NATIONAL UNIVERSITY-SUDAN

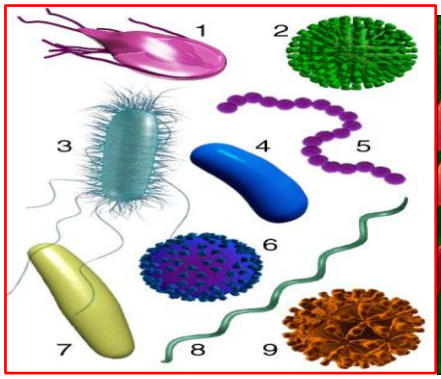
Faculty of Medical Laboratory Sciences

Student Practical Manual- Microbiology Department

Second year, Semester (4)
Basic Microbiology (MLS-BMIC-224)

Student Name: ……...

ID: ……**Batch**…….....…...



Contents

References:

1. **Mackie and MacCartney,** Practical Medical Microbiology
2. ***Cheesbrough Monica,*** *District Laboratory Practice In Tropical Countries, part 2 or volume 2.*

Practical No. (1):

Demonstration of Lab Equipment's

1. **Item:** .....

Uses: .....

2. **Item:** .....

Uses: .....

3. **Item:** .....

Uses: .....

4. **Item:** .....

Uses: .....

5. **Item:** .....

Uses: .....

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10. **Item:** .....

Uses: .....

11. **Item:** .....

Uses: .....

Practical No. (2)

Preparation and Fixation of Smears

Aim: To prepare dry fixed smear for staining.

Requirements: Cultured plate, Slides, Wire loop, Lead pencil, Normal saline, and Flame.

Method:

A. From cultured plate

1. In dry, clean slide but small drops of normal saline.
2. Emulsify a small portion of bacterial culture in the sterile saline and spread to make thin smear.
3. Left the smear air dry.
4. Fix the smear by passing it three times over the flame.

B. From broth culture:

- Place a drop of mixed broth and spread to make a thin smear.

Comment:

Questions:

Complete

1. Absolute alcohol used to fix ……

2. 70 % alcohol used to fix ……..……

3. Potassium permanganate used to fix …….……

4. Heat used to fix ……..……

Practical No. (3)

Methylene blue stain

Aim: To demonstrate bacterial shape.

Principle: Basic stains are attracted to the negative bacteria

Requirements: Cultured plate, Loop, Sterile saline, Methylene blue stain, Microscope, oil, Flame, and Staining rack.

Method:

1. Prepare a well, dried, fixed smear.
2. Cover the smear with methylene blue.
3. Allow for 60 seconds.
4. Tip the slide so the excess stain drops into the sink.
5. Wash with tap-water
6. Dry the smear in the air, and examine using the oil-immersion lens

Result: Bacterial cells appear blue.

Comment:

Questions:

- List different shapes of bacteria ..……...……
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- List different arrangements of bacteria ……...……
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Practical No. (4)

Negative stain (Nigrosine)

Aim: To demonstrate the bacterial morphology and capsule.

Principle: Negative stains are repelled from the bacteria, and stain back ground.

Requirements: Cultured plate, Loop, Nigrosine, Microscope, oil, Flame, and Staining rack.

Method:

1- On clean dry slide place a large loop full of Nigrosin clean dry slide.
2- Emulsify a very small portion of solid bacterial culture in the Nigrosin and spread to make smear.
3- Let it to dry then put one drop of oil and examine with X100 (oil immersion lens).

Result:

- Bacteria appear shine against dark background.
- Capsule appears as clear zone around the bacterial cell on dark back ground.

Comment:……

Questions:

1. Mention other methods for capsule detection. ……
   ……..……
2. Name 3 bacteria that have a capsule as a virulence factor. ……
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Practical No. (5)

Gram stain (1)

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 a high concentration of lipids (fats) that dissolve in the decolorizer and stain by counter stain.

Requirements: Cultured plate, 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 1 min. Wash well with water.
3. Apply Gram's iodine, for 2 mins. Wash well with water.
4. Flood 95% alcohol and wash off within 5-10 seconds. Wash well with water.
5. Apply safranin, for 1 min. Wash well with water.
6. Blot dry the back of slide with filter paper and leave to dry.
7. Examine using 100X oil immersion lens.

Result:

- Gram positive bacteria appear violet.
- Gram negative bacteria appear light pink.

Report: Gram reaction, Shape and Arrangement.

Comment:……

Practical No. (6)

Gram stain (2)

Report**:**

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Comment:

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Questio**ns**:

1. When gram positive bacteria appear red? ….……...……

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2. When gram negative bacteria appear violet? ..……..……

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3. List important bacteria that do not stain with the Gram stain reagents ..……

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Practical No. (7)

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.

Report:

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Comment:

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Questions:

- List 3 ZN positive bacteria ……

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Practical No. (8)

Staining of Spores (Schaeffer-Fulton Method)

Aim: To demonstrate bacteria spore and position.

Principle: Hot Malachite green retention by thick wall spore, while bacilli stain by Safranin.

Requirements: Cultured plate, Loop, Sterile saline, malachite green, safranin or 0.05% basic fuchsin Microscope, oil, Flame, and Staining rack.

Method:

1. Prepare a well, dried, fixed smear.
2. Cover the smear with 5% aqueous solution of malachite green.
3. Heat till vapour arise (avoid boiling of stain) let it for 5 - 10 min. Wash with clean water.
4. Apply 0.5% safranin or 0.05% basic fuchsin for 2 min.
5. Wash, dry, put one drop of oil and see with X100 (oil immersion lens).

Result:

- Spores appear ……. green.
- Vegetative bacilli ……red.

Report:

Comment:

Question:

Define spores, mention their types, and set example for each type? ……..…..

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Practical No. (9)

Sterilization

The effect of the moist heat on the bacteria:

1- Pasteurization:

1. Inoculate a tube containing 5 ml milk with Salmonella, mix well and inoculate a plate of MacConkey agar with the milk, incubate at 37^0C for 24 h.
2. Incubate a tube on water path at 63^oC for 30 sec, inoculate a plate of MacConkey agar with the pasteurilized milk, incubate at 37^oC for 24 hrs.
3. Comment on the growth of the bacteria on the two plates.

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2- Moist heat at 100oc (boiling):

1. Inoculate two tubes of nutrient broth with E.coli and Bacillus.
2. Incubate the tubes on water path at 100^oC for 10 mins, inoculate a plate of MacConkey agar with the two organisms, incubate at 37^oC for 24 hrs.
3. Comment on the growth of the bacteria on the two plates.

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3- Moist heat above 100oC (Autoclave):

1. Inoculate two tubes of nutrient broth with E.coli and Bacillus.
2. Autoclave at 121^oc for 15 mins, inoculate a plate of MacConkey agar with the two the organism and incubate at 37^oC for 24 hrs.
3. Comment on the growth of the bacteria on the two plates.

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Practical No. (10)

Disinfections

The effect of concentration and time on the action of disinfections:

Requirements:

1. Cultured plates of *Escherichia coli*, and *Staphylococcus aureus*.
2. Three plates of MacConkey agar.
3. Sterile distilled water
4. Concentrated ethyl alcohol.
5. Sterile graduated pipettes.
6. Sterile universal container.

Method:

1. Make serial dilution from concentrated alcohol on sterile universal containers using the distilled water as follows:

Distilled water (ml)	4	3	1.5
Alcohol (ml)	1	2	3.5
% of dilution	20%	40%	70%

2. Label each plat by the one of these dilutions and divide the plate to 4 parts, everyone has a time begin from zero, 10, 20, and 30 minutes respectively.
3. Select 2 colonies from the given strains and inoculate them in the diluted alcohol starting with dilution 10%, mix well and inoculate in the part of zero time do the same with other dilution.
4. Inoculates every dilution (on periods) according to the time of every part.
5. Incubate at 37^{o}C for 24 hrs and comment on the growth of the bacteria on the plates.

Comment:……

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Practical No. (11)

Collection and transportation of Microbiological specimens (1)

1. blood for culture

Site of collection: peripheral venous or arterial

Time: When body T^{o} begin arise.

Technique:

- Select the vessel (site).
- Disinfect the skin by swabbing with 70% alcohol.
- disinfect again with 2% tincture iodine.
- Allow iodine to dry completely before performing venipuncture. This should take 1 - 2 minutes.
- Decontaminate the rubber stopper of blood culture bottle with 70% alcohol.
- Collect 5 - 20 ml of blood using a sterile syringe and needle.
- Insert needle through the rubber of the bottle cap and add the blood aseptically

2. Sputum

- give the patient clean (doesn't need to be sterile), dry wide nicked leak-proof container, and request him cough deeply to produce sputum specimen.
- **Time:** early morning specimen before any mouth wash is the best.
- If Mycobacterium tuberculosis is suspected, three early morning sputum specimens are require.

- When pneumonia or bronchopneumonia is suspected, process sputum as fast as possible due to presence of fastidious organisms .
- Transportation: Transfer a purulent part of sputum to a cotton-wool swab and insert it into a container of Amies transport medium and send to micro-lab within 6 hrs.

3. Cerebrospinal fluid (CSF)

- Advise the laboratory before collection
- CSF is obtains through a lumbar puncture between L4 and L5
- Lay the patient on his side, with the knees pull up toward the chest.
- Disinfect the back and inject a local anesthesia into the lower spine.
- Insert a spinal needle into the lower back area and collect 1 - 2 ml first sample for microbiology and 2- 3 ml second sample for other investigations in sterile transparent screw caped container.
- Remove the needle, clean the area, and place a bandage over the needle site.
- Ask the patient to lie down for a short time.
- Transportation: CSF must be cultured immediately, when a delay is unavoidable keep CSF at 37^{o}C

4- Throat and mouth swab:

1. In a good light , use the tongue depressor to examine inside the mouth.

- **With sore throat:** look for the inflamed tonsil (large with yellow spots).
- **With diphtheria:** look for greyish-yellow green black membrane extending forwards over the soft palate and backwards onto the pharyngeal wall.

- **With Candidiasis (oral thrush):** look for patches with white exudate.
- With Vincent's angina: look for ulceration in mouth lips or throat.

2. Swab the affected area using a sterile cotton-wool swab.
    - Taking care don't contaminate the swab with saliva.
    - For 8 hrs before swabbing the patient mustn't be treated with antibiotic or antiseptic mouth wash
- **Transportation:** Insert the swab in a container of Amies transport medium and send to micro-lab within 6 hrs, or in tube containing silica gel for up to 3 days .

5. Pus from wound and abscesses:

- Using a sterile technique aspirate or collect from a draining tube up to 5ml, and transfer to leak-proof sterile container.
- when pus is not being discharged us a sterile cotton-wool swab to collect sample from infected site.
- Transportation: Transport in Amies transport medium to micro-lab within 6 hrs.

✓ Collect the sample before antiseptic dressing is applied.

✓ Avoid contaminating the specimen with skin flora.

✓ Collection from abscesses is best done by sterile syringe, or after rupturing naturally or surgically.

Practical No. (12)

Collection and transportation of Microbiological specimens (2)

6. Urethral discharge from male patients:

- Cleans around the urethral opening using a swab moistened with sterile physiological saline
- Gently massage the urethra from above downwards
- Using a swab collect a sample of discharge.
- In case of urethral swab insert a swab 20 mm into urethra and rotate to collect sample.
- Collect sample at least after 2 – 4 hrs after urination
- Transportation: Transport in Amies transport medium to micro-lab within 6 hrs.

7. Cervical specimens from female patients:

- Moist the sterile speculum with sterile warm water.
- Clean the cervix using a swab moistened with sterile physiological saline
- Pass a sterile cotton-wool swab 20-30mm into the endocervical canal and gently rotate the swab against endocervical wall to obtain the specimen .
- Transportation: Transport in Amies transport medium to micro-lab within 6 hrs.

8- Faeces:

- Give the patient a clean, dry, leak-proof, disinfectant free, bedpan, transparent, wide mouth container (doesn't need to be sterile) for passing the specimen.

- Transfer a portion of specimen that contains mucus pus and or blood into another container.
- Transportation: Transfer a portion of specimen to Cary-Blair transport medium and transport to micro-lab within 48 hrs.

9. Urine

- Male:
- Wash hands with soap and water and dried with a paper towel.
- Pass a first voided urine and collect about 20 -30 ml a mid stream urine (MSU) into a sterile, transparent wide-necked screw-cap plastic cup.
- Female:
- Wash hands with soap and water and dried with a paper towel.
- Cleans the area around the urethral opening with clean water from front to back. and dried with a paper towel.
- With one hand spread labia and keep continuously apart until the urine is voided into a sterile screw-cap container.
- Transportation: Refrigerate at 4oC for up to 6hrs, or add (1%w/v) boric acid to container and transport to micro lab within 48 hrs.

10- Effusions (Synovial, Pleural, Pericardial, Peritoneal, and Hydrocele)

- After aspiration dispense 1 - 2 ml for microbiology and 2- 3 ml for other investigation into sterile transparent screw-capped container.

Practical No. (13)

Preparation of culture media (1)

Aim:

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Requirements:

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Method:

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Comment:

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Practical No. (14)

Preparation of culture media (2)

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Method:

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Comment:

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