminated

Sterile pyuria

2

Renal tuberculosis

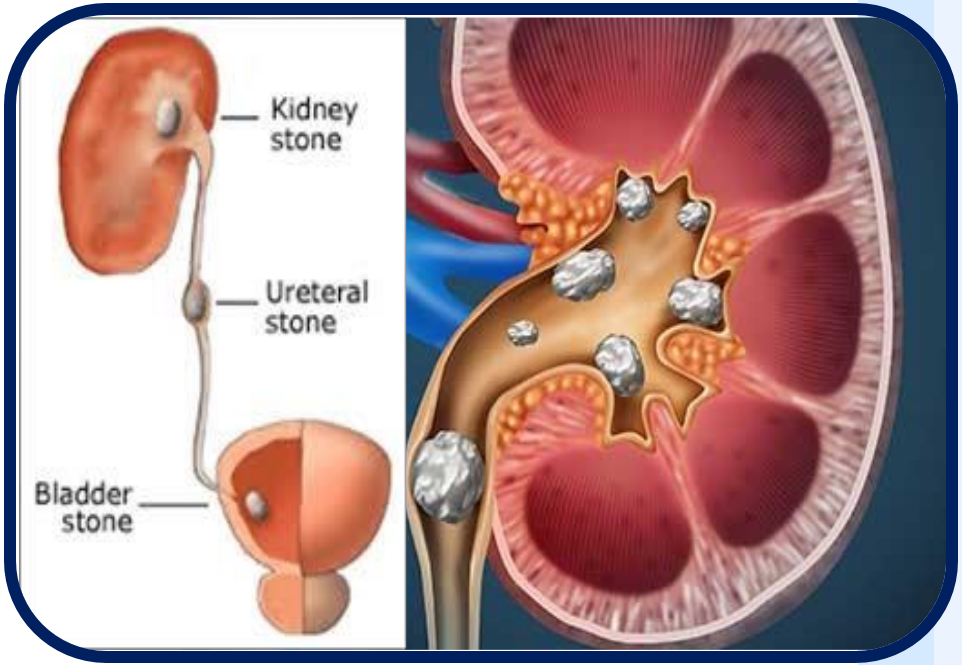
Responsible of the Pus (cells)

Sterile pyuria

3

Renal stones

Responsible of the Pus (cells)
= there's no bacterial infection



Sterile pyuria

4

Organism not grow on ordinary media

Petri dish \ nutrients media

Needs different, specific media :

- **Mycoplasma**
- **L-form bacteria**
- **Anaerobic infection**

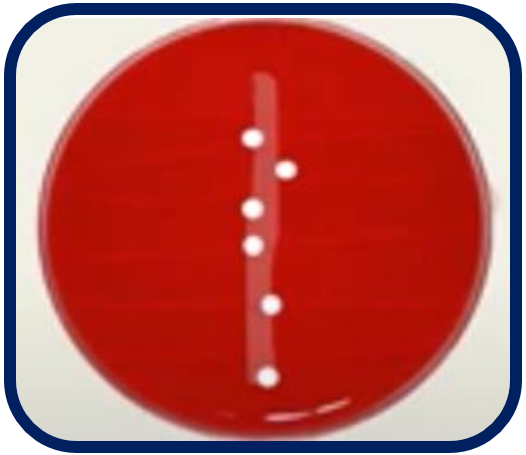
Extra info :
staph aureus (golden in Latin) can be Primarily identified **macroscopically** from its golden color \ cocci shape \ G+

Interpretation

10^3 = not significant bacteriuria

(No UTI)

Although there is pyuria



Why ?

1) **Prostatitis** — **Male** with Inflammation of the prostate gland
2) **Vaginitis** — Female → Inflammation of the vagina
3) **Cervicitis** — Female → Inflammation of the cervix
4) **Malignancy**
5) **Renal calculi** — renal stones

Interpretation

Suprapubic aspiration

Even in the presence of a single colony

10^3

Any growth is significant bacteriuria

Because it's directly taken from the bladder = there's no contamination

Interpretation

Suprapubic aspiration

1X1000= 1000 CFU/ml

10^3

Any growth is

significant bacteriuria

Interpretation

Same thing here :

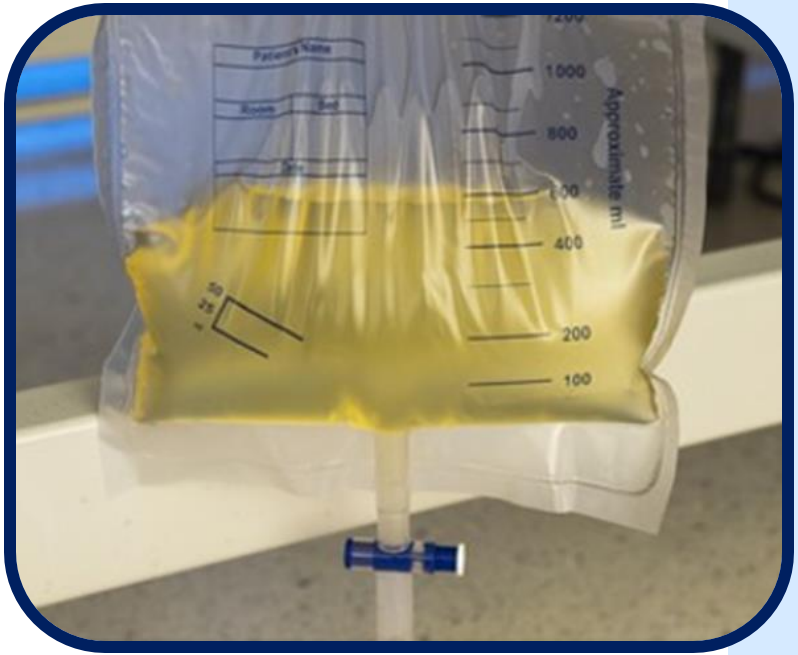
Catheterization

Even in the presence of a single colony

10^3

Any growth is

significant bacteriuria



Interpretation

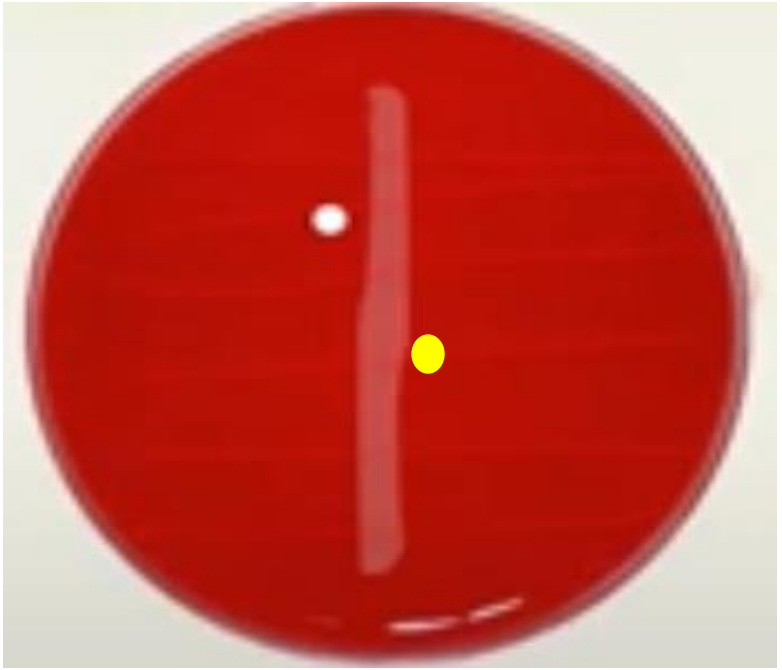
Catheterization

1X1000= 1000 CFU/ml

10^3

Any growth is

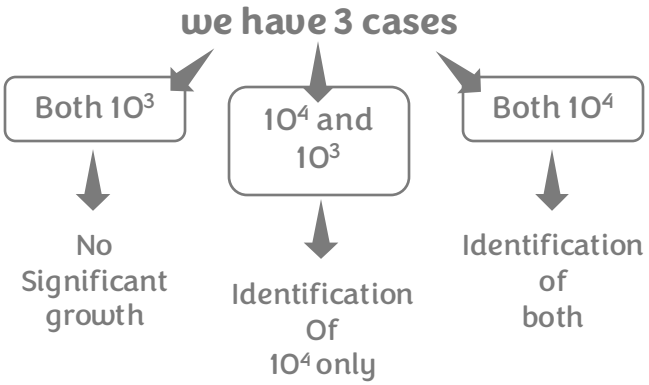
significant bacteriuria



Urine culture

Interpretation

Two pathogen



Interpretation

Case no. 1:

Count 1 $\leq 10^3$ CFU/ml

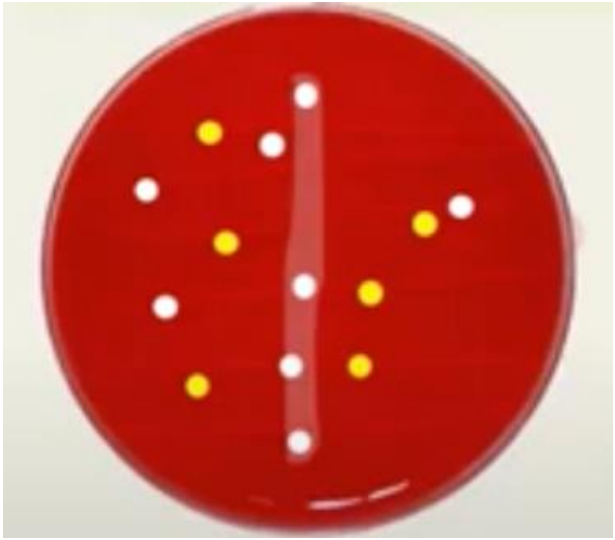
8X1000= 8000 CFU/ml = 10^3

Count 2 $\leq 10^3$ CFU/ml

6X1000= 6000 CFU/ml = 10^3

Both 10^3

No significant growth



Interpretation

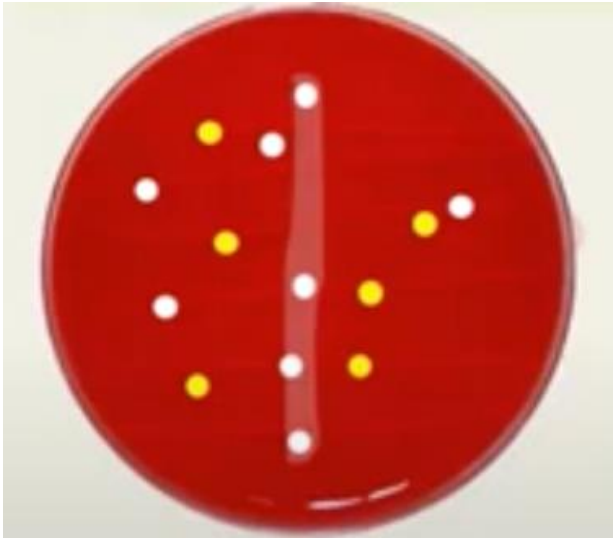
Case no.2 :

Count 1 $\leq 10^4$ CFU/ml

13X1000= 13000 CFU/ml = 10^4

6X1000= 6000 CFU/ml = 10^3

Continue with higher & ignore the other



Interpretation

Case no. 3:

Count 1 $\leq 10^4$ CFU/ml

16X1000= 16000 CFU/ml = 10^4

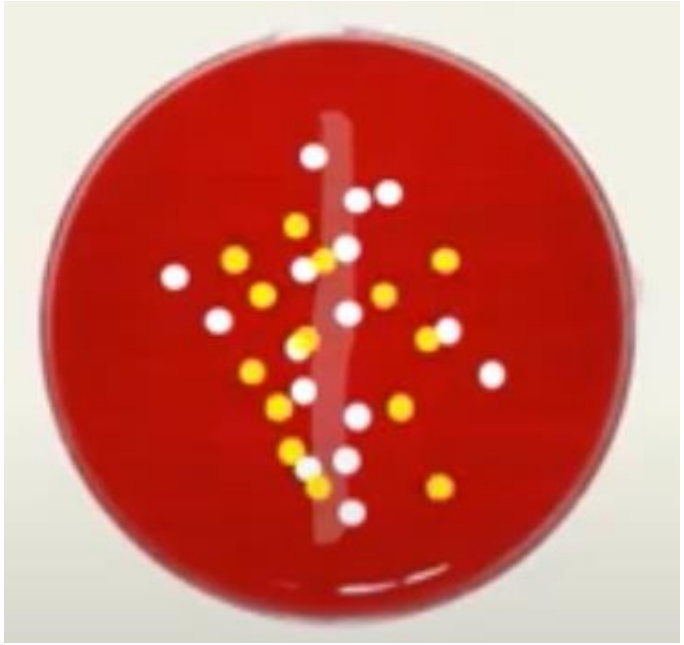
Count 2 $\leq 10^4$ CFU/ml

14X1000= 14000 CFU/ml = 10^4

Both 10^4

Identification for both

AST for both



Blood culture

Purpose

Specimen

Method

Purpose

Why ?

To check if there's

Bacteremic infections → bacteremia = a pathogen or microorganism in blood

Causing different diseases :

Typhoid fever

Endocarditis

Puerperal sepsis

Brucellosis

Specimen

1

3ml blood to

30 ml broth

For child

3ml of blood taken from a child

BACTEC system

BACTEC tube contains nutrient broth

Specimen

1

10 ml blood to 30 ml broth for Adult (aerobic)

10ml of blood taken from an adult

Specimen

1

10 ml blood to 40 ml broth for Adult (anerobic)

Blood draw procedure occurs under septic conditions

Specimen

Purposes of the broth :

10 ml blood & 30 ml broth

1.Dilutes antibacterial antibodies

2.Provides good nutrient (organism present in small number)

to increase their number

Always remember! **Purposes of culture**

1.Detection

Bacteria present = bacterial infection

No bacteria = no bacterial infection

2. Identification

Identify the causative agent ؛that's why we grow them

3. AST

Method

Incubation

5 to 21 days

Specially in brucellosis

Very long period

In this case we should prioritize the patient's life and give an antibiotic even if there was no bacteria (AB is abused)
As the benefits outweigh the risks

Method

Organism present

⇩

Consume nutrients

⇩

CO_2 released

⇩

CO_2 reacts with sensor

In the bottle

⇩

Light appears

Method

Then we should

Subculture & incubate at

$37^{\circ}C$ for 24h .

⇩

Identification

⇩

Susceptibility test

For any feedback, scan the code or click on

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	1. Slide #10 2. Slide #37-38-39 3. Slide #51	1. the MIC is determined to be 16 µg/mL of this antibiotic. -extra info added (grey) 2. less than or equal (≤) 3. Greater than or equal ($\geq 10^3$)	1. the MIC is determined to be 8 µg/mL of this antibiotic. MIC = the lowest concentration (in µg/mL) of an antibiotic that inhibits the growth of a given strain of bacteria. (not necessarily 100% inhibition) 2. Greater than or equal (≥) 3.less than or equal ($\leq 10^3$)
V1 → V2	Slide #22	$\leq 10^5$ CFU/ml	$\leq 10^5$ CFU/ml

Additional Resources:

رسالة من الفريق العلمي:

Extra References for the Reader to Use:

1. Vitek System Demonstration :)
2. Lab 🧪 Urine Culture Method (1:33 min)
3. 4 streaking for isolation-quant urine culture

اللهم اكسر بنا شوكتهم ، اللهم نكّس بنا رايتهم ، اللهم أذّل بنا قادتهم ، اللهم حطّم بنا هيبتهم ، اللهم أزل بنا دولتهم ، اللهم أنفذ بنا قدرك فيهم ، بالزوال والتدمير والتتبير يا رب العالمين

اللهم استخدمنا ولا تستبدلنا، انفعنا وانفع بنا، اصطفينا واصنعنا على عينك واصطنعنا لنفسك، سُدّ بنا ثغور أمتك واكفنا شرّ الرياء وابعد عنا التخاذل والتقاعس، وجازنا بما أنت أهله، ولا تفتنّا ولا تستبدلنا ولا تحرمنا يا الله

Best of Luck :) 🐓🧣