Bacteriology lab

Submitted to:
493 MIC
by:
Sahar Ali Alshowiman
437201143

Introduction

Bacteriology lab include different benches, stool bench, urine bench, blood bench, respiratory bench and general bench.

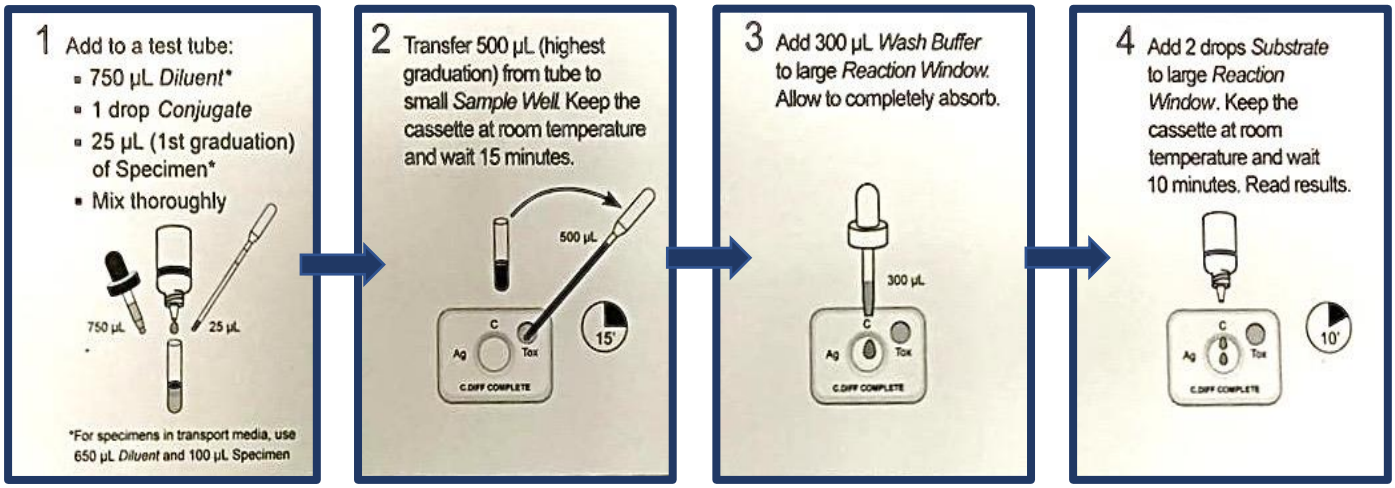
- **Stool bench:**

The bacteria found in stool are representative of the bacteria that are present in the digestive system (gastrointestinal tract).
The species we look for are:
Salmonella –Shigella – Yersinia – Campylobacter – Clostridium.

Once we receive samples there is a toxin test for gram positive bacteria (clostridium test) and culture for gram negative species.

Types of media used:
Xylose lysine deoxycholate agar (XLD) – Selenite broth – Campy agar (for *Campylobacter*) - (CIN) Yersinia agar (for *Yersinia*) - Hektoen enteric agar.

Toxin test (clostridium test)



Results:

culturing

- Use one plate for each two samples.

- Culture the samples on (Xylose lysine deoxycholate XLD - Yersinia agar - Campy agar - Hektoen agar - Selenite broth) use cotton swap.

- Incubate for 24h except Hektoen 48h.

Reading procedure:

- XLD: looking for (*Salmonella* - *Shigella)*, witch appearance: *Salmonella* pink colonies and black center and *Shigella* pink colonies. Do oxidase test to differentiate between: *Pseudomonas* & *Enterobacteriaceae*. If it's negative result will appear colorless need to do urease to differentiate between: *Enterobacteriaceae* & proteus. *Enterobacteriaceae* will be negative and appear yellow.
- Yersinia agar: red center colon, but it's very rare.
- Campy agar: *Campylobacter* appearance colorless shiny, water drop colony.
- Hektoen agar: look for salmonella and Proteus produce black center on plate.
- Selenite broth: depend on turbidity.

Devices required to be used: Microscan + Maldi-Tof.

Blood bench:

Blood normally do not contain any bacteria, so any bacteria found there are significant.

They receive blood samples in bottles. There are two types of bottles:

1- Aerobic (use for adult + paediatric)
2- Anerobic (use only for adult)
*Should Receive two type of bottles (10ml) from adult but paediatric only one (3-5ml).

BACTEC Fluorescent

Microorganisms if present in blood samples consume the nutrients in the bottle and release CO_2 into the medium or use oxygen in the medium. The fluorescent scanner shines a flash to the bottle sensor that measures the amount of CO_2 production or oxygen use. Analysis of the rate and amount of CO_2 produced or O_2 used enable to determine whether the result is positive or negative.

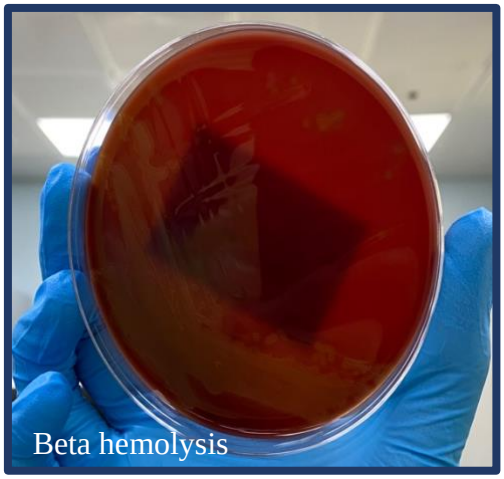
- Load bottles inside the machine
- After 5days if there is no growth it's be negative result (green light).
- If there is growth (red light) will show. Do culture on different types of media:
  2Blood agar (aerobic & anerobic) + 1chocolate agar + 1MAC.
- Also do gram stain and make a report.

Reading procedure:

Blood agar

The type of hemolytic reaction on blood agar has long been used to classify the streptococci.

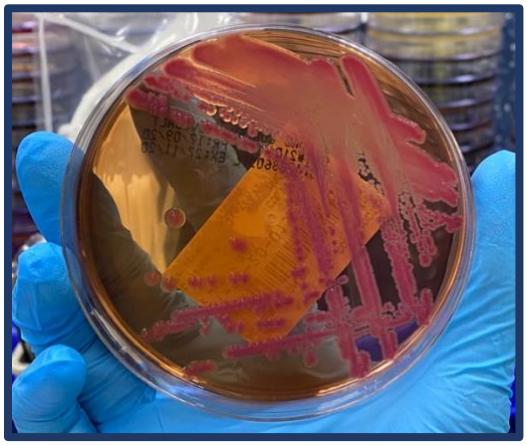
1- Beta hemolysis: complete lysis RBCs leading to formation of clear zone around the colonies ex: *S. pyogenes + S. agalacteae.*
2- alpha-hemolysis: partial hemolysis (Greenish Discoloration) associated with reduction of red cell hemoglobin ex: *S. pneumoniae + S. viridans*
3- gamma-hemolytic: Non hemolytic colonies.

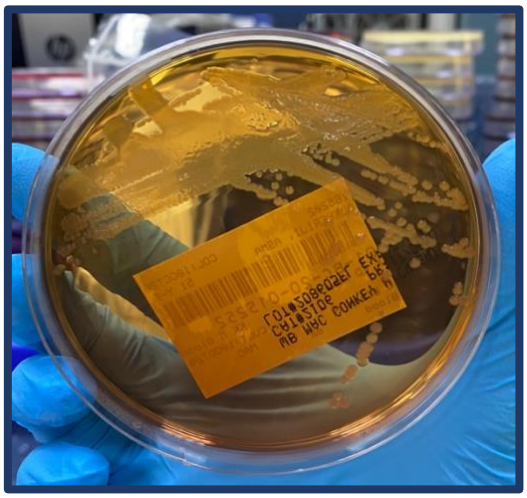


Beta hemolysis

MacConkey's agar

It is a selective medium for gram's -ve bacteria.
Contain bile salt and crystal violet to inhibit the growth of gram's +ve bacteria.
Differential for lactose fermenters and non.





Chocolate agar

Contain X + V factors required for *H.influenza* growth
Most times no growth on it.

* Devices required to be used: Microscan + Maldi-Tof + vitek.

* Chemical tests required:

1- Catalase to distinguish between: strept(-) & staph(+).
2- Coagulase to distinguish between staph species: *S.aureus* (+).

General bench:

General bench receiving a lot and different samples each day.
Types of samples:

1- Swabs: wound + ear + eye + throat + vaginal (high vaginal -HVS- & low vaginal -LVS-)
2- Body fluids: CSF + synovial + PD
3- Tissues.
4- Catheter.
5- Water from different machines.

Culturing

Swabs:

- Wound swabs culture on:
  1- Blood agar.
  2- MAC.
  3- columbia cna agar -anaerobic-.
  4- Gram-negative broth (GN) -anaerobic-.
  5- cooked meat agar (RCM) -anaerobic-.

- Eye & Ear swabs:
  1- Blood agar.
  2- MAC.
  3- Chocolate agar.
  4- SAB.

- Vaginal swabs:
  1- Blood agar.
  2- Thyer martin.
  3- SAB.

- Throat swabs:
  1- Blood agar.

Others:

- Tissue:
  1- Blood agar.
  2- Chocolate agar.
  3- MAC.
  4- RCM.

- Catheter (should be sterile):
  1- Roll it on Blood agar.
- Body fluids:
  First: Macroscopic examination (clear – turbid – clotted).
  Second: if not clotted do cell count (RBC + WBC).
  Do gram stain if there isn't WBC.
  Culture on:
  1- Blood agar.
  2- MAC.
  3- Chocolate agar.
  4- columbia cna agar -anaerobic-
  5- Gram-negative broth (GN) -anaerobic-.

Reading procedure:

- Growth from wound, throat, eye and ear swabs:
  If there is any growth do microscan.
  Chemical tests required:
  1- Catalase & Coagulase.
  2- Oxidase: the reagent from colorless to purple color, *Pseudomonus* (+) which is clinically important in this site.

- Growth from vaginal swabs:
  1- SAB for yeast if growth , do gram stain to make sure it's yeast (yeast may grow on TM sometimes).
  2- Blood agar for strept group B (beta haemolysis).
  3- TM for *N.gonorrhea*.
  4- if mix it's normal flora.

- Growth from CSF: Any growth is significant.

* Devices required to be used: Microscan + Maldi-Tof

- **Urine bench:**

Samples come to urine bench are midstream urine or urine bag and catheter. analysis of urine depending on: chemistry & microscopic analysis.

- Chemistry analysis such as: Ph, Glucose, Protein, Ketones, Nitrate.

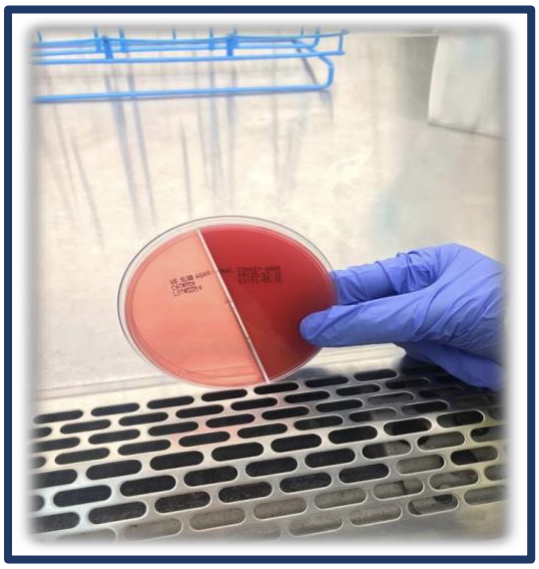
- Microscopic analysis is: WBC , RBC's , bacteria, yeast crystals (high salts), casts (high protein) , squamous cells

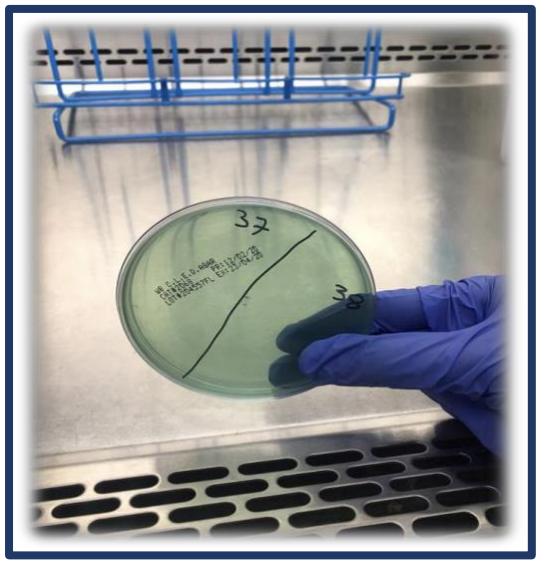
And that's all done by IRIS machine. It produces quantitative results for specific gravity, semi- quantitative results for glucose, blood, leukocyte esterase, bilirubin, urobilinogen, pH, protein, ketones and ascorbic acid; and qualitative results for nitrites, color and clarity. This called UA process.

Culturing

culture from the UA tubes on:

1- Plate half MAC and half BA.
2- CLED.





Reading procedure:

CLED

LF:

- *E.coli* (small).
- *Klebsiella* (large, mucid).

NLF:

- *Pseudomonus* large , mucoid , fruity smell , produce pigments).
- *proteus* (Large colourless)

MacConkey's agar

It is a selective medium for gram's -ve bacteria.

Blood agar

Usually use for subculture and examine hemolysis.

* 10 colonies significant growth (100,000), not necessary 10,000 only is the significant , catheter should be sterile. Any growth is significant.
* Yeast expect to grow aslso.
* gram stain required.
* Devices required to be used: Microscan + Maldi-Tof + vitek.

Respiratory bench:

At respiratory bench we look for microorganisms (and we mean by microorganisms bacteria and yeast) that infect lower and upper respiratory tract.

Types of samples received: Sputum – Endotrachial - BAL.

Culturing

Once samples received we culture it on three types of media: B.A - MAC - CA.
Also make a smear on a slide for gram stain.

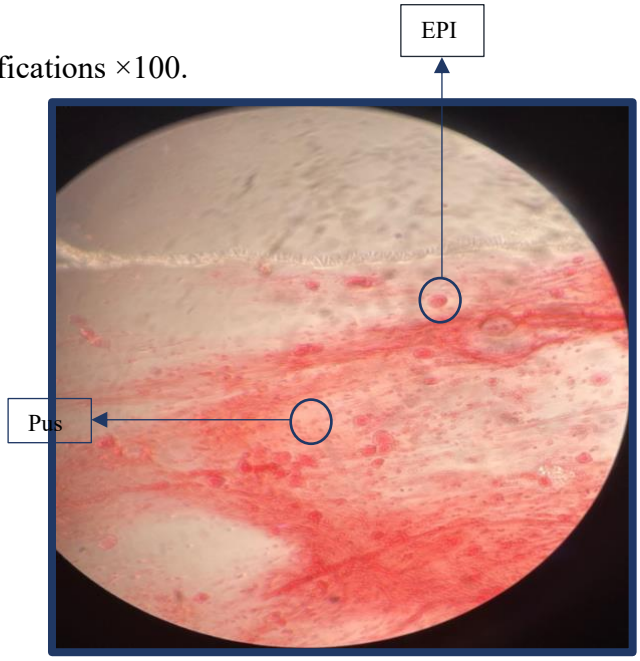
After culturing incubate plates for 24h or 48h (it depends on the growth)

Microscopic examination

After gram stain we do microscopic examination and we look for:

1- pus and epithelial cells see at magnifications ×10.
2- microorganisms (bacteria and yeast) see at magnifications ×100.

First: we measure the Pus and epithelial cells
1- 1-10 =+
2- 10-20 =++
3- 20-35 =+++
Epi shouldn't be +2 or more on one field,
and if it's reject the sample because it's salivary.
Second: look at the organism:
1- The stain (positive or negative)
2- Shape of the organism
3- Type of the organism (bacteria or yeast)
4- Sometimes it will be no organism, because the Patient collect the sample while take antibiotic.



Reading procedure:

BA

While it's enrichment media, both of organism will growth (yeast and bacteria) also gram positive and negative bacteria. Two species of gram positive bacteria are clinically important (*S.aureus* + *S. Pneumonae*)

MAC

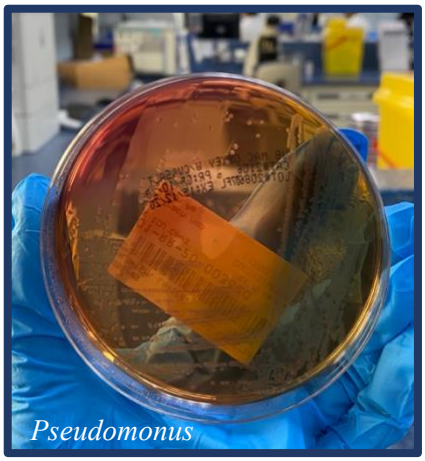
Only G- will growth.
And there's ferment of lactose.
Species which clinically important in RT:

LF :

-Klebsiella

NLF:

-Pseudomonus
-Acinetobacter


Pseudomonus

CA

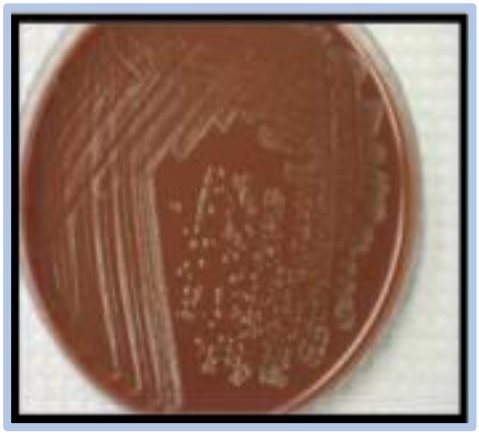
It's enrichment media, but which is clinically important is just *H.influenza*

- See the growth after 24h in all 3 plates, sometimes will need more incubation time.
- Compare the growth with microscopic examination results, and calculate Q-score system (Epi – Pus).
- If there is a growth on MAC do microscan, because most pathogens are G-, and G+ most times is normal flora. If there isn't chose B.A - *H.influenza* is rarely growth-.
- If it's yeast subculture in SAB.
- Maldi-Tof will be required, also Vitek sometimes.

Media used in bacteriology lab

Chocolate Agar

• An Enriched Media environment where most bacteria grow
• It contains factors X and V resulting from the breakdown of red blood cells RBCs, through which the growth of Heamophilus spp. These types of fastidious bacteria are considered.



Thayer martin (GC agar)

It is contains the antibiotic colicetin (kills Gram-negative bacteria in the gastrointestinal tract), vancomycin (kills Gram-positive), nystin (kills fungi).
Use to detect *N.gonorrhoeae.*

Mannitol salt agar

it is selective media because it contains a high level of salt and some *Staphylococci* species can grow well in this medium, and differential because it contain menthol, which causes the fermented bacteria to change the color from pink to yellow.

Sabouraud Agar media

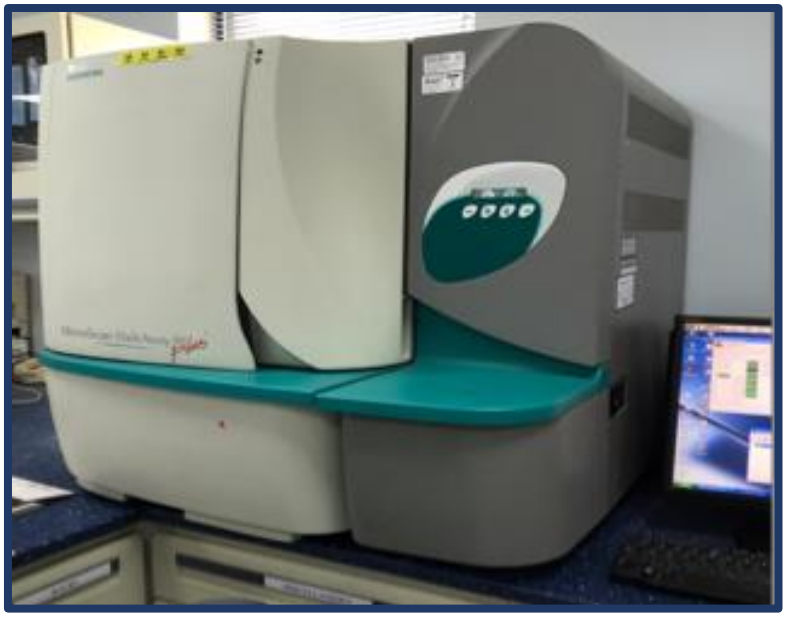
SDA is primarily used for the selective cultivation of yeasts, molds and aciduric bacteria. The medium is often used with antibiotics for the isolation of pathogenic fungi from material containing large numbers of other fungi or bacteria. This medium is also employed to determine microbial contamination in food, cosmetics, and clinical specimens.

Devices used in bacteriology lab

- **Microscan:**

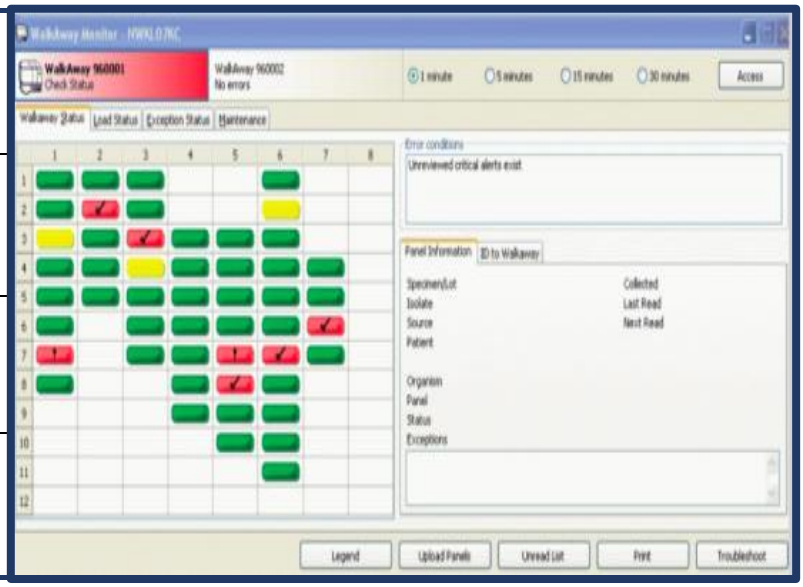
It is used to identify bacteria and their sensitivity to antibiotics. Principle of work API test. It is an incubator in which a plate should place inside it. Plate contains 96 well, the front part of it is biochemical tests, and the back is a test for antibiotic sensitivity. It takes 24 hours for the results to appear.

First: should do a suspension by taking a single colony then mix it with the solution.
Second: took from the suspension and culture it on BA for gram +ve and MAC for gram -ve.
Third: pour the suspension on device's plate.
Last step: place the plate in the machine then run it.



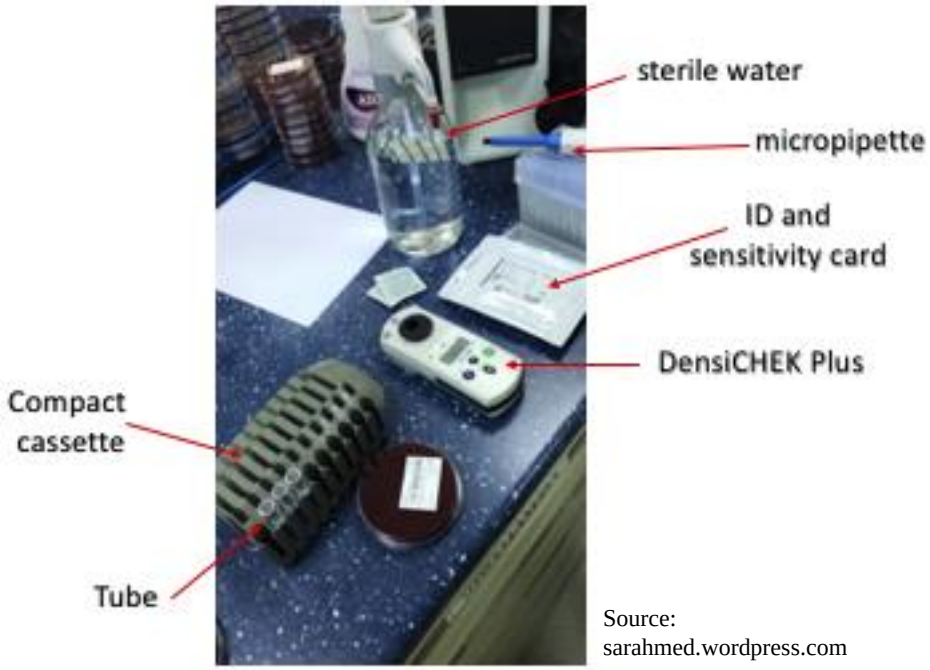
Results:

white	processing
green	Finish
red	Alarm- further test or any thing
yellow	Need more time



- **Vitek:**

chromogenic & depend on biochemical tests. Antibiotics with different concentrations. For critical samples like body fluid , blood or if microscan failed to Identify More specific , faster than microscan. Sometimes result done with five hour.



Source: sarahmed.wordpress.com

First: each sample should have two tube. One for ID and the other one for sensitivity. put 3ml of water in tubes.
Second: make suspension by taking a single colony then mix it with water, make sure it 0.5 – 0.63 McFarland.
Third: transfer from tube one to the other tube by micropipette.
Fourth: put the cards (ID cards & sensitivity cards).
Last step: log in the rack number and barcodes, place the rack in the machine.

- **Maldi-Tof**

  MALDI methodology is a three-step process. First, the sample is mixed with a suitable matrix material and applied to a metal plate. Second, a pulsed laser irradiates the sample,
  triggering ablation and desorption of the sample and matrix material. Finally, the analyte molecules are ionized by
  being protonated or deprotonated in the hot plume of ablated gases, and then they can be accelerated into whichever mass spectrometer is used to analyse them.

  How put samples in slide?
  -each microbe has different QC.
  -most times use E.coli.
  -use a lop to take from the plate single colony then put it in the slide.
  -put drop from formic acid (if it's yeast only).
  - after formic acid dry put matrix.
  -then load it in the maldi tof