NATIONAL UNIVERSITY-SUDAN

Faculty of Medical Laboratory Sciences

Student Practical Manual- Microbiology Department

Fourth year, Semester (7)
Bacteriological Techniques (MLS-MICR-412)

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

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

Instructions

- Wear lab coat
- Wear Gloves
- Avoid swallow any chemical
- Follow the procedures provided
- Write your results in this manual

Content

Practical NO	content	Page
1	**Blood culture**	1
2	**Central nervous s**	7
3	**Upper respiratory tract infection**	11
4	**lower respiratory tract infection**	15
5	**lower respiratory tract infection (pulmonary TB)**	19
6	**Stool examination**	21
7	**Urine examination**	26
8	**Wound infection**	33
9	**Ear infection**	36
10	**Eye infection**	39

Practical No. (1):

Blood culture

Day one

Blood for culture is requested in case of Bacteraemia (Enteric fever, Brucellosis, Meningitis, Osteomyelitis, Arthritis, Bacterial Pneumonia, or Occult abscess), Septicaemia, Endocarditis Pyrexia of Un Known Origin (PUO).

Collection:.

Blood should be collected.

- Before antimicrobial treatment has started.
- When temperature begins rise.
- Tow specimens to increase the chances of isolating pathogens.
- A septic technique.
- Site of collection: peripheral venous or arterial

Technique:

- Select the vessel (site).
- Disinfect the skin by swabbing with 70% alcohol.
- Disinfect again with 2% tincture iodine.
- Allow the 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.

Prevention of clotting can be done by:

- Adding Sodium Polyanethole Sulphonate (SPS) (It also inhibits the antibacterial effect of serum, antimicrobial agents, and phagocytes (Neutralize component)).
- Adding a sufficient volume (50 ml) of broth and thoroughly mixed to prevent (dilution1:10 or more) (It also inhibits the antibacterial effect of serum, antimicrobial agents, and phagocytes (Neutralize component)).

Media:

- Brain heart infusion broth (aerobic).
- Columbia agar and Columbia diphasic media (aerobic).
- Thioglycollate broth (anaerobic).

Incubation:

Incubate blood-culture bottles at 35 – 37°C for signs of microbial growth.

Sterile culture usually shows:

A layer of sedimented red blood covered by a pale yellow transparent broth.

Growth is evidenced by:

- A floccular deposit on top of the blood layer.
- Uniform or subsurface turbidity.
- Haemolysis.
- Coagulation of the broth.
- A surface pellicle.
- Production of gas.
- White grains on the surface or deep in the blood layer.

Sub culture:

Whenever visible growth appears, open the bottle aseptically, aseptically remove a small amount of broth with a sterile syringe aseptically.

- Subcultures from aerobic culture on blood agar, heated blood agar and MacConkey agar. Incubate blood agar, and MacConkey aerobically at 35 – 37°C over night, Incubate heated blood agar in CO_2 at 35 – 37°C over night.
- Subcultures from anaerobic culture on blood agar, incubate anaerobically at 35 – 37°C over night,
- Examine Gram-stain smear for the presence of microorganisms.

Blind subcultures:

Some microorganisms may grow without producing turbidity or visible alteration of the broth. Routine subcultures after 18 - 24 hours, 72 hours of incubation, and at the end of 7 days of incubation.

Incubation time:

For routine examinations, it is not necessary to incubate blood cultures beyond 7 days. In some cases, incubation may be prolonged for an additional 7 days, e.g. if Brucella or other slow growing organisms are suspected, or in cases of endocarditis.

Day two

Colonial morphology:

	Blood agar	Chocolate BA	MacConkey agar

Indirect Gram stain result:

………………………………………………………………………………

……………………………………………………………………………….

Biochemical tests:

Name of the test	Requirement	Method of inoculation and incubation

Day three

Biochemical tests result

Name of the test	Comment	Result

Final report of the isolated organism:

…………………………………………………………………………………………………

…………………………………………………………………………………………………

…………………………………………………………………………………………………

Name and signature of the instructor:………………………………………………………

Date:………………………………………………………………………………………

Practical No. (2):

Microbiological examination of Cerebrospinal Fluid (CSF)

Day (1)

Specimen: CSF

Collection:

- Aseptically by experienced medical.
- A sterile wide bore needle is inserted between 4th and 5th lumber vertebrae and allowed to drip into two dry $sterile containers
- Sample 1 (about 1ml) for culture and sample two (about 2-3 ml) for cells count, sugar and proteins estimation.

Macroscopical examination:

Report the appearance of the CSF whether the fluid:

- Is **clear**: like distilled water indicates normal CSF
- **Purulent:** indicates presence of pus suggestive of acute pyogenic meningitis.
- **Contains blood**: May due to traumatic lumber puncture or less commonly to haemorrhage in CNS. When due to a traumatic lumber puncture, sample 1 will usually contain more blood than sample two. Following subarchnoid haemorrhage the fluid may appear xanthochromic.
- **Contains clots**: indicates high protein concentration with increased fibrinogen or when there is spinal constriction.

Results of macroscopy:

....
....
....
....

Processing:

Depending on the appearance of the CSF proceed as follow:

Purulent or cloudy CSF

- Immediately make and examine a Gram stained smear for bacteria and polymorphnuclear neutrophilis (fixation should be done by using ethanol instead of heat)
- Culture the CSF on blood agar and heated blood agar.

Slightly turbid or clear CSF:

- Perform a cell count and note whether there is an increase in white cells (neutrophils or lymphocytes).

When the cells predominantly pus cells:

- Examine a Gram stained smear for bacteria
- Examine a wet preparation from centrifuged sample for motile amoebae
- Inoculate the specimen on blood agar, heated blood agar

When cells predominantly lymphocytes

- Measure the concentration of protein.
- Measure the concentration of glucose.
- Examine a wet preparation for encapsulated yeast cells (C.pneuformans), and trypanosomes.

Report the CSF as normal when it appears clear, contains no more than 5×10^6 WBC

Microscopical examination:

Cell count:

……
……
……

Gram smear and stain:

- Mix sample 2 and centrifuge at 1000 g for 5- 10 min
- Transfer several drops of sediment to a slide; don't make the preparation too thick because this will make it difficult to decoluorize.
- Fix the smear with ethanol and stain with Gram stain
- Examine the smear microscopically for pus cells and bacteria using the 40X and 100X objectives.
- Pus cells are reported as many, moderate, or few number.
- Do not centrifuge a purulent fluid

Report:……………………………………………………………………………………………………
……...
...
………
…

Inoculation and incubation:

- On heated blood agar, and blood agar. Incubate both plates in a CO2 at 37 0C.
- Add an optochin disc on blood agar to assist the identification of S. pneumoniae
- When patient is a newborn infant inoculate the specimen, also on MacConkey.

	Appearance	Cells/L	Glucose mg/dl	Proteins mg/dl
Normal CSF	Clear	< $5X10^6$ (pus)	45 – 72	**15 – 40**
Pyogenic meningitis	Purulent	200 - 20,000 (lymph)	Very low < 40	**High > 50**
Viral meningitis	Clear or slightly turbid	100 – 1000 (lymph)	Usually normal	**Moderately high**
Cryptococcal meningitis	Clear or slightly turbid	100 – 1000 (lymph)	Low	**High**
Tuberculous meningitis	**Usually clear**	**25 – 20000 (lymph)**	**Low**	**High**

Common causes of bacterial and fungal meningitis

1. **In neonates (from birth to 2 months):** *Escherichia coli, Listeria monocytogenes,* Other Enterobacteriaceae (*Salmonella spp., Citrobacter spp*) and *Streptococcus agalactiae* (group B).
2. **In all other age groups:** *Haemophilus influenzae* (capsular type b)[a] , *Neisseria meningitides, Streptococcus pneumonia, Mycobacterium tuberculosis, Listeria monocytogenes* [b], *Cryptococcus neoformans* [b], Staphylococci [c].

a Uncommon after the age of 5 years.
b In immunocompromised patients.
c Associated with neurosurgery and postoperative drains.

Day (2)

Colonial morphology:

	Blood agar	Chocolate BA	MacConkey agar

Indirect Gram stain result:

…………………………………………………………………………………

…………………………………………………………………………………..

Biochemical tests:

Name of the test	Requirement	Method of inoculation and incubation

Day three

Biochemical tests result

Name of the test	Comment	Result

Final report of the isolated organism:

……………………………………………………………………………………………………

……………………………………………………………………………………………………

……………………………………………………………………………………………………

Name and signature of the instructor:……………………………………………………………

Date:………………………………………………………………………………………………

Practical No(3)

Upper Respiratory tract infections`

1.Pharyngitis, sometimes involving tonsillitis, and giving rise to a "sore throat"

Causative agents: (80% viruses 20% bacteria):

- *Streptococcus pyogenes* (> 50% of all cases in children, about 10% in adults . Symptoms: pain of throat- fever, headache, neutrophilia, difficulty in swelling. Can be complicated to post streptococcal infection.
- Groups B, C and G β haemolytic streptococci.
- Coliforms, *S. aurues* and *Pseudomonas spp.* if present in predominant growth.
- Viruses (rhinoviruses and corona viruses (common cold viruses), denoviruses, herpes simplex, parainfluenza viruses, influenza viruses, coxsackie virus A, Epstein–Barr virus, cytomegalovirus)

Normal flora of the pharynx includes:

- Viridans (a-haemolytic) streptococci (60%)and non capsulated pneumococci.
- Nonpathogenic *(Neisseria spp*., *Haemophilus spp* and *Moraxella catarrhalis)*.
- Staphylococci (*S. aureus, S. epidermidis*).
- Diphtheroids (with the exception of *C. diphtheriae*).
- Yeasts (*Candida spp.*) in limited quantity
- Various strictly anaerobic G +ve cocci and G -ve rods, Spirochetes and filamentous forms.

Tonsillitis (Throat swab):

Collection: In a good light , use the tongue depressor to examine the inside the mouth look for the inflamed tonsil (large with yellow spots). Swab the affected area using a sterile cotton-wool swab. Taking care not to contaminate the swab with saliva. For 8 hrs before swabbing the patient must not 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 .

Culture: Inoculate swab on blood agar media (make also few stabs). IF swab received in silica gel moisten first with sterile nutrient broth and inoculate on blood agar media. Add 0.05 unit bacitracin disc to the plate, and incubate plate (preferably anaerobically) over night at 37°C.

2. Diphtheria

- *Corynebacterium diphtheriae* cause a typical form of infection, characterized by a greyish-white membrane at the site of infection (pharynx, tonsils, nose, or larynx).
- Diagnosis is made on the basis of clinical findings, culture request for confirmation.
- **Culture:** Inoculate tellurite blood agar, Identify, and perform toxigenicity test (Elek)

3. **Vincent angina**:

A necrotic ulceration of the pharynx with or without formation of a pseudomembrane. It is associated, at the site of infection, with a heavy mixed flora of strict anaerobes dominated by Gram-negative fusiform rods and spirochaetes, generally referred to as *Fusobacterium spp*. and *Treponema vincentii*, and possibly others. Although both species belong to the normal mouth flora, their presence in large numbers in a Gram-stained smear of ulcerated lesions should be reported as a "fusospirochaetal complex". This microscopic diagnosis need not be confirmed by anaerobic culture, which is difficult and time-consuming. However, the

presence of this complex does not exclude the need to search for other pathogens, particularly *S. pyogenes*.

4. **Oral thrush:**

   Although small numbers of *C. albicans* or other Candida species may be part of the normal oral flora, oral candidiasis r esults when the number of organisms increases considerably in certain pathological conditions, e.g. in malnourished premature babies, in immunodeficient adults (e.g. patients with HIV/AIDS), or in patients who have received broad-spectrum antimicrobials or cancer therapy. The affected area - tongue, tonsils, throat o r buccal mucosa - may be extremely red, or covered with white patches or a confluent grey- white membrane (thrush). The diagnosis of candidiasis is best made by finding numerous yeast cells, some of them forming long mycelium-like filaments, in a Gram-stained smear of the exudate.
   Culture: Inoculate swab on blood agar media or SDA.
5. **Otitis media**
6. **Sinusitis**
7. **Epiglottitis:**

   cause by *H. Influenzae* serotype B it's mainly infection of children less than 5 years. Rare and serious because it can obstruct the air passage & cause severe septicaemia-reduced b vaccine. Can be caused by other: *Haemophilus spp, S. pyogens, S. Pneum oniae, S. aureus*

Swabs from the URT to detect a pathogen in a healthy subject, a pharyngeal or a nasal (carrier):

• *Staphylococcus aureus* (MRSA) (20–40%)..
• *Neisseria meningitides* (20% or more)
• *Streptococcus pyogenes* (20–30%).
• *Corynebacterium diphtheria* (Carrier rate high in non-vaccinated populations).

Identification:

- Look for small beta haemolytic colonies sensitive to bacitracin.
- Do gram stain, catalase test and Lancefield grouping.
  - *S. aureus*, other beta haemolytic streptococci, some coliform and *Pseudomonas spp*. can cause tonsillitis
  - **Colonial morphology:**

	Blood agar	Chocolate BA	MacConkey agar

- **Indirect Gram stain result:**
- ………………………………………………………………………………………
  ……………………………………………………………………………………..
- **Biochemical tests:**

Name of the test	Requirement	Method of inoculation and incubation

- **Day three**

- **Biochemical tests result**

Name of the test	Comment	Result

- **Final report of the isolated organism:**
- **……………………………………………………………………………………………**
  ……………………………………………………………………………………………
  ……………………………………………………………………………………………

Name and signature of the instructor:…………………………………………………………

Date:………………………………………………………………………………………………

Practical No. (4)

Lower respiratory tract infections (Sputum Culture)

Collection:

- Give the patient clean (need not be sterile), dry wide nicked leak-proof container, and request him to cough deeply to produce sputum specimen and should be sent to the laboratory without delay. If the sputum is allowed to stand after collection, overgrowth of contaminating bacteria may take place.
- Time: early morning specimen before any mouth wash is the best.
- Amies transport media can be used (send the swab and sputum specimens within 6 hours).

Macroscopy:

- Colour: green, yellow, grey, white, or blood-stained.
- Consistency: purulent (green looking (mainly pus)), muco-purulent, mucoid (mainly mucus), frothy, or watery (i.e. only saliva present), muco-salivary. Salivary or muco-salivary samples should be rejected

Report of macrosopical examination:

…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………

Microscopy:

- A portion of the purulent or mucopurulent sputum should be used for the preparation of a Gram-stained smear and ZN stained smear.
- Reject any specimen that contains fewer than 10 polymorphonuclear neutrophils per epithelial cell.
- **Guidance of Gram stain:**
  - Gram-positive diplococci surrounded by an empty space (unstained capsules) (*S. pneumoniae*).
  - Gram-positive cocci in chains (*S. pyogens*).
  - Small Gram-negative coccobacilli (*H. influenzae*).
  - Gram-negative diplococcic, or coccobacilli, intracellular and extracellular (*M. catarrhalis*).
  - Gram-positive cocci in grape-like clusters (*S. aureus*).
  - Gram-negative rods (Enterobacteriaceae or *Pseudomonas spp*.).
  - large Gram-positive yeast-like cells, often with mycelia (*Candida spp*.).

Rreport of microscopical examination:

…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………

Culture:

- Select a purulent material, wash with 5 ml sterile normal saline to reduce contamination, (liquefy mucoid sputum with dithiothreitol).
- Using a sterile swap or loop inoculate: blood agar, chocolate agar with an optochin disc and MacConkey agar.
- Incubate blood agar and MacConkey aerobically, and chocolate agar in CO_2 at $37^{O}C$ over night.
- If clusters of Gram-positive cocci were present in the smear add (MSA), incubate O_2 at $37^{O}C$.
- If Gram positive, yeast seen in smear add Sabouraud dextrose agar, incubate O_2 at $37^{O}C$ for 72 hrs.

Day two:

- **Colonial morphology:**
- **Guide of interpretation of colonial morphology:**

Interpretation of growth:

- Flat, clear colonies with concave centres and zones of green (α) haemolysis, (optochin sensitive) on chocolate agar may be *S. pneumoniae*.
- Very small (β) haemolytic colonies on blood agar may be *S. pyogens*.
- Tiny, water-drop colonies growing as non-haemolytic chocolate agar and very small colonies on blood agar, suggest the presence of *H. influenza*, confirm this by X and V factor dependence tests.
- Brittle, dry, grey-white colonies on blood agar and chocolate agar plates that can be moved intact with a loop may indicate *M. catarrhalis*. Confirm by a set of sugar tests (all results negative).
- Medium-sized, golden-buff colonies are formed by *S. aureus*.
- Colonies on MacConkey suggest that Enterobacteriaceae, *Pseudomonas spp*., or *Acinetobacter spp*.
- Whitish, round, matt colonies on the blood agar and chocolate agar may be *Candida albicans* also on Sabouraud dextrose agar.++++

- **Colonial morphology:**

	Blood agar	Chocolate BA	MacConkey agar

- **Indirect Gram stain result:**
- ……………………………………………………………………………………
  ……………………………………………………………………………………

- **Biochemical tests:**

Name of the test	Requirement	Method of inoculation and incubation

- **Day three**

- **Biochemical tests result**

Name of the test	Comment	Result

-
- **Final report of the isolated organism:**
- ……………………………………………………………………………………………
  ……………………………………………………………………………………………
  ……………………………………………………………………………………………
- **Name and signature of instructor:**……………………………………………………………
- **Date:**…………………………………………………………………………

Practical No. (5)

Lower respiratory tract infections (Pulmonary tuberculosis)

Collection:

- Give the patient clean, dry wide nicked leak-proof container, and request him to cough deeply to produce sputum specimen and should be sent to the laboratory without delay. If the sputum is allowed to stand after collection, overgrowth of contaminating bacteria may take place.
- Time: early morning specimen before any mouth wash is the best.

Macroscopy: Salivary or mucosalivary samples should be rejected

Microscopy:

- **Zeihl Neelsen stain.**
- **Auramine phenol fluorochrome stains.**
- **Sodium hypochlorite (bleach) (concentrated Zeihl Neelsen stain) (for microscopy only):**

1. Transfer two ml sputum to screw cap centrifuge tube (15 - 20 ml capacity) and add equal volume of concentrated sodium hypochlorite, mix well, and leave at room temperature for 15 min, shake at intervals to breakdown the mucus in sputum.
2. Add 8 ml of distilled water and mix well, centrifuged at 3000 rpm for 15 min, remove supernatant mix the sediment, and spread to make a thin smear and allow to air dry.
3. Heat Fix and stain by ZN staining technique and examine microscopically for AFB.

Culture:

Concentration, digestion, and decontamination:

This procedure liquifies mucoid sputum while destroying the contaminating organisms. However, sodium hydroxide is also toxic for mycobacteria, and care must be taken to ensure that:

- the final concentration of NaOH does not exceed 2%;
- the tubercle bacilli are not exposed to sodium hydroxide for more than 30 minutes, including centrifugation time.

1. Mix equal volumes of sputum and 4% sodium hydroxide 40 g/l (previously sterilized by autoclaving) in a sterile, leak-proof, 50-ml glass bottle or jar, or plastic conical centrifuge tube.
2. Incubate at room temperature (25 - 30°C) for 15 minutes, shaking the mixture carefully every 5 minutes using a mechanical shaker. In hot climates some cooling may be needed or the reaction time may be reduced to 10 - 15 minutes.
3. Centrifuge immediately or dilute the mixture to the 50 ml mark with distilled water or phosphate buffer (pH 6.8) to stop the action of the NaOH.

4. After 15 minutes, centrifuge the specimen at 3000g for 15 minutes. Discard the supernatant carefully into a splash-proof container filled with a suitable disinfectant (phenol - or glutaraldehyde-based).
5. Neutralize the sediment drop by drop with a 2 mol/l HCl solution containing 2% of phenol red, combined with shaking, until the colour changes persistently from red to yellow. Alternatively add one drop of indicator solution and then add HCl drop by drop while shaking continuously.
6. If the media is to be inoculated immediately, suspend the neutralized deposit in 1 - 2 ml of sterile 0.85% NaCl or sterile distilled water. Otherwise, suspend the sediment in 1 - 2 ml of sterile bovine albumin fraction V.

Inoculation:

1. Inoculate 3 drops (about 0.1 ml) of the sediment onto at least three plates of Löwenstein–Jensen medium or equivalent.
2. Determine the contamination rate of the incubated media regularly and record the number of contaminated plates.
3. The rate of contamination should be 3 - 5%. Excessive contamination (over 5%) of the Löwenstein–Jensen cultures usually indicates that the decontamination procedure was not effective enough. Contamination rates of <3% suggest that the decontamination procedure was too vigorous and mycobacteria present in the samples may fail to grow.

Interpretation of cultures for *M. tuberculosis*

The tubes containing the Löwenstein–Jensen medium should be incubated for 2 - 3 days at 35 - 37°C in a horizontal position, with the tops loosened half a turn. The culture tubes should then be stored at 37°C for six weeks and inspected for growth at weekly intervals. During these weekly inspections, the growth of any colonies of bacteria on the surface should be noted. A smear should be carefully made and stained by the Ziehl–Neelsen procedure. If the organisms are not acid-fast bacilli, then the culture may be recorded as contaminated.

Typical human strains of *Mycobacterium tuberculosis* are "rough, tough and buff", and can sometimes be seen after 2 - 3 weeks of incubation (but seldom earlier). Bovine strains (*M. bovis*) are generally smooth and whitish-cream in colour. Other, generally nonpathogenic, mycobacterial species may grow more quickly (sometimes after only several days) and may or may not produce pigmented growth (red, yellow, or orange). If an isolate has the typical colonial appearance and the Ziehl–Neelsen stained smear from a colony is also typical, it should be reported as "*Mycobacterium spp*., probably *M. tuberculosis"*; the isolate should be process for identification and susceptibility testing.

Practical No. (6)

Microbiological Examination of Stool

A 2 -years- child was admitted to KTH with diarrhea containing mucus and blood. You are provided with stool specimen taken from this patient, proceed to isolate and identify the causative agent

Collection:

- **Faeces:** Give the patient a clean, dry, leak-proof, disinfectant free, bedpan, transparent, wide mouth container (need not to be sterile) to pass a specimen. Transfer a portion of specimen that contain mucus pus or blood into another container and proceed within 2 hrs.
- **Rectal swab:** Moisten a cotton-tipped swab with sterile water. Insert the swab through the rectal sphincter, rotate, and withdraw. The number of swabs to be collected will depend on the number and types of investigations required.
- **Transportation:** Transfer a portion of specimen into sterile swab to Cary-Blair transport medium and transport to micro-lab within 48h (Salmonella, Shigella, Vibrio, and Yersenia survive well for up to 48 hours, and Capmylobacter for up to 6 hours).Alkaline peptone water: when cholera is suspected. Viruses: Use virus transport media (VTM). Campylobacter: campy - thioglycolate broth.

Macroscopical examination: Examine the stool sample visually and record the following:

- Consistency (formed, unformed (soft) or liquid).
- Colour (white, yellow, brown or black).
- Presence of any abnormal components (e.g. mucus, blood, segments, and or worms).
- Unformed or semi-formed containing pus mucus mixed with blood: The suspected infections are: shigellosis, EIEC dysentery, Campylobacter enteritis, amoebic dysentery (acid pH), shistosomiasis and salmonella infection
- Watery diarrhea: ETEC, EPEC diarrhea, cholera (rice watery), rotavirus, enteritis, cryptosporidiosis and salmonella infection.
- Bloody diarrhea without pus cells: EHEC 0157 infection.
- Fluid stool with pH bellow 6: lactose deficiency.
- Black unformed: GIT bleeding, Hook-warms disease or iron therapy.

Report of macroscopical examination:

………………………………………………………………………………
………………………………………………………………………………
………………………………………………………………………………

Microscopical examination:

1. Wet preparation:

- Place a small portion of the stool specimen or rectal swab in a drop of normal saline on a clean slide and mix thoroughly.`
- Place a cover slide on the suspension, avoiding the formation of air bubbles.
- Examine the slide under the microscope using the high-power objective lense.
- Report: pus cell, RBCs, motile bacteria (*V. cholera* and *Campylobacter*), protozoa, ova and eggs of parasites.

2. Methylene blue stain for faecal leucocytes:

- Place a small portion of the stool specimen or rectal swab in a drop of 0.05% methylene blue solution on a clean slide and mix thoroughly.
- Place a cover slide on the stained suspension, avoiding the formation of air bubbles, wait 2 - 3 mins.
- Examine the slide under the microscope using the high-power objective lenses.
- Report cells that can be clearly identified as mononuclear or polymorphonuclear; ignore degenerated cells.
- Clumps of polymorphonuclear leukocytes (>50 cells per high-power field), macrophages and erythrocytes are typical of shigellosis.
- Smaller numbers of polymorphonuclear leukocytes (<20 cells per high power field) are found in salmonellosis, and invasive *E. coli*.
- In amoebic dysentery the cells are mostly degenerated (ghost cells).
- Leukocytes and erythrocytes are also found in about half the cases of diarrhoea due to *Campylobacter sp*.
- Few leukocytes (2 – 5 cells per high-power field) are present in cases of cholera, enterotoxigenic and enteropathogenic *E. coli*, and viral diarrhoea.

3. Diluted Carbol fuchsin stain for Campylobacter:

- Make a thin dry fixed smear from stool specimen on a clean slide.
- Stain the smear with diluted carbol fuchsin stain (1 in 20) for 2–3 minutes.
- Examine the slide under the microscope using the high-power objective.
- Report the sea gull shape of campylobacter if present.

4. Motility and immobilization test for *V. cholera:*

Enrichment Media

- Selenite F or tetrathionate broths are recommended for the enrichment of S*almonella sp*.
- Alkaline peptone water (APW) for the enrichment of *V. cholerae.*
- Enrichment is not required for *Shigella sp., Campylobacter sp*., *Yersinia enterocolitica* and *Clostridium difficile*.

Report of direct microscopical examination:

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

Inoculation of enrichment media:

- Suspend 1 g of the stool sample in a tube containing 1 ml of sterile saline. If the stool sample is liquid, saline is not needed to be added. Rectal swabs received fresh or in Cary–Blair medium should be rinsed thoroughly in 1 ml of saline. Before removing the swab, press it against the side of the tube to express any remaining fluid.
- Add three or more loopfuls of faecal suspension to the enrichment broth.
- Incubate selenite F broth for 18 hours and APW for 6 – 8 hours.
- Subculture by streaking a loopful of broth on selective stool media.

Media for enteric pathogens:

- Blood agar (supportive media) supports the growth of any bacteria causing GIT infection (Yesinia, Aeromonas, and Plesidomonas).
- Xylose–lysine–deoxycholate (XLD) agar for the isolation of *Shigella sp.* and *Salmonella sp.*
- Deoxycholate citrate agar (DCA), or Salmonella – Shigella (SS) agar is suitable alternatives.
- *S. dysenteriae* type 1, *S. sonnei* and enteroinvasive *E. coli* do not grow well on SS agar.
- *For Campylobacter sp*: (Blaser, Butzler, Skirrow) containing different antimicrobial supplements.
- Thiosulfate citrate bile salts sucrose (TCBS) agar is selective for *V. cholerae* O1 and non-O1 and for *V. parahaemolyticus*.
- ETEC, EPEC: MacConkey Agar (for small children less than 2 years).
- EHEC: Sorbitol Mac. A (either NSF or late Sorbitol for mental).

Incubation of plates:

- After inoculation, incubate the plates for the isolation of Salmonella, Shigella,*Yersinia sp*. and *V. cholerae* at 35°C aerobically. Incubate the plates for

Campylobacter sp. at 42°C in microaerophilic atmosphere with 10% CO_2 for 24 h.

- **Colonial morphology:**

	DCA agar	XLDagar	SS agar

-
- **Indirect Gram stain result:**
- …………………………………………………………………………………
…………………………………………………………………………………
………………………………………………………………………………..

- **Biochemical tests:**

Name of the test	Requirement	Method of inoculation and incubation

- **Day three**

- **Biochemical tests result**

Name of the test	Comment	Result

-

- **Final report of the isolated organism:**

………………………………………………………………………………………………………

………………………………………………………………………………………………………

………………………………………………………………………………………………………

Name and signature of the instructor:………………………………………………………

Date:…………………………………………………………………………………………

Practical No. (7):

Urinary Tract Infection **(Microbiological Examination of urine)**

Day (1)

Collection of voided urine for culture ("clean-catch" techniques)

Specimen: Mid stream urine(MSU) in aplstic universal container.

Collection:

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 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.

Children and young infants:

- Urine from bag held with adhesive tape over genitalia (contamination)
- Suprapubic aspiration of urine directly from bladder.
- None invasive method by stmulating urine flow in the baby by tapping just above the pubis with fingers at one hour after feed.

catheter:

- A sterile, polyurethane catheter tube is inserted into the urethra and passed up into the bladder. The urine drains through the catheter tube and is collected in a sterile specimen container

Transportation:

1. Refrigerate at 4°C for up to 6hrs,or
2. add (1%w/v) (0.1g/ml of urine) boric acid which inhibits bacterial

a. growth in the sample for up to 12 hours at room temperature hrs. To preserve bacteria without multiplying, white cells, red cells and cast are also preserved there is no interference in themeasurement of urine protein and glucose.

3. Urine for culture must not be preserved with a bactericidal chemical such as thymol, bleach, hydrochloric acid, acetic acid, or chloroform.

- **Causes of specimens rejection:**
- Bedpans
- bags of catheterized patients
- leaky containers
- unlabelled specimens
- unrefrigerated, unpreserved specimens over 2 hours old
-

Macroscopical examination:

- **Colour**
- **Turbidity.**

Report of Macroscpical examination:

……………………………………………………………………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

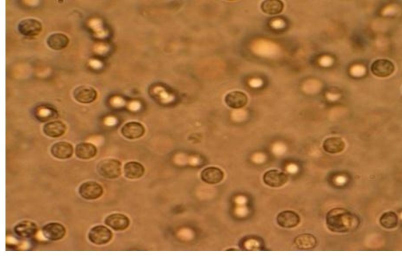
……………………………………………………………………………………………

……………………………………………………..

Microscopical examination (Un-centrifuged wet preparation):

- Place a well mixed drop of urine on slide, cover with cover glass and examine using low power field.
- One cell/low power field = 3 cells/µl, significant pyuria ≥10 cells or more/µl ($\geq 10^4$ pus cells/ml)

Results:



Culture:

- On CLED, Incubate plate O_2 at 37 ^{0}C for 24 hrs.
- Or on blood agar, and MacConkey agar . Incubate both plates in O_2 at 37 ^{0}C for 24 hrs.

Significant bacteruria:

Bacterial count:

- Normal urine specimen may contain small number of bacterial less than 10^4/ml of urine
- The approximate number of bacteria per ml of urine can be estimated by using calibrated loop or measured a piece filter paper.
- Both methods are based on the accepting that a single colony represents one organism.

Interpretation of urine cultures:

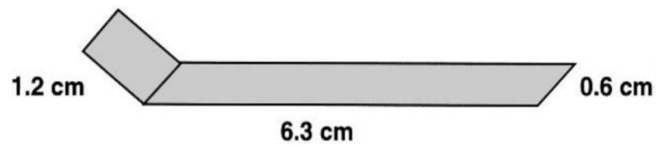
- The presence of bacteria is urine is called bacteruria. Significant bacteruria is defined by Kass as the presence of 10^5 or more colony forming units of bacteria per millilitre of urine (cfu/ml) on culture and it is a stro ng evidence of UTIs.
- If found between 10^4 and 10^5 cfu/ml on culture, interpret as significant doubtful and further specimen should be obtained.
- If found less than 10^4 cfu/ml, this regard as contamination (unless the patient is on treatment for a known UTI). The contamination of specimen is characterized by growth of two or more bacterial species accounting for less than 10^7 bacteria / L and the presence of squamous epithelial cells from the peri-urethra.

Counting using calibrated loop

- The calibrated loop size may be 1 µl (0.001 ml), 2 µl (0.002 ml), or 4 µl (0.004 ml).
- If 100 colonies are counted and a 1µl calibrated loop was used , the approximate number of CFU per ml of urine: 1000 x 100 = 10^5CFU per ml. Such account will be reported as significant.

Counting using Filter-paper dip-strip method:

- Prepare a filter paper as shown below and sterilize by autoclaving
- Mix urine sample, insert the strip to be absorbed and withdrawn excesses urine immediately.
- Place filter paper in contact with a gar for 2 – 3 seconds, incubate 37°C overnight.



Conversion from number of colonies to bacteruria:

Gram negative bacteria

> 25 colonies   > 10^5 CFU/ml, 25 colonies = 10^5CFU/ml, 5 – 24 colonies = $10^4 - 10^5$, 0 – 4 colonies < 10^4

Gram positive bacteria

> 30 colonies > 10^5 CFU/ml, 30 colonies = 10^5CFU/ml, 10 – 29 colonies = $10^4 - 10^5$, 0 – 9 colonies < 10^4

Day two

Report of estimated colonies:

………………………………………………………………………………………………

………………………………………………………………………………………………

………………………………………………………………………………………………

………………………………………………………………………………………………

……………….

- **Colonial morphology:**

	Blood agar	macConkey agar	CLED agar

- **Indirect Gram stain result:**

  …………………………………………………………………………………

  …………………………………………………………………………………

  …………………………………………………………………………………..

- **Biochemical tests:**

Name of the test	Requirement	Method of inoculation and incubation

- **Day three**

- **Biochemical tests result**

Name of the test	Comment	Result

-

- **Final report of the isolated organism:**

…………………………………………………………………………………………………

…………………………………………………………………………………………………

…………………………………………………………………………………………………

Name and signature of the instructor:………………………………………………………

Date:…………………………………………………………………………………………

Practical No. (8):

Wound Infections, Bacteriological Examination of wound swab

Day (1)

Specimen: Pus.

Collection:

- 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 use a sterile cotton-wool swab to collect sample from infected site (2 swabs required).
- Collect 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.

Transportation: Insert swab in Amies transport medium and transport within 6 hrs.

Macroscopical examination: Look for presence of granules (size, shape and colour).

Report of Macroscopical examination:

……………………………………………………………………………………………

……………………………………………………………………………………………

…………………………………………………………………………………………..

Inoculation and incubation: Inoculate blood agar, and MacConkey agar, and incubate both plates O2 at 37 0C for 24 hrs. If anaerobic infection is suspected inoculate a plate of neomycin BA and incubate anaerobically at 37 0C for 24 hrs.`

- **Colonial morphology:**

	Blood agar	macConkey agar	CLED agar

- **Indirect Gram stain result:**
- ……………………………………………………………………………
  ……………………………………………………………………………
  …………………………………………………………………………….
- **Biochemical tests:**

Name of the test	Requirement	Method of inoculation and incubation

- **Day three**

- **Biochemical tests result**

Name of the test	Comment	Result

- **Final report of the isolated organism:**

………………………………………………………………………………………………………

………………………………………………………………………………………………………

………………………………………………………………………………………………………

Name and signature of the instructor:……………………………………………………………

Date:……………………………………………………………………………………………

Practical No(9)

Microbiological examination of ear infection

Day (1)

Specimens:

Ear swabs

1) Break off the top part of the stick without touching the tip of the tube and tighten the screw cap firmly.
2) Repeat the procedure with a tube containing the appropriate viral transport medium.
3) Label the specimens.
4) Specimens for viral detection are transported at 4-8°C in virus transport medium. Swabs in viral transport medium may also be frozen in liquid nitrogen.

Microscopy:

Smear should be done on site if possible **before insertion on transport media stain with:**

1- **Gram stain.**
2- **Wet preparation with 20%KOH**

Report of:

1- **direct gram stain.**

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

2- **Report of wet preparation.**

………………………………………………………………………………
………………………………………………………………………………
………………………………………………………………………………

Inoculation and incubation:

- On heated blood agar, and blood agar. Incubate both plates in a CO2 at 37 0C
- MaCconkey Incubate both plates in O2 at 37 0C

Day (2)

- **Colonial morphology:**

	Blood agar	Chocolate BA	MacConkey agar

- **Indirect Gram stain result:**
- ……………………………………………………………………………………
  ……………………………………………………………………………………

- **Biochemical tests:**

Name of the test	Requirement	Method of inoculation and incubation

- **Day three**

- **Biochemical tests result**

Name of the test	Comment	Result

-
- **Final report of the isolated organism:**
- ……………………………………………………………………………………………
  ……………………………………………………………………………………………
  ……………………………………………………………………………………………
- **Name and signature of instructor:**……………………………………………………
- **Date:**……………………………………………………………………

Practical No. (10):

Microbiological examination of eye infection

Day (1)

Spceimens:

Conjunctival and corneal swabs and smears

Dry swab is not suitable it my absorb the delicate organism

Collection by:

- Cotton tip alumonium
- Platinum loop
- Rounded tip of glass or plastic rod
- Tip of thin serum coated swab

Corneal scrapings must only be collected by an ophthalmologist or other trained person.

Label all specimens

Method of collection of conjunctiva swabs

Clean the skin around the eye with a mild antiseptic.

Moisten a swab in sterile saline and roll over the conjunctiva in a c ircular manner.

Insert the swab into a sterile screw-cap tube containing the appropriate transport media (Amies Transport Medium or Amies Transport Medium)

Break off the top part of the stick without touching the tip of the tube and tighten the screw cap firmly.

Repeat the procedure with a tube containing the appropriate viral transport medium.

Label the specimens.

Specimens for viral detection are transported at 4-8°C in virus transport medium. Swabs in viral transport medium may also be frozen in liquid nitrogen.

Microscopy:

Smear should be done on site if possible **before insertion on transport media** two smears **stain with:**

Direct Gram stain report:

……………………………………………………………………………………
……………………………………………………………………………………
……………………………………………………………………………………

Gimesa stain Report:
………………………………………………………………………………………
………………………………………………………………………………………
………………………………………………………………………………………
………………………………………………………………………………………

Inoculation and incubation:

- On heated blood agar, and blood agar. Incubate both plates in a CO2 at 37 0C.
- Modified New York City (MNYC).
- Loffler serum agar or Dorset egg medium

Day (2)

- **Colonial morphology:**

	Blood agar	Chocolate BA	MacConkey agar	others

- **Indirect Gram stain result:**
- ………………………………………………………………………………………
………………………………………………………………………………………

- **Biochemical tests:**

Name of the test	Requirement	Method of inoculation and incubation	Others

- **Day three**

- **Biochemical tests result**

Name of the test	Comment	Result

-

- **Final report of the isolated organism:**
- **……………………………………………………………………………………………… ……………………………………………………………………………………………… ………………………………………………………………………………………………**
- **Name and signature of instructor:………………………………………………………**
- **Date:…………………………………………………………………………**

.