General Bench

CLS 417: Clinical Practice in Microbiology

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Topics to be covered in this lecture:

1. Types of Specimen
2. Each type:

- ✓ Collection & transport of specimens
- ✓ Most common pathogens
- ✓ Processing of specimens: macroscopy, microscopy, and culture

Types of Specimens

1. **Sterile fluids:**

   a. Blood b. CSF c. Effusions d. Bone marrow

2. **Non-sterile Fluids:**

   a. Pus b. Ulcer material

3. **Swabs:**

   a. Eye & Ear b. Mouth & Throat c. Urogenital

   d. Bite wound

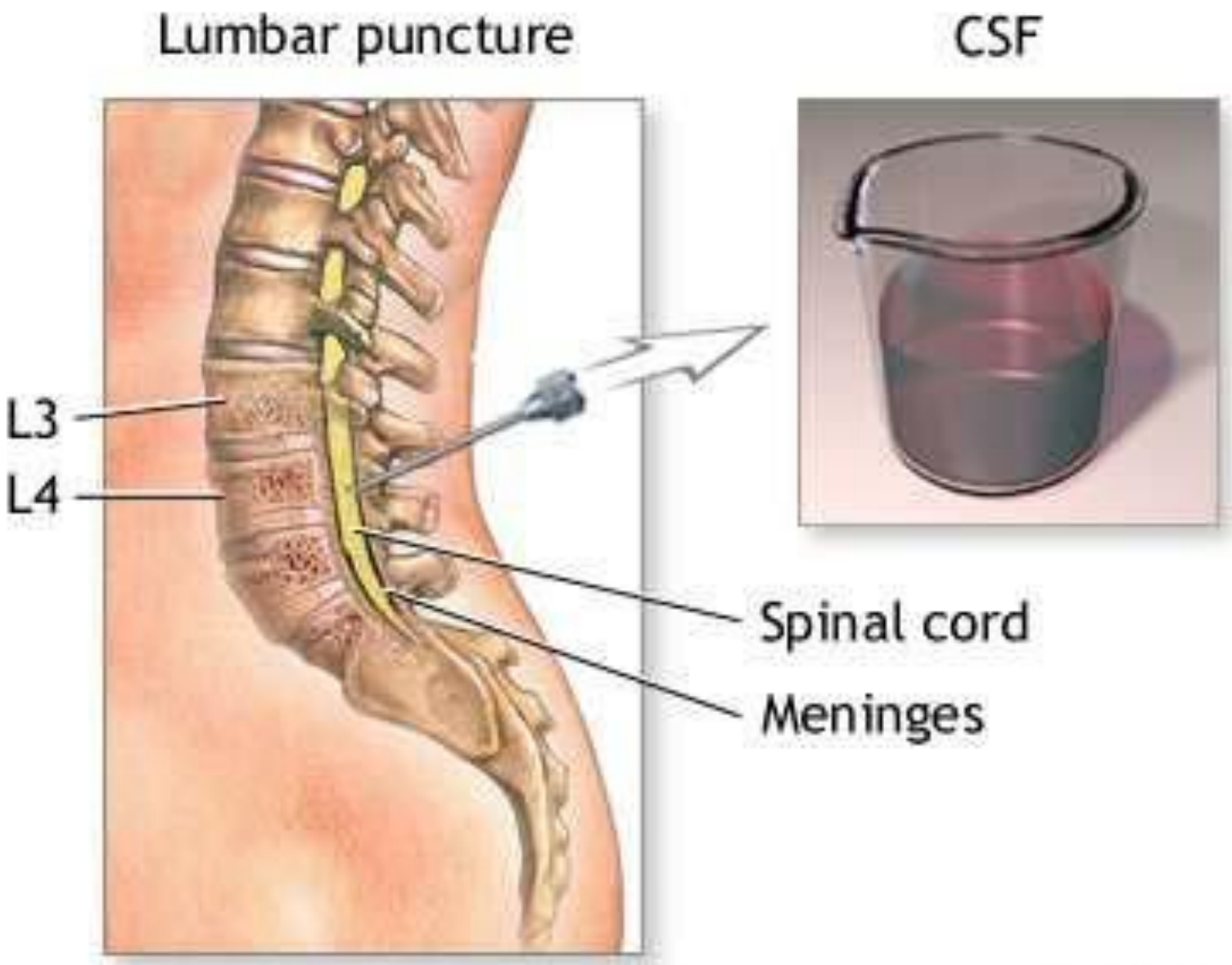
4. **Skin, tissues, transplant, prosthetic devices, and autopsy specimens**

5. **Catheter**

CSF

Collection of CSF

- Cerebrospinal fluid *must be collected by an* ***experienced*** medical officer or health worker.
- It must be collected ***aseptically*** *to prevent organisms being* introduced into the CNS.
- The fluid is usually collected from the ***arachnoid space.*** A sterile wide-bore needle is inserted between the 4th and 5th lumbar vertebrae and the CSF is allowed to drip into a dry sterile container.
- A ***ventricular puncture*** is sometimes performed to collect CSF from infants.
- When the CSF is to be examined for trypanosomes, it is usually collected after treatment.


Lumbar puncture
CSF
L3
L4
Spinal cord
Meninges
A.D.A.M.

CSF Collection Technique

IMPORTANT: Advise the laboratory before performing a lumbar puncture so that staff are prepared to receive and examine the specimen immediately.

Collection of CSF:

1. Take two sterile, dry, screw-capped containers and label one No. 1 (first sample collected, to be used for culture), and the other No. 2 (second sample collected, to be used for other investigations).
2. Collect about 1 ml of CSF in container No. 1 and about 2–3 ml in container No. 2.
3. Immediately deliver the samples with a request form to the laboratory.

Delay in examining CSF will:

1. Reduces the chances of isolating a pathogen.
2. Lower cell count due to WBCs being lyzed.
3. Falsely low glucose value due to glycolysis.

Most Common Pathogens Isolated from CSF

BACTERIA

Gram positive

Streptococcus pneumoniae

Streptococcus agalactiae
(Group B)

Listeria monocytogenes

Streptococcus suis

Gram negative

Neisseria meningitidis

Haemophilus influenzae B

E. coli

Pseudomonas aeruginosa

Proteus species

Salmonella serovars

Flavobacterium meningosepticum

Also *Mycobacterium tuberculosis* and *Treponema pallidum.*

Most Common Pathogens Isolated from CSF

VIRUSES

- Coxsackieviruses, echovirus, and arboviruses.
- Also, herpes simplex 2 virus, varicella zoster virus, and lymphocytic choriomeningitis virus (LCM).
- Rarely polioviruses.

FUNGI AND ACTINOMYCETES

- *Cryptococcus neoformans (AIDS* patients)
- Less commonly *Aspergillus species.*

PARASITES

- *Trypanosoma species and Naegleria fowleri.*
- *Rarely* the larvae of *Angiostrongylus cantonensis, Dirofilaria immitis*
- Also *Toxoplasma gondii (AIDS patients).*

NO NORMAL FLORA IN THE CSF

Macroscopic Examination of CSF

Report whether the fluid is:

- **Clear:** normal.
- **Slightly turbid, cloudy or definitely purulent (looking like pus):** indicates presence of pus cells, suggestive of acute pyogenic bacterial meningitis.
- **Contains blood:** may be due to a traumatic (bloody) lumbar puncture or less commonly to haemorrhage in the central nervous system. When due to a traumatic lumbar puncture, sample No. 1 will usually contain more blood than sample No. 2. Following a subarachnoid haemorrhage, the fluid may appear xanthrochromic, i.e. yellow-red (seen after centrifuging).
- **Contains clots:** Indicates a high protein concentration with increased fibrinogen, as can occur with pyogenic meningitis or when there is spinal constriction.

WBC Count

- Perform a WBC count and note whether there is an increase in white cells and whether the cells are mainly neutrophils or lymphocytes.
- **Normal CSF contains:** up to $5x10^6$ cells/litre (higher in neonates).
- **When no WBCs are seen, report the count as:** Below $5x10^6$cell/l.

BIOCHEMICAL TESTING OF CSF

1. **Measurement of CSF glucose-** Normal CSF glucose: 2.5–4.0 mmol/l (45–72 mg%).
2. **Measurement of CSF total protein and globulin test-** Normal CSF protein: 0.15–0.40 g/l (15–40 mg%).
3. **Pandy's test for CSF globulin:** is a screening test which detects rises in CSF globulin. Only traces of globulin are found in normal CSF.

Testing the CSF

Depending on the appearance of the CSF, proceed as follows:

Normal CSF

Report the CSF as 'Normal' when:

- It appears clear.
- Contains no more than 5 WBC x 10^6/l.
- CSF protein concentration is normal (or Pandy's test is negative).
- CSF glucose concentration is normal.

Purulent or Cloudy CSF

- Immediately make and examine a Gram stained smear for bacteria and neutrophils (pus cells).

➢ **Issue the report without delay.**

- Then culture the CSF.

Slightly Cloudy or Clear CSF

- Report if there is an increase in WBC count.
- When cells predominantly neutrophils:
  - Examine a Gram stained smear for bacteria.
  - Examine a wet preparation (sediment from centrifuged CSF) for motile amoebae which could be Naegleria (rare).
  - Culture the CSF
- When cells predominantly lymphocytes: this could indicate viral meningitis, tuberculous meningitis, cryptococcal meningitis, trypanosomiasis encephalitis.

Cont. Slightly Cloudy or Clear CSF

- CSF protein is raised in most forms of meningitis and meningoencephalitis.
- CSF glucose is helpful in differentiating viral meningitis, normal CSF glucose, from tuberculous meningitis and other conditions in which the CSF glucose is reduced.
- Examine a wet preparation for encapsulated yeast cells that could be *C. neoformans.*
- Examine a wet preparation for trypanosomes and a Giemsa stained smear for morula (Mott) cells when late stage trypanosomiasis is suspected.

Gram Staining: Making a Smear

1. Mix No. 2 CSF sample and centrifuge at approximately 1000 g for 5–10 minutes (leave a small amount of uncentrifuged CSF for a cell count should this be required).
2. Transfer the supernatant fluid to another tube (to be used for glucose and protein tests should these be required).
3. Mix the sediment. Transfer several drops of the sediment to a slide, but do not make the preparation too thick because this will make it difficult to decolorize adequately. Allow the preparation to air-dry.
4. Alcohol-fix the preparation and stain it by the Gram technique.

- **Do not centrifuge a purulent CSF fluid.**

Reporting Gram Stain

Examine the smear microscopically for pus cells and bacteria using the 40 and 100 objectives.

1. **Pus cells:** Report as many, moderate number, or few. Pus cells will be found mainly in pyogenic bacterial meningitis and in amoebic-meningoencephalitis (rare).
2. **Bacteria:** Look in well stained (not too thick) areas for:

- ***N. meningitidis:*** Gram negative intracellular diplococci.
- ***S. pneumoniae:*** Gram positive diplococci or short streptococci. It is often possible to see the capsules as unstained areas around the bacteria.
- ***H. influenzae:*** Gram negative rods, especially if filamentous or other polymorphic forms are seen.
- ***E. coli or other coliforms:*** Gram negative rods, especially when the CSF is from a newborn infant.

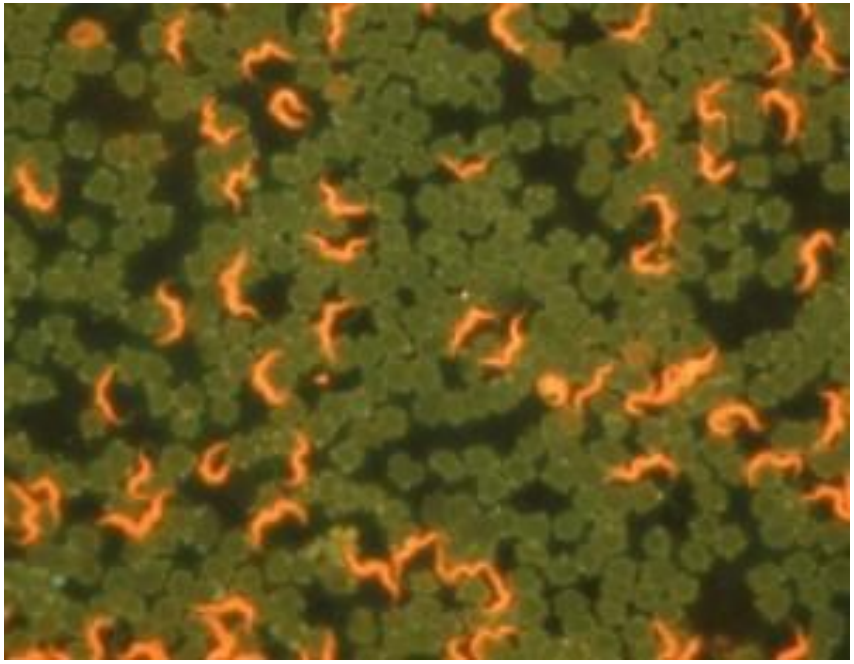
Cont. Reporting Gram Stain

3. **Yeast cells:** unevenly stained irregular in size (some showing budding), suggestive of *C. neoformans*. The large capsule that surrounds the cell does not stain. It is best seen in an India ink preparation. The smear will usually contain lymphocytes.

- ***Important: Advise the medical officer immediately*** **if the Gram smear contains bacteria, pus cells, or yeast cells**
- **When bacteria and pus cells are seen in the Gram smear,** culture the CSF. There is no need to perform a cell count or measure the protein or glucose.
- Patient taking antibiotics (usually as emergency treatment) are difficult to detect bacteria in the Gram smear and in culture.

Reporting Acridine Orange

- For *Mycobacterium tuberculosis* and *Treponema pallidum.* Bacteria stain bright orange and cells and debris stain green or yellow. The organisms can be detected using the 40 objective.



Immunological Diagnosis of acute Bacterial Meningitis

- Direct antigen testing of CSF may provide a rapid diagnosis of acute bacterial meningitis, particularly when the patient has been treated with antimicrobials and bacteria cannot be detected in a Gram smear or by culture.
- Tests are available to detect *N. meningitidis groups A, B, C, Y and W135 (Group B* reagent cross-reacts with *E. coli K1 antigen), H. influenzae* type b, *S. pneumoniae, and S. agalactiae*.

CULTURING CSF

- Use CSF sample No. 1. If it is not clear or slightly cloudy, centrifuge the sample in a sterile capped tube for about 15 minutes, and use the sediment to inoculate the culture media.
- CSF must be cultured as soon as possible after collection. When a delay is unavoidable, the fluid should be kept at 35–37°C (not refrigerated).

CULTURING CSF

- Inoculate the specimen on **chocolate agar, blood agar, and on MacConkey agar.**
- If Gram positive diplococci are seen in the Gram smear, add an optochin disc to the blood agar plate to assist in the identification of *S. pneumoniae.* Incubate both plates in a CO_2 enriched atmosphere at 35–37°C for up to 48 hours.
- Check for growth after overnight incubation.

Examine and Report the Cultures

Chocolate Agar and Blood Agar Cultures

Look especially for colonies that could be:

- *Neisseria meningitidis (growing on chocolate agar* and blood agar, oxidase positive).
- *Streptococcus pneumoniae* (sensitive to optochin).
- *Haemophilus influenzae* (growing only on chocolate agar).
- *Cryptococcus neoformans* (Gram stain the colonies).

MacConkey Agar Culture

Look especially for colonies that could be:

- *E. coli or other coliform.*
- *Strep agalactiae.*
- *Listeria monocytogenes.*
- Other bacteria that cause neonatal meningitis.

PUS, Ulcer Material and Skin

Most Common Pathogens Isolated from PUS

BACTERIA

Gram positive

S. aureus

Strep pyogenes

Enterococcus sp.

Anaerobic Strep

Other Strep

Clostridium sp.

Actinomycetes

Gram negative

Pseudomonas aeruginosa

Proteus sp.

E. coli

Bacteriodes sp.

Klebsiella sp.

Pasteurella sp.

Also *Mycobacterium tuberculosis*

Most Common Pathogens Isolated from PUS

FUNGI

- *Histoplasma capsulatum & duboisii.*
- *Candida albicans.*
- Fungi that cause mycetoma.

PARASITES

Entamoeba histolytica.

COMMENSALS

Any commensal organisms found in pus are usually those that have contaminated the specimen from skin, clothing, soil, or from the air if an open wound.

Most Common Pathogens Isolated from Ulcer Material and Skin

BACTERIA

Gram positive

S. aureus

Strep pyogenes

Enterococcus sp.

Anaerobic Strep

Bacillus anthracis

Gram negative

Pseudomonas aeruginosa

Proteus sp.

E. coli

Yersenia pestis

Vincent's organisms

Also *Mycobacterium sp.* and *Treponema sp.*

Most Common Pathogens Isolated from Ulcer Material and Skin

VIRUS

Poxviruses and herpes viruses

FUNGI

- Dermatophytes
- *Malassezia furfur*
- Fungi that cause chromoblastomycosis
- *Candida albicans*

PARASITES

- *Leishmania sp.*
- *Onchocerca volvulus*
- *Dracunculus medinensis*

COMMENSALS

Commensals of the skin

Collection & Transport of PUS& Ulcer

- Specimens should be collected by a medical officer or an experienced nurse.
- Pus from an abscess is best collected at the time the abscess is incised and drained, or after it has ruptured naturally. When collecting pus from abscesses, wounds, or other sites, avoid contaminating the specimen with NF.
- As far as possible, a specimen from a wound should be collected before an antiseptic dressing is applied.
- Using a sterile technique, aspirate using a sterile needle and syringe or collect from a drainage tube up to 5 ml of pus. Transfer to a leak-proof sterile container, label, send with request form ASAP.
- Or use a sterile cotton-wool swab (with transfer media) to collect pus sample.

Culture of PUS & Ulcer

- ***Incase of swab sample, inoculate the culture media first before making smears.***
- **2 Blood agar**
- **MacConkey agar**
- **Cooked meat medium (or thioglycollate broth)**

➢ Incubate BA (aerobic, anaerobic) in CO_2, MAC aerobically, and cooked meat medium at 35–37°C for up to 72 hours.

➢ U can add neomycin to 2nd BA to inhibit most of anaerobic bacteria.

Microscopic Examination of Pus &Ulcer

- **Gram smear:** for pus cells and bacteria.
- **Ziehl-Neelsen smear:** for Tb or M. ulcerans.
- **Giemsa or Wayson's smear:** for *Yersenia pestis.*
- **Polychrome methylene blue:** for cutaneous anthrax.
- **Dark-field microscopy:** for *Treponemes.*
- **KOH preparation:** for fungus.

EFFUSIONS

Types of Effusions

An effusion is fluid which collects in a body cavity or joint.

Exudate: is fluid which collects due to an inflammatory process. It is important to investigate whether the inflammatory process is an infective one (septic) or caused by a non-infective process, e.g. malignancy.

Transudate: is fluid which forms due to a non-inflammatory condition. When the fluid is a transudate, no further microbiological testing is required.

Effusions sent to the laboratory for investigation include:

Fluid	*Origin*
Synovial	From a joint
Pleural	From the pleural cavity (Space between the lungs and the inner chest wall)
Pericardial	From the pericardial sac (Membranous sac surrounding the heart)
Ascitic (peritoneal)	From the peritoneal (abdominal) cavity
Hydrocele	Usually from the sac surrounding the testes

Other Sterile Fluids

- **Pleural (Thoracentesis/ Empyema) Fluids**:

  Infection of the pleural space may result in severe morbidity and mortality. Therefore rapid and accurate microbiological assessment is required. Any organism found in pleural fluid must be considered significant (although specimen contamination may occur during collection).

- **Peritoneal (Ascites) Fluids:**

  Peritonitis may be classified as primary (spontaneous), secondary or tertiary. Primary peritonitis usually occurs in someone with pre-existing ascites (e.g. patients with chronic liver disease) in which there has been no entry into the abdominal cavity. Secondary and tertiary peritonitis occur after surgery or trauma to the abdomen. Although enteric Gram negative organisms are the most common isolates associated with these types of infections, polymicrobial infection is common with a mixture of both Gram positives and negatives including anaerobes.

- **Synovial (Joint) & Pericardial Fluids**:

  These are normally sterile fluids. Infection of these fluids may be due to a variety of different organisms as a result of direct infection, contamination at the time of surgery/trauma or hematogenous spread.

- **Amniotic Fluids**:

  Amniotic fluid is that fluid which surrounds the developing fetus in uterus. As with other normally sterile fluids, infection of the amniotic fluid may result in severe morbidity and mortality to the mother and fetus. Any organism isolated must be considered significant (although contamination may occur during collection).

- **Other Fluids**:

  Infection of normally sterile body fluids may result in severe morbidity and mortality. Any organism isolated must be considered significant (although specimen contamination may occur during collection). Specimens include tympanocentesis fluid (behind ear drum), intraocular fluid, hydrocele fluid, cyst fluid, etc.

Most Common Pathogens Isolated

SYNOVIAL FLUID

(Synovitis and Infective arthritis)

Gram positive	***Gram negative***
Staphylococcus aureus	*Neisseria gonorrhoeae*
Streptococcus pyogenes	*Neisseria meningitidis*
Streptococcus pneumoniae	*Haemophilus influenzae*
Anaerobic streptococci	*Brucella* species
Actinomycetes	*Salmonella* serovars
	Escherichia coli
	Pseudomonas aeruginosa
	Proteus
	Bacteroides

Also *Mycobacterium tuberculosis.*

PLEURAL AND PERICARDIAL FLUIDS

(Empyema and Purulent Pericarditis)

Gram positive	***Gram negative***
Staphylococcus aureus	*Haemophilus influenzae*
Streptococcus pneumoniae	*Bacteroides*
Streptococcus pyogenes	*Pseudomonas aeruginosa*
Actinomycetes	*Klebsiella* strains
	Other enterobacteria

Also *Mycobacterium tuberculosis,* fungi, and viruses especially coxsackie B virus.

Most Common Pathogens Isolated

ASCITIC FLUID

(Ascites and Peritonitis)

Gram positive	***Gram negative***
Enterococcus species	*Escherichia coli*
Streptococcus pneumoniae	*Klebsiella* strains
Staphylococcus aureus	Other enterobacteria
Streptococcus pyogenes	*Pseudomonas aeruginosa*
Streptococcus agalactiae	*Bacteroides*
Viridans streptococci	
Clostridium perfringens	

Also *Mycobacterium tuberculosis* and *Candida* species.

HYDROCELE FLUID

Occasionally *Wuchereria bancrofti* microfilariae and rarely *Brugia* species can be found in hydrocele fluid.

Commensals

The small amounts of fluid which surround the joints and can be found in the pleural cavity, pericardial sac, and peritoneal cavity, have no normal microbial flora.

Collection and Transport of Effusions

- Collection of effusions is carried out by a medical officer.
- After aspiration, aseptically dispense the fluid as follows:
- 2–3 ml into a dry, sterile, screw-cap tube or bottle to observe for clotting.
- 9 ml into a screw-cap tube or bottle which contains 1 ml of sterile anticoagulant (tri-sodium citrate) and mix.
- Label, deliver ASAP with a completed request.

Macroscopic Examination of Effusion

Describe and report:

- **Color.**
- **Clarity:**
  - ❖ Clear = Normal
  - ❖ Cloudy or purulent (like pus) = Gram stain and proceed like pus sample.
- **Presence of blood:** Gram stain and culture.
- **If clotted** (sample without anti-coagulant).

WBC Count & Protein level

	*Transudate**	*Exudate*
Appearance	Clear, pale yellow	Purulent, cloudy, or blood-stained
Clotting	Does not clot	Often clots
Cells	Few cells	*Purulent*: Many cells, mostly neutrophils *Non-purulent*: Few or many cells, mostly lymphocytes
Protein	Less than 30 g/l	More than 30 g/l

Culture of Exudate

- Culture the fluid when it contains more than a few WBCs and more than 30 g/l of protein, or when it appears blood-stained.
- Centrifuge the citrated sample in a sterile tube at high speed for about 20 minutes to sediment the bacteria. Remove the supernatant fluid (do not discard) and re-suspend the sediment.
- **Culture on:**

- **Chocolate agar (in CO_2), Blood agar, MacConkey agar, and fastidious Anaerobic broth at 35–37°C for up to 72 hours.**
- **Fastidious Anaerobic Agar in anaerobic conditions at 35–37°C for up to 48 hours.**

Microscopic Examination of Exudate

- **Gram smear:** Look for pus cells and bacteria.
- **Ziehl-Neelsen:** Look for AFB.
- **Wet preparation for crystals:** When gout or pseudogout is suspected (Monosodium urate and Calcium pyrophosphate crystals from joint fluid only).
- **Cytology smear:** When malignancy is suspected.

Swabs from non-sterile sites

Eye & Ear Swabs

Most Common Pathogens Isolated from EYE Swabs

BACTERIA

Gram positive

Staph aureus

Strep pneumoniae

Strep Group A

Moraxella catarrhalis

Moraxella sp.

Gram negative

Haemophilus influenzae

Pseudomonas aeruginosa

Neisseria gonorrhea

Most Common Pathogens Isolated from EAR Swabs

BACTERIA

Gram positive

Staph aureus

Strep pneumoniae

Strep Group A

Gram negative

Pseudomonas aeruginosa

FUNGI

Candida albicans and other yeasts.

Collection and Transport of Eye& Ear Swabs

- The specimen should be collected by a medical officer or experienced nurse using a clean, sterile swab and sent in Amies transport medium.
- Eye specimen include: Eye / conjunctival / lid swabs. It is preferable that both eyes be swabbed, even if the infection is unilateral.
- If a delay in transport or processing is anticipated, the specimen should be kept at $4^{o}C$.

Microscopic Examination of Eye &Ear Swabs

- **Gram smear:**
  - Pus cells.
  - Bacteria.

Culture of Eye & Ear Swabs

- **Blood Agar, Chocolate Agar:** Incubate in CO_2 at 35–37°C for up to 48hours.
- **MacConkey Agar, Colistin Nalidixic Acid Agar (CNA):** Incubate aerobically at 35–37°C for up to 48hours.
- **Martin-Lewis Agar (ML):** for *N. gonorrhea*, Incubate in CO_2 at 35–37°C for up to 72hours.

Throat & Mouth Swabs

Most Common Pathogens Isolated from Throat & Mouth

BACTERIA

Gram positive

Strep pyogenes

Corynebacterium diphtheria

Corynebacterium ulcerans

Gram negative

Vincent's organisms

VIRUSES

Respiratory viruses, enteroviruses and HSV-1.

FUNGI

Candida albicans and other yeasts.

Commensals

BACTERIA

Gram positive

Strep viridans

Non-haemolytic Strep

Strep pneumoniae

Staph aureus

Micrococci

Lactobacilli

Diphtheroids

Gram negative

Maroxilla catarrhalis

Neisseria pharyngitidis

Fusobacteria

Coliforms

Bacteroides species

Haemophilus influenzae

Also various spirochaetes, actinomycetes, aerobic and anaerobic spore-bearers, and yeasts.

Collection and Transport of Throat & Mouth Swabs

- Whenever possible throat and mouth swabs should be collected by a medical officer or experienced nurse.
- In a good light and using a tongue depressor, examine the inside of the mouth. Look for inflammation, and the presence of any membrane, exudate, or pus.
- Swab the affected area using a sterile cotton wool swab. Taking care not to contaminate the swab with saliva.
- Within two hours of collection, deliver the swab with a completed request form to the lab.

Culture of Throat & Mouth Swabs

- **Blood agar**

– Add a bacitracin disc

– Incubate, preferably anaerobically (or in CO_2)

- **MTM (Modified Tinsdale medium), or TBA (Tellurite blood agar):** *C. diphtheria*

Microscopy of Throat & Mouth Swabs

- **Gram smear:**
  - Pus cells
  - Gram negative Vincent's organisms
  - Gram positive pleomorphic rods (diphtheria)
  - Gram positive yeast cells (thrush)
- **Giemsa or Wayson's smear:** for *C. diphtheria*

Urogenetal Specimens

Types of Urogenital Specimens

1. Urethral Swabs
2. Cervical Swabs
3. Vaginal Swabs
4. Genital Ulcer Specimens

Most Common Pathogens Isolated from Urogenital Specimen

URETHRAL SWABS

- *Neisseria gonorrhoeae*
- *Chlamydia trachomatis* (serovars D-K)
- *Ureaplasma, Mycoplasma, and Trichomonas vaginalis*

CERVICAL SWABS

From women with puerperal sepsis or septic abortion:

- *Strep pyogenes*
- *β-haemolytic* strep
- *Staph aureus*
- *Enterococcus sp.*
- *Anaerobic cocci*
- *Clostridium sp.*
- *Bacteroides*
- *Proteus, E. coli*
- *Listeria monocytogenes*

From non-puerperal women:

- *Neisseria gonorrhoeae*
- *Chlamydia trachomatis*
- *Strep pyogenes*
- *HSV*

Most Common Pathogens Isolated from Urogenital Specimen

VAGINAL SWABS

- *Trichomonas vaginalis*
- *Candida sp.*
- *Gardnerella vaginalis with* anaerobes

GENITAL ULCER SPECIMENS

- *Treponema pallidum*
- *Haemophilus ducreyi*
- *Klebsiella*
- *Chlamydia trachomatis* (serovars L1, L2, L3)
- *HSV*

Commensals

- ***Urethral swabs:*** *Diphtheroids, Acinetobacter* sp., and enterobacteria. Skin commensals may also be present.
- ***Cervical swabs:*** the cervix is normally sterile.
- ***Vaginal swabs from puberty to menopause (acid pH):*** *Lactobacilli, anaerobic or* microaerophilic strep, *Clostridium species, Bacteroides. Acinetobacter species, fusobacteria, G. vaginalis, mycoplasma,* and small numbers of *diphtheroids* and yeasts.
- ***Vaginal swabs after menopause (alkaline pH):***

  Diphtheroids, micrococci, *S. epidermidis, Strep viridans*, enterobacteria, *C. albicans* and other yeasts.

Collection and Transport of Urogenital Specimens

- Urogenital specimens should be collected by a medical officer or an experienced nurse.
- Amies transport medium in a cool box.
- The area should be cleaned with sterile saline. Using a swab, collect a sample, label, and transport ASAP with a request form.

Culture of Urogenital Specimen

- **Modified New York City (MNYC) or Thayer Martin medium:** for isolating *N. gonorrhoeae.* Incubate in CO_2 at 35–37°C for up to 48hours.
- **Blood agar (aerobic and anaerobic), MacConkey agar, and cooked meat medium:** Incubate aerobically at 35–37°C overnight.

Microscopical Examination

- **Gram smear:**
  - **Urethral:** intracellular Gram negative diplococci
  - **Vaginal:** Yeast cells (candidiasis) Clue cells (bacterial vaginosis)
  - **Vaginal/cervical:** pus cells and bacteria associated with puerperal sepsis and septic abortion
- **Wet preparation:** motile *T. vaginalis*
- **Dark-field:** *T. pallidum* (Syphilis)
- **Giemsa smear:** *K. granulomatis* infection (donovanosis)
- **Cervical smear(s) sent to histology/cytology laboratory:** for malignancy

Tissues, transplant, prosthetic devices, and Biopsy

- **Direct Examination:** Not indicated.
- **Culture:** in fastidious anaerobic broth in O_2 at 35–37°C for up to 5 days. Examine the broth daily for evidence of growth. If there is evidence of growth, then perform Gram stain and subculture onto BA, MAC, CHOC

Catheter

- Intravascular Catheter Tips
- **Direct Examination:** Not indicated.
- **Culture:** on Blood agar incubate in CO_2 at 35–37°C for up to 48hours.
- **Reporting Results**
- Negative Report: "No growth" **For non-significant organisms:**

  Report as TEST Comment: "<15 colonies of (list morphotypes of non-significant organisms)". No susceptibility required.

  Report as TEST Comment: ">15 colonies of (list morphotypes of mixed non-significant organisms)". No susceptibility required.

- Positive Report: **For significant organisms:**

  Report as ISOLATE: "<15 colonies of (organism name)" or "≥15 colonies of (organism name)". Report with appropriate susceptibilities.