# NATIONAL UNIVERSITY-SUDAN

## Faculty of Medical Laboratory Sciences

Student Practical Manual- Microbiology Department

## Third year, Semester (5)
## Clinical Microbiology-2 (MLS-CMIC-315)

**Student Name:** ……………………………………………………………………...

**ID:** ………………………………………**Batch**……………………….............…...

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Practical No. (1):

## *Escherichia coli*

1. **Colonial Morphology:**

| | Nutrient Agar | CLED | MacConkey Agar | Eosin Methylen blue media | Blood agar |
|---|---|---|---|---|---|
| **Size** | | | | | |
| **Shape** | | | | | |
| **Elevation** | | | | | |
| **Consistency** | | | | | |
| **Colour** | | | | | |
| **Fermentation** | | | | | |
| **Heamolysis** | | | | | |

2. **Indirect Gram stain:**

**Aim:** To differentiate between gram positive and gram negative bacteria as an aid to their identification.

**Principle:** Gram-positive bacterial walls are rich in tightly linked peptidoglycans that enable cells to resist decolorization and keep the basic stain. Gram-negative bacterial walls have thin layer of peptidoglycans and a high concentration of lipids (fats) that dissolve in the decolorizer and stain by counter stain

**Requirements:** Cultured plate,wire Loop, Sterile saline, Crystal violet, or Methyl violet (basic stain), Gram's iodine (mordent), 95% Alcohol, Acetone alcohol, or Acetone (decolourizer), Safranin or Neutral red stain (counter stain), Microscope, Oil, Flame, and Staining rack.

**Method:**

1. Prepare a well, dried, fixed smear.
2. Apply crystal violet, for 30-60 sec. Wash well with water.
3. Apply Gram's iodine(lugol's iodine ), for 30-60 sec. Wash well with water.
4. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water.
5. Apply safranin, for 1-2 min .Wash well with water.
6. Blot dry the back of slide with filter paper and leave it to dry.
7. Examine using 100X oil immersion lens.

**Report:** (Gram reaction, and Shape )

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## 3. Identification tests:

**a. Rapid test:**

- **Oxidase test:**

**Aim**: The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain

**Principle**: A piece of filter paper is soaked with a few drops of oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride ). A colony of the test organism is then smeared on the filter paper using wooden stick. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple color (indophenols)

**Requirements**: oxidase disc, Wooden stick,

**Method**:

1. Place oxidase test in a clean petri dish , Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and smear it on the filter paper.
2. Look for the development of a blue-purple color within 10 seconds.

**Results:**

Blue-purple colour . . . . . . . . . . Positive oxidase test (within 10 seconds)

No blue-purple colour . . . . . Negative oxidase test (within 10 seconds)

Note: Ignore any blue-purple colour that develops after 10 seconds.

**Comment:**

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**b. 24 h tests :**

**1. Indole test :**

**Aim** : Indole test is used to determine the ability of an organism to split amino acid tryptophan to form the compound indole.

**Principle :** Tryptophan is hydrolysed by tryptophanase to produce three possible end products – one of which is indole. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylamino benzaldehyde, this reacts with indole to produce a red coloured compound.

**Requirements:** 3ml peptone water containing tryptophan ,wire loop

**Procedure :**

1. Inoculate the test organism in test tube containing 3 ml of sterile peptone water containing tryptophan.
2. Incubate at 35–37 C for up to 24 h.
3. Test for indole by adding 0.5 ml of Kovac's reagent. Shake gently. Examine for a red colour in the surface layer within 10 minutes.

- ✓ Precaution to be taken :
  - The result must observe within 10 second
  - Use wooden stick or platinum wire loop
  - Nickle-base (iron or chromium) loop can cause false positive result .
  - Must be perform from media without sugar fermentation .acid produce from sugar may cause false negative result )

***Results***

Red surface layer . . . . . . . . . . . . Positive indole test.

No red surface layer . . . . . . . . Negative indole test .

**Comment:**

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## 2. SPOT INDOLE TEST (assignment 1)

- Aim:

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- **Principle**

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- **Requirement and Procedure :**

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- **Result**

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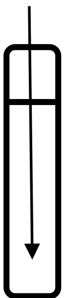
## 3. Motility test

**Aim :**

Motile bacteria move about with structures called flagella (a few exceptional bacteria move with the help of axial filaments, which cannot be seen in the microscope). In semi-solid agar media, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye.

**Principle:** Motility Test Agar is a semisolid medium designed to detect bacterial motility. Its agar concentration is reduced from the typical 1.5% to 0.4%—just enough to maintain its form while allowing movement of motile bacteria. It is inoculated by stabbing with a straight transfer needle. Motility is detectable as diffuse growth radiating from the central stab line. Freshly prepared medium containing 1% glucose can be used for motility tests on anaerobes.

**Requirements**: Cultured plate, Semi-solid medium, Straight wire loop and Flame.



**Procedure:** inoculate tube contain semisolid medium. Use a sterile straight wire to inoculate a stab medium. Stab through the center of the medium till the 2/3 of the tube, taking care to withdraw the wire along the line of inoculum without making further stab lines. Incubate at 37$^{o}$C overnight.

**Result:** Diffusion of the organism around the line of the inoculation indicate positive test.

- Report:

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## 4. Citrate utilization test:

**Aim:** Citrate utlilization test is used to determine the ability of bacteria to utilize sodium citrate as its only carbon source and inorganic (NH4H2PO4) is the sole fixed nitrogen source.

**Principle** When an organic acid such as citrate (*remember Krebs cycle)* is used as a carbon and energy source, alkaline carbonates and bicarbonates are produced ultimately. In addition, ammonium hydroxide is produced when the ammonium salts in the medium are used as the sole nitrogen source.

Citrate = oxaloacetate + acetate

oxalacetate = pyruvate + $CO_2$

The carbon dioxide that is released will subsequently react with water and the sodium ion in the medium to produce **sodium carbonate, an alkaline compound** that will raise the pHGrowth usually results in the **bromothymol blue indicator, turning from green to blue**

**Requirements :** Simmon's citrate agar slope , straight loop

**Procedure :**

1. Inoculate simmons citrate agar by zig zagging on the slant surface using the tip of loop to a colony that is 18 to 24 hours old.
2. Incubate at 35 C for 24 hours. Look for a bright blue colour in the medium

**Result :**

Bright blue . . . . . . . . . . . . . . . . . Positive citrate test

No change in colour . . . . . . . . Negative citrate test

**Comment :**

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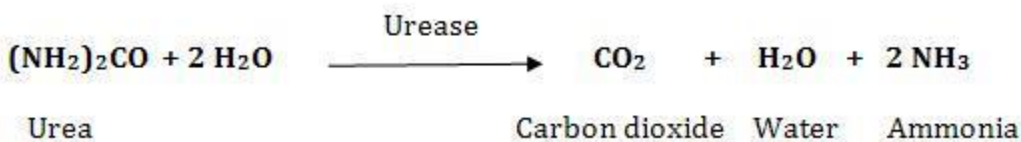
## 5. Urease test (urea utilization test )

**Aim :** Testing for urease enzyme activity

**Principle :**

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease-producing, the enzyme will break down the urea (by hydroly-sis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.



**Requirement :** Christensen's urea media slope or broth ,straight loop

**Procedure :**

3. Inoculate Christensen's urea agar by zig zagging on the slant surface using the tip of loop to a colony that is 18 to 24 hours old.
4. Incubate at 35 C for 24 hours. Look for red colour in the medium

**Result :**

Red/purple colour . . . . . . . ..... Positive urease test Yellow/orange
. . . . . . . . . .Negative urease test

**Comment :**

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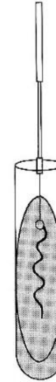

## 5. Methyl red (MR)/ Voges-Proskauer test (VP test )

**Principale** :

Used to determine the ability of microbes to produce non acidic or neutral end product (acetyl-methylcarbinol or acetoin ) from glucose fermentation .to overcome the buffering capacity of the system .

**Requirement : glucose 6 phosphate media , wire loop , pure culture organism**

**Method :**

1. Inculate 5ml G6P broth with the organism
2. Incubate at 37C for 24-48 h

   **For MR test :**
3. Add 5 or 6 drop of methyl red reagent per 5ml of broth
4. Read the reaction

   MR test result

   Bright Red color ……………….positive

   Yellow color ……………….negative
5. **For VP test :**
6. **Add 6 drops of α-naphthol solution and 2 drops of 40% KOH solution .**

7. **Shake well after addition of each reagent**
8. **Observe for 5 min .**

**VP test result :**

Red color ……………….positive

Yellow color ……………….negative

**Comment :**

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## 6. Kliglar iron agar(KIA):

**Principle :**

- KIA contains the 2 sugars: **glucose and lactose in a ratio of 1:10**.
- The medium is used in the form of slants +ferrous sulphate + phenol red indicator
- Since lactose is a disaccharide (glucose and galactose) if lactose is fermented then glucose must be fermented too.
- Thus distinction is made between fermentation of one sugar (glucose) or both sugars (glucose and lactose).
- The production of gas in these reactions is made visible by cracks or displacement of the agar in the medium.
- Production of $H_2S$ is made visible by the addition of ferrous salts that precipitate the $H_2S$ and form a black precipitate

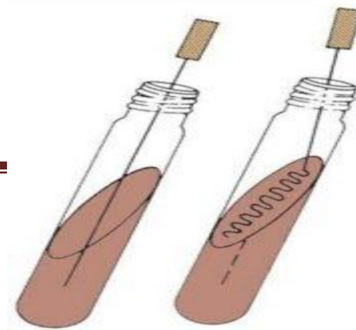
**Requirement:**

KIA media , straight loop , culture organism

**Procedure :**

1. With straight loop inculcate KIA by stabbing through the center of medium to the bottom of the tube and then streaking the slant
2. Cap the tube tightly and incubate at $37C^0$ for 24 h

Result :



Possible reactions:

- Red \yellow

Gulcose ferment \non lactose ferment

- yellow \yellow

Glocose and lactose fermented

- Red \red

Non lactose non glucose ferment

- Crack ------- gas production
- Black coloration – $H_2S$ produced

**Final report :**

**The isolate organism is (species and genus)**

**………………………………………………………………………………….**

Instructor Name: ……………………………………….

Instructor Signature: ………………………………

Date :………………………………….

Practical No. (2):

## *Klebsiella spp*

1. **Colonial Morphology:**

| | **Nutrient Agar** | **CLED** | **MacConkey Agar** |
|---|---|---|---|
| **Size** | | | |
| **Shape** | | | |
| **Elevation** | | | |
| **Consistency** | | | |
| **Colour** | | | |
| **Fermentation** | | | |

2. **Indirect Gram stain:**

   **Method :**

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   **Report :**

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3. **Capsule stain :**

   **Aim : to demonstrate the capsule around the bacterial cell**

**Materials and reagents required**

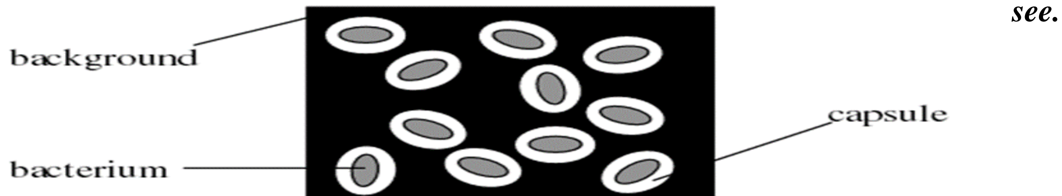
- **Test bacteria: 36-48 hour culture , Stain solution (Crystal violet, India ink,or Nigrosin) ,Microscopic slide, Inoculating loop**

**Procedure :**

1. **Place a single drop of India ink on a clean microscope slide, adjacent to the frosted edge.**
2. **Using a flamed loop and sterile technique, remove some *organism* from culture plate and mix it into the drop of India ink. Be sure there are no large clumps of organism, but try to spreading the drop.**
3. **Allow the film to air dry *(will take 5-7 minutes). DO NOT heat or blot dry! Heat will melt the capsule!***
4. **Saturate the slide with crystal violet for 1 minute and rinse slightly & very gently with water.**
5. **Let the slide air dry for a few minutes. *DO NOT blot the slide***
6. **Observe the slide under oil immersion.**

**Results:**

**Look for purple cells surrounded by a clear halo on a dark background. The halo is the capsule. *You may need to decrease the amount of light in order to make the capsule easier to see.***




**Capsule stain result :**

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4. **Oxidase test :**

**Procedure :**

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**Result :**

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5. **KIA test**

**Result :**

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6. **Urease test**

**Result :**

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7. **Citrate utilization test**

**Result**

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8. **Indole test**

**Result :**

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9. **Motility test :**

   **Result :**

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10. **MR /VP test**

**Result**

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**Final report :**

**The isolate organism is (species and genus)**

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**Assignment (1)**

**Eosin methylene blue media :**

**What is the type of this media ?**

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**What is the component of EMB media ?**

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**What is the uses of this media ?**

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**What is the colonial morphology of E.coli and klebsilla in this media and why they produce this colonial morphology ?**

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Instructor Name: ……………………………………….

Instructor Signature: ………………………………

Date :………………………………….

Practical No.( 3):

# *Salmonella spp*

| | | MacConkey Agar | XLD media | DCA |
|---|---|---|---|---|
| S .typhi | **Size** | | | |
| | **Shape** | | | |
| | **Elevation** | | | |
| | **Consistency** | | | |
| | **Color** | | | |
| | **Fermentation** | | | |

1. **Colonial Morphology:**

| | | MacConkey Agar | XLD media | DCA |
|---|---|---|---|---|
| *S .para A* | **Size** | | | |
| | **Shape** | | | |
| | **Elevation** | | | |
| | **Consistency** | | | |
| | **Color** | | | |
| | **Fermentation** | | | |

| | | **MacConkey Agar** | **XLD media** | **DCA** |
|---|---|---|---|---|
| *S .para B* | **Size** | | | |
| | **Shape** | | | |
| | **Elevation** | | | |
| | **Consistency** | | | |
| | **Color** | | | |
| | **Fermentation** | | | |

2. **Indirect Gram stain:**

**Method:**

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**Report:** (Gram reaction, and Shape )

1. ***S.typhi***

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2. ***S.paratypi A:***

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3. ***S.paratyphi B***

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### 3. Identification tests:

c. **Rapid test:**

- **Oxidase test:**

**Requirements**:

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**Method**:

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**Result :**

***S.typhi***

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***S.paratyphi A:***

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***S.partyphi B***

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**24h test :**

1. **KIA test**

   **Procedure:**

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2. **Urease test**

**Procedure:**

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3. **Citrate utilization test**

**Procedure:**

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4. **Indole test**

**Procedure:**

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5. **Motility test :**

**Procedure:**

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**Result:**

| | KIA | Urease | Citrate | Indole | Motility |
|---|---|---|---|---|---|
| **S.typhi** | | | | | |
| **S.para A** | | | | | |
| **S.para B** | | | | | |

**6. The carbohydrate fermentation test(set of sugar )**

**Aim :**

**used to determine whether or not bacteria can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species.**

**Principle :**

- **When microorganisms ferment carbohydrate an acid or acid with gas are produced. Depending up on the organisms involved and the substrate being fermented, the end products may varies. Common end-products of bacterial fermentation include lactic acid, formic acid, acetic acid, butyric acid, butyl alcohol, acetone, ethyl alcohol, carbondioxide and hydrogen.**
  **The production of the acid lower the pH of the test medium, which is detected by the color change of the pH indicator. Color change only occurs when sufficient amount of acid is produced, as bacteria may utilize the peptone producing alkaline end products. Phenol red is commonly used as a pH indicator in carbohydrate fermentation tests. Other pH indicators such as bromocresol/bromocresol purple (BCP), bromothymol/bromothymol blue (BTB), and Andrade's can be used.**
- **Durham tubes are inserted upside down in the test tubes to detect gas production. If the test organism produce gas, the gas displaces the media present inside the tube and get trapped producing a visible air bubble.**
- **Based on the characteristics reactions observed, bacteria can be classified as:**
- **Fermenter with acid production only**
- **Fermenter with acid and gas production**
- **Non-fermenter**

**Requirement:**

**10% sugar (glucose, lactose , mannitol , sucrose , maltose ) broth with phenol red indicator + Durham tube , wire loop**

**Procedure :**

1. **Aseptically inoculate each test tube with the test microorganism using an inoculating needle or loop.**
2. **Incubate tubes at 35-37°C for 18-24 hours.**

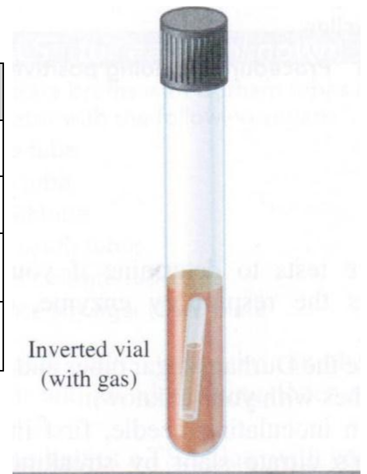
**Result**

- **Positive: …………………….yellow**
- **Negative: …………………..red.**

- **Gas Production**
  - **Positive: A bubble (small or big depending up the amount of gas produced) will be seen in the inverted Durham tube.**
  - **Negative: There won't be any bubble in the inverted Durham tube**

**Result :**

| | **Lactose** | **Mannitol** | **Glucose** | **Sucrose** |
|---|---|---|---|---|
| *Shigella* species | | | | |
| *Salmonella* Typhi | | | | |
| *Salmonella para A* | | | | |
| *Salmonella para B* | | | | |



Durham fermentation tube

**Final report :**

**The isolate organism is (species and genus)**

**……………………………………………………………………………………**

Instructor Name: ………………………………………

Instructor Signature: ………………………………

Practical No. (4):

# *Shigella spp*

| | MacConkey Agar | XLD media | DCA media | SS media |
|---|---|---|---|---|
| **Size** | | | | |
| **Shape** | | | | |
| **Elevation** | | | | |
| **Consistency** | | | | |
| **Color** | | | | |
| **Fermentation** | | | | |

4. **Indirect Gram stain:**

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**Report:** (Gram reaction, and Shape )

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5. **Identification tests:**

**Rapid test:**

- **Oxidase test:**

**Requirements**:

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**Method**:

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**Result :**

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## 24h tests

7. **KIA test**

   **Procedure:**

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8. **Urease test**

   **Procedure:**

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9. **Citrate utilization test**

**Procedure:**

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10. **Indole test**

**Procedure:**

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11. **Motility test :**

**Procedure:**

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**Result:**

| | KIA | Urease | Citrate | Indole | Motility |
|---|---|---|---|---|---|
| **Shigella** | | | | | |

12. **The carbohydrate fermentation test(set of sugar )**

**Aim:**……………………………………………………………………………………

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**Procedure :**

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**13. ONPG test (O-Nitrophenyl-β-D-galactopyranoside )**

**Aim :**

**differentiate between non lactose ferment and late lactose ferment organisms**

**Principle :**

**Lactose fermenting bacteria posses both lactose permease and β-galactosidase, two enzymes required for the production of acid in the lactose fermentation test. The permease is required for the lactose molecule to penetrate the bacterial cell where the β-galactosidase can cleave the galactoside bond, producing glucose and galactose.Non-lactose fermenting bacteria are devoid of both enzymes and are incapable of producing acid from lactose. Some bacterial species appear to be non-lactose fermenters because they lack permease, but do possess β-galactosidase and give a positive ONPG test, So called late lactose fermenters may be delayed in their production of acid from lactose because of sluggish permease activity. In these instances, a positive ONPG test may provide a rapid identification of delayed lactose fermentation.**

**Requirement :**

**Sodium phosphate buffer, 1 M, pH 7.0, O-Nitrophenyl-β-D-galactopyranoside (ONPG), 0.75 M Physiologic saline, Toulene**

**Procedure :**

1. **A loopful of bacterial growth is emulsified in 0.05mL of physiologic saline to produce a heavy suspension**
2. **One drop of toluene is added to the suspension and vigorously mixed for a few seconds to release the enzyme for bacterial cells.**
3. **An equal quantity of buffered ONPG solution is added to the suspension.**
4. **The mixture is placed in a 37$^{o}$C water bath**

**Results and Interpretations:**

- **yellow color ……………………. Positive**
- **colorless …………………………. Negative**

- **Final report :**
- **The isolate organism is (species and genus)**
- **……………………………………………………………………………**

Instructor Name: ……………………………………….

Instructor Signature: ………………………………

Date :………………………………….

Practical No. (5):

# *Proteus species*

| | MacConkey Agar | Blood agar | CLED | Nutrient agar |
|---|---|---|---|---|
| **Size** | | | | |
| **Shape** | | | | |
| **Elevation** | | | | |
| **Consistency** | | | | |
| **Color** | | | | |
| **Fermentation** | | | | |
| **Heamolysis** | | | | |

1. **Indirect Gram stain**

**Report:** (Gram reaction, and Shape )

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## Identification tests:

**Rapid test:**

- **Oxidase test:**

**Requirements**:

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……………………………………………………………………………………………………….

**Result : ……………………………………………………………………………………………**

# 24h tests

2. **KIA test :**

**Requirement :**

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**Procedure:**

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3. **Urease test**

**Requirement :**

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**Procedure:**

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4. **Citrate utilization test**

**Requirement :**

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**Procedure:**

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5. **Indole test**

**Requirement :**

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**Procedure:**

………………………………………………………………………………

6. **Motility test :**

**Requirement**

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**Procedure:**

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**Result:**

| | KIA | Urease | Citrate | Indole | Motilitu |
|---|---|---|---|---|---|
| ***P .mirabilis*** | | | | | |
| ***P .vulgaris*** | | | | | |

**Final report :**

**The isolate organism is (species and genus)**

………………………………………………………………………………

**Assignment(2) :**

1. **phenyl pyruvic acid test (PPA test ):**

**aim :**

…………………………………………………………………………………………………

…………………………………………………………………………………………………

**requirements:**

…………………………………………………………………………………………………

**Principle :**

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**Procedure :**

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**Result :**

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2. **What are the different methods used to prevent *Proteus* swarming on culture media?**

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   ………………………………………………………………………………………

Instructor Name: ……………………………………….

Instructor Signature: ………………………………

Date :………………………………….

Practical No. (6):

# *Pseudomonas species*

| | MacConkey Agar | Blood agar | Nutrient agar |
|---|---|---|---|
| **Size** | | | |
| **Shape** | | | |
| **Elevation** | | | |
| **Consistency** | | | |
| **Color (pigment)** | | | |
| **Fermentation** | | | |
| **Heamolysis** | | | |

1. **Indirect Gram stain:Report:** (Gram reaction, and Shape )

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## Identification tests:

**Rapid test:**

- **Oxidase test:**

**Requirements**:

……………………………………………………………………………………………

……………………………………………………………………………………………

**Method**:

……………………………………………………………………………………………

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**Result :**

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## 24h tests

7. **KIA test :**

**Requirement :**

…………………………………………………………………………………………………

**Procedure:**

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8. **Urease test**

**Requirement :**

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**Procedure:**

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9. **Citrate utilization test**

**Requirement:**

…………………………………………………………………………………………………

**Procedure:**

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10. **Indole test**

**Requirement:**

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**Procedure:**

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11. **Motility test :**

**Requirement**

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**Procedure:**

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**Biochemical test Results :**

| | KIA | Urease | Citrate | Indole | Motility |
|---|---|---|---|---|---|
| ***Pseudomonas*** | | | | | |

## The oxidative-fermentative (OF) test:

- Aim : used to determine if gram-negative bacteria metabolize carbohydrates oxidatively, by fermentation, or are nonsacchrolytic (have no ability to use the carbohydrate in the media).
- Principle:

  The oxidative-fermentative test determines if certain gram-negative rods metabolize glucose by fermentation or aerobic respiration (oxidatively). During the anaerobic process of fermentation, pyruvate is converted to a variety of mixed acids depending on the type of fermentation. The high concentration of acid produced during fermentation will turn the bromthymol blue indicator in OF media from green to yellow in the presence or absence of oxygen .Certain nonfermenting gram-negative bacteria metabolize glucose using aerobic respiration and therefore only produce a small amount of weak acids.

Requirement: :

Hugh and Leifson's OF medium(semi solid media ), sterile mineral oil or liquid paraffin , straight loop .

- Procedure

1. Inoculate two tubes of OF test medium with the test organism using a straight wire by stabbing "half way to the bottom" of the tube.
2. Cover one tube of each pair with 1 cm layer of sterile mineral oil or liquid paraffin (it creates anaerobic condition in the tube by preventing diffusion of oxygen), leaving the other tube open to the air.

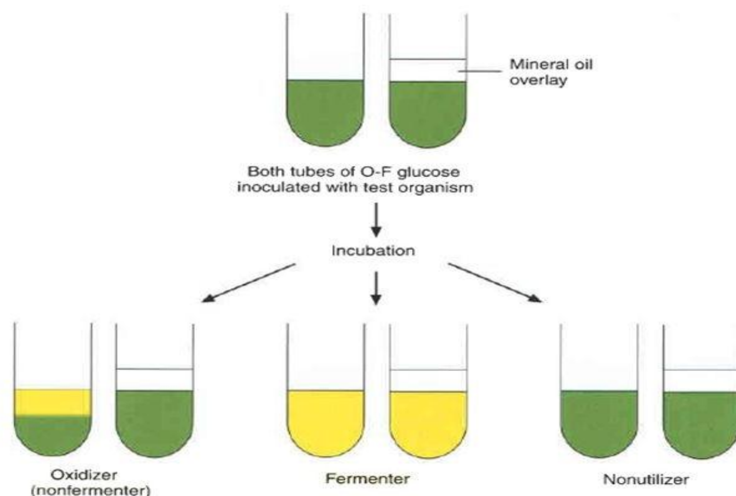
3. Incubate both tubes at 35oC for 48 hours (Slow growing bacteria may take 3 to 4 days before results can be observed)

- Result

- Oxidative result: Acid production (yellow color )in the open tube (aerobic) and not the oil-covered tube (anaerobic) indicates an oxidative result.

- Fermentative result : Acid production in the open tube (aerobic) and the oil-covered tube (anaerobic) indicates an fermentative result.

- Non saccharolytic (Negative OF result): Nonsacchrolytic bacteria give a negative OF result. The negative result is indicated by no color change in the oil-covered tube and open tube.



**Assignment :**

**List the biochemical test used to identify Vibrio cholera in TCBS media :**

## Final report :

## The isolate organism is (species and genus)

……………………………………………………………………………………

Instructor Name: ……………………………………….

Instructor Signature: ………………………………

Date :………………………………….

Practical No.( 7)

# *Vibrio spp*

| | MacConkey Agar | Blood agar | Nutrient agar | TCBS Agar |
|---|---|---|---|---|
| **Size** | | | | |
| **Shape** | | | | |
| **Elevation** | | | | |
| **Consistency** | | | | |
| **Color (pigment)** | | | | |
| **Fermentation** | | | | |
| **Heamolysis** | | | | |

2. **Indirect Gram stain:**

   **Report:** (Gram reaction, and Shape )

…………………………………………………………………………………………………

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## Identification tests:

**Rapid test:**

- **Oxidase test:**

**Requirements**:

…………………………………………………………………………………………………

…………………………………………………………………………………………………

**Method**:

……………………………………………………………………………………………….

**Result :**

……………………………………………………………………………………………

……………………………………………………………………………………………

# 24h tests

1. **KIA test :**

**Requirement:**

……………………………………………………………………………………………

**Procedure:**

……………………………………………………………………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

2. **Urease test**

**Requirement :**

……………………………………………………………………………………………

**Procedure:**

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3. **Citrate utilization test**

**Requirement:**

……………………………………………………………………………………………

**Procedure:**

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4. **Indole test**

**Requirement:**

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**Procedure:**

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5. **Motility test :**

   **Requirement**

   **:**……………„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„„…

   **Procedure:**

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**Biochemical test Results :**

| | KIA | Urease | Citrate | Indole | Motility |
|---|---|---|---|---|---|
| ***V.Cholarea*** | | | | | |

**Final report :**

**The isolate organism is (species and genus)**

**……………………………………………………………………………**

Instructor Name: ………………………………………

Instructor Signature: ………………………………

Date :…………………………………

## Practical No. (7)

## Mycobacterium species

# Ziehl - Neelsen technique (acid fast stain)

# (Hot technique)

**Aim:** Used to stain Mycobacterium tuberculosis and Mycobacterium leprae as an aid to their identification.

**Principle:** When smear stained with carbol fuchsin, applied with heat or in a concentrated solution, the stain can penetrate the lipid cell wall and reach the cytoplasm. Once the cytoplasm is stained, it resists decolorization with acid-alcohol, which cannot dissolve and penetrate the mycobacterial lipid wall (acid fast). Other bacteria whose cell walls do not contain high concentrations of lipid are readily decolorized by acid alcohol after staining with basic stain and stain by counter stain (non acid fast)

**Requirements:** Fixed sputum smear, Carbol fuchsin (basic stain), 3% Acid alcohol or 20% $H_2SO_4$ (decolourizer), Malachite green, or Methylene blue (counter stain) Microscope, Oil, Flame, and Staining rack.

**Method:**

1. Cover the smear with carbol fuchsin stain.
2. Heat the stain until vapour just being to rise, allow for 5 mins, and wash the stain with clean water.
3. Cover the smear with 3% acid alcohol or 20% $H_2SO_4$, for 5 mins, and wash well with clean water.
4. Counter stain with malachite green or methylene blue for 2 mins, and wash the stain with clean water.

5. Place to air dry, and examine using 100X oil immersion.

## Result:

- AFB: Red or curved bacilli occurring singly or in small groups
- Back ground: green or blue.

## *Reporting of sputum smears:*

- 1 - 9 AFB/100 fields report the exact number (Scanty)
- 10 - 99 AFB/100 fields report (+)
- 1- 10 AFB/1 field report (++)
- More than 10 AFB/1 field report (+++)
- No acid fast bacilli seen when examine more than 300 field and no AFB found.

## Comment :

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## Report :

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**Assignment**

1. **Mention the other methods of ZN stain techniqes :**

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2. **Regarding Löwenstein–Jensenmedium**
   a. **List the componante of LJ media**

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   b. **The colonial morphology of Mycobacterium Tuberculosis**

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Instructor Name: ……………………………………….

Instructor Signature: ………………………………

Date :………………………………….

Practical No. (8):

# Urine culture

**Specimen collection and storage :**

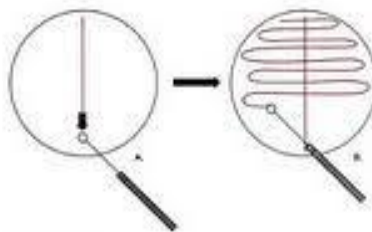
**Urine collected in sterile specimen container must be processed within 2 hours, or refrigerated and processed within 24 hours • Urine collected in sterile specimen container with borate preservative (boric acid )should be processed within 24 hours (no refrigeration required)**

**Culture media :**

**MacConkey agar , blood agar , and CLED media (media of choice for urine culture )**

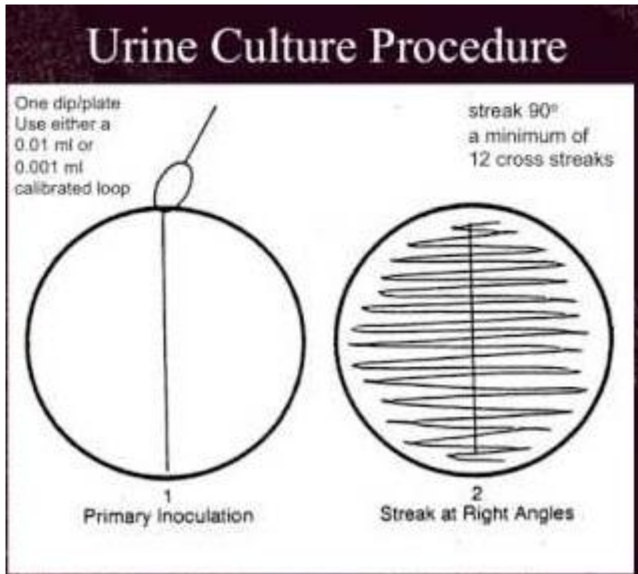
**Inculation :**

- Flame the calibrated wire loop and allow it to cool without touching any surface. Alternatively, aseptically remove a plastic calibrated loop from its package.
- Mix the urine thoroughly and remove the top of the container.
- Insert the loop vertically into the urine to allow urine to adhere to the loop.
- Spread the loopful of urine over the surface of the agar plate (see figure below) .



- Without reflaming, insert the loop vertically into the urine again for transfer of a loopful to a second plate. Repeat for each plate.
- Incubate plates for at least 24 hours at $35^{0}$C to $37^{0}$C in air. Colonies are counted on each plate. The number of CFUs is multiplied by 1000 (if a 0.001 mL loop was used) to determine the number of microorganisms per milliliter in the original specimen.

- Because antimicrobial treatment or other factors may inhibit initial growth, reincubate plates with no growth or tiny colonies for an additional 24 hours before discarding plates.




**Interpretation of result :**

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**Final report :**

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Instructor Name: ……………………………………….

Instructor Signature: ………………………………

Date :…………………………………..

**Reading assignments :**

1. **Sorbitol MacConkey agar**
2. **Biken test**
3. **Quelling test**
4. **Weil Felix test**
5. **Cetrimide agar**
6. **Vibrio O/129 differential disc**
7. **Salmonella serology (O and H antigens )**
8. **Sugar utilization test for shigella**
9. **Multiple antibiotics resistance in pseudomonas**

## Chart 7.10 Biochemical reactions of some enterobacteria and other enteric organisms

| | | | | | | | | | | | | | KIA Medium | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Urea** | **VP** | **ONPG** | **Lact** | **Man** | **Glu** | **Suc** | **Ox** | **Cit** | **Mot** | **Ind** | **LDC** | **Slope** | **Butt** | **$H_2S$** | **Gas** |
| *Escherichia coli* | – | – | + | + | + | + | d | – | – | +[5] | +[2] | + | Y[6] | Y | – | +[2] |
| *Shigella* species | – | – | –[7] | – | d | + | –[1] | – | – | – | d | – | R | Y | – | –[3] |
| *Salmonella* Typhi | – | – | – | – | + | + | – | – | – | + | – | + | R | Y | + Weak | – |
| *Salmonella* Paratyphi A | – | – | – | – | + | + | – | – | – | + | – | – | R | Y | – | + |
| Most other salmonellae | – | – | – | – | + | + | – | – | + | + | – | + | R | Y | +[2] | d |
| *Citrobacter freundii* | d | – | + | + Late | + | + | d | – | + | + | –[3] | – | R or Y | Y | d | + |
| *Klebsiella p. pneumoniae* | + slow | + | + | + | + | + | + | – | + | – | –[3] | + | Y | Y | – | + |
| *Enterobacter* species | – | + | + | + | + | + | d | – | +[2] | + | – | d | Y | Y | – | + |
| *Serratia marcescens* | d | + | + | d | + | + | + | – | + | + | – | + | R or Y | Y | – | d |
| *Proteus vulgaris* | + | – | – | – | – | + | + | – | d | + | + | – | R | Y | + | d |
| *Proteus mirabilis* | + | d | – | – | – | + | d | – | +[2] | + | – | – | R | Y | + | + |
| *Morganella morganii* | + | – | – | – | – | + | – | – | – | +[5] | + | – | R | Y | – | d |
| *Providencia* species | d | – | – | – | d | + | d | – | + | + | + | – | R | Y | – | d |
| *Yersinia enterocolitica*[4] | + slow | – | + | – | + | + | + | – | – | + | d | – | R | Y | – | – |
| *Vibrio cholerae* | – | d | + | – 24h | + | + | + | + | d | + | + | + | R | Y | – | – |
| *Vibrio parahaemolyticus* | –[3] | – | + | – | + | + | – | + | d | + | + | + | R | Y | – | – |

**Key: LDC** = Lysine decarboxylase, **VP** = Voges-Proskauer, **ONPG** = *beta*-galactosidase, **Lact** = Lactose, **Man** = Mannitol (mannite), **Glu** = Glucose, **Suc** = Sucrose, **Ox** = Oxidase test, **Cit** = Citrate test, **Mot** = Motility, **Ind** = Indole test, **Urea** = Urease, **$H_2S$** = Hydrogen sulphide (blackening), R = Red-pink (alkaline reaction), Y = Yellow (acid reaction), d = different strains give different results.

**Notes**

1 *S. sonnei* ferments sucrose slowly.
2 A minority of strains give a negative result.
3 A minority of strains give a positive result.
4 Tests should be incubated at 20–28 °C.
5 A few strains are non-motile.
6 A few strains give reactions similar to *Shigella* species.
7 *S. sonnei* is ONPG positive.

**References:**

1. **Mackie and McCartney**: **Practical medical microbiology**. Eds J. G. Collee, J. P. Duguid, A. G. Fraser & B. P. Marmion, 13th edition; Churchill Livingstone (1989) .
2. **District Laboratory Practice in Tropical Countries**, Part **2**. Front Cover. **Monica Cheesbrough**. Cambridge University Press, Mar **2**, 2006 ).