LECTURE -6&7.

Laboratory Diagnosis of Infectious Diseases

Infectious diseases are disorders caused by organisms such as bacteria, viruses, fungi and parasites. Many organisms live in and on our bodies. They're normally harmless or even helpful, but under certain conditions, some organisms may cause disease.

Some infectious diseases can be passed from person to person. Some are transmitted by bites from insects or animals. And others are acquired by ingesting contaminated food or water or being exposed to organisms in the environment.

The methods that used for identification of microorganisms include:

1- Phenotypic (morphology)
   - Direct exam
   - Isolation of pathogen (culture)

2- Biochemical tests

3-Immunological (serological analysis)

4-Genotypic (genetic techniques)

Blood Stream Infections (BSI)

Blood stream infection (BSI) is a serious problem that needs immediate attention and treatment. It is a cause of high mortality especially if caused by multidrug resistant bacteria.

Blood culture

Blood is cultured to detect and identify bacteria or other cultivable microorganisms (yeasts, filamentous fungi). The presence of such organisms in the blood is called bacteraemia or fungaemia, and is usually pathological. In healthy subjects, the blood is sterile.

Blood collection

blood should be taken before antibiotics are administered. The best time is when the patient is expected to have chills or a temperature spike. It is recommended that two or preferably three blood cultures be obtained, separated by intervals of approximately 1 hour (or less if treatment cannot be delayed). The advantages of repeated cultures are as follows:

— the chance of missing a transient bacteraemia is reduced.

— the pathogenic role of "saprophytic" isolates (e.g. *Staphylococcus epidermidis*) is confirmed if they are recovered from multiple venepunctures.

- Because the number of bacteria per millilitre of blood is usually low, it is

important to take a reasonable quantity of blood: 10 ml per venepuncture for adults; 2–5 ml may suffice for children.

- Two tubes should be used for each venepuncture: the first a vented tube for optimal recovery of strictly aerobic microorganisms, the second a non-vented tube for anaerobic culture.
- The skin at the venepuncture site must be meticulously prepared using a bactericidal disinfectant.

Blood-culture media

The blood-culture broth (brain heart infusion broth) and tryptic soy broth (TSB) should be able to support growth of all clinically significant bacteria.

The **blood should be mixed with 10 times its volume of broth (5 ml of blood in 50 ml of broth) to dilute any antibiotic present and to reduce the bactericidal effect of human serum.**

Incubation time

Blood-culture bottles should be incubated at 35–37 C for 7 days. In some cases, incubation may be prolonged for an additional 7 days, e.g. if *Brucella* or other fastidious organisms are suspected, in cases of endocarditis, or if the patient has received antimicrobials. Asterile 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.
Whenever visible growth appears, the bottle should be opened aseptically, a small amount of broth removed with a sterile loop or Pasteur pipette, and a Gram-stained smear examined for the presence of microorganisms.

Subcultures are performed by streaking a loopful on appropriate media: MacConkey agar, Blood agar and chocolate agar.
Some microorganisms may grow without producing turbidity or visible alteration of the broth. Other organisms, e.g. pneumococci, tend to undergo autolysis and die very rapidly. For this reason some laboratories perform routine subcultures on chocolate agar after 18–24 hours of incubation.

Chocolate agar **Blood agar**

MacConkey Agar

<u>A blind subculture</u> may be made at the end of 7 days of incubation, by transferring several drops of the well-mixed blood culture (using a sterile

Pasteur pipette) into a tube of thioglycollate broth, which in turn is incubated and observed for 3 days ,as in *Brucellae.*

Common causes of bacteraemia:

Gram-negative organisms	Gram-positive organisms
Escherichia coli	*Staphylococcus aureus*
Klebsiella spp.	*Staphylococcus. epidermidis*
Enterobacter spp.	a-Haemolytic (viridans) streptococci
Pseudomonas aeruginosa	*Streptococcus pneumoniae*
Salmonella typhi	*Enterococcus. faecalis*
Salmonella spp. other than *S. typhi*	*Streptococcus pyogenes*
Proteus spp.	*Streptococcus agalactiae*

Cerebrospinal fluid (CSF) Infections

The examination of cerebrospinal fluid (CSF) is an essential step in the diagnosis of bacterial and fungal meningitis and CSF must always be considered as a priority specimen that requires prompt attention by the laboratory staff.
Normal CSF is sterile and clear, and usually contains three leukocytes or fewer per mm3 and no erythrocytes. The chemical and cytological composition of CSF is modified by meningeal or cerebral inflammation, i.e. meningitis or encephalitis.

Collection and transportation of specimens

Approximately 5–10 ml of CSF should be collected in two sterile tubes by lumbar puncture performed by a physician. Part of the CSF specimen will be used for cytological and chemical examination, and the remainder for the microbiological examination.
The specimen should be delivered to the laboratory at once, and processed immediately, since cells disintegrate rapidly. Any delay may produce a cell count that does not reflect the clinical situation of the patient.

Common causes of bacterial and fungal meningitis

In neonates (from birth to 2 months):-
Escherichia coli, *Listeria monocytogenes*, *Streptococcus agalactiae*, Other Enterobacteriaceae, *Salmonella* spp. and *Citrobacter* spp.

In all other age groups:-

Haemophilus influenza, Neisseria meningitides, Streptococcus pneumonia, Mycobacterium tuberculosis, Listeria monocytogenes, Staphylococci and *Cryptococcus neoformansb*.

- *Haemophilus influenza* the main cause meningitis in children.
- *Neisseria meningitides* and *Streptococcus pneumonia* the main cause meningitis in adult.

Microscopic examination

Preparation of specimen

If the CSF is purulent (very cloudy), it can be examined immediately without centrifugation. In all other cases, the CSF should be centrifuged in a sterile tube. Remove the supernatant and transfer it to another tube for chemical and/or serological tests. Use the sediment for further microbiological tests.

Direct microscopy

Examine one drop of the sediment microscopically for:

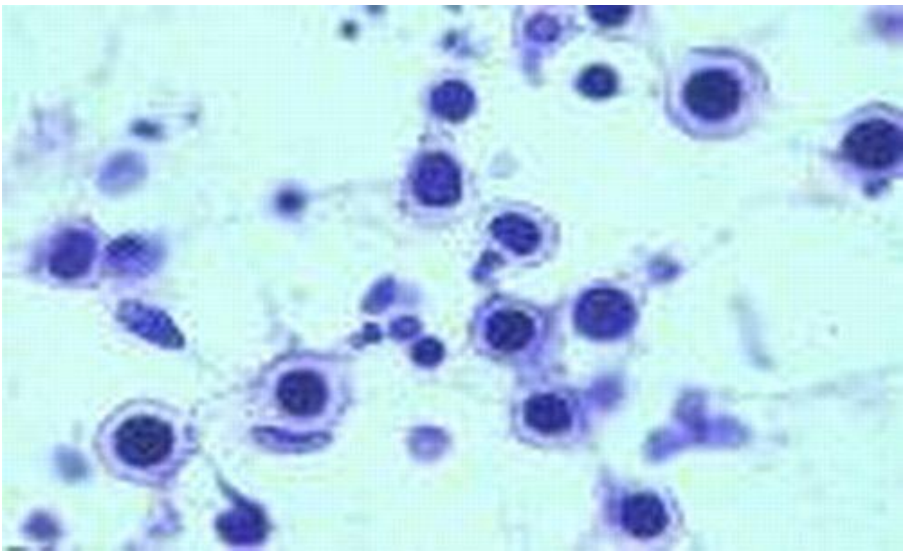
— leukocytes (polymorphonuclear neutrophils or lymphocytes)

— erythrocytes

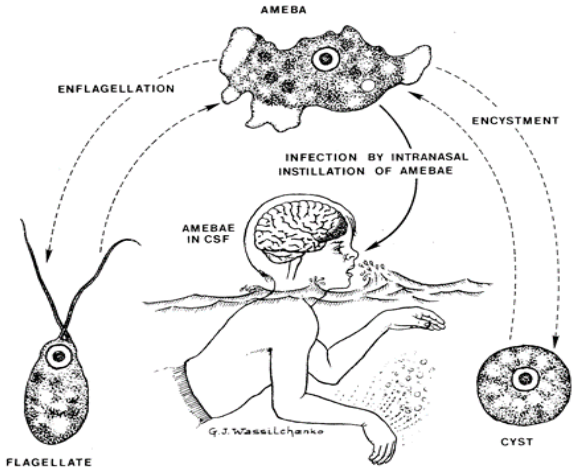
— bacteria

— yeasts.

If the yeast-like fungus *Cryptococcus neoformans* is suspected, mix a loopful of the sediment with a loopful of India ink on a slide, place a coverslip on top, and examine microscopically for the typical, encapsulated, spherical, budding yeast forms.



A rare and generally fatal type of meningitis is caused by free-living amoebae found in water (***Naegleria fowleri*)** which enter through the nose and penetrate the central nervous system. They may be seen in the direct wet preparation as active motile amoebae**.**



Naegleria fowleri

- **Pyogenic (purulent):** when the CSF contains mainly polymorphonuclear neutrophils (pus cells), as in acute meningitis caused by *N.meningitids*, *H.influenzae*, and *S.pneumoniae.* Pus cells are also found in the CSF in acute amoebic meningo-encephalitis.
- **Lymphocytic:** when the CSF contains mainly lymphocytes, as in meningitis caused by viruses, *M. tuberculosis* and *Cryptococcus neoformans.* Lymphocytes are also found in the CSF in trypanosomiasis meningoencephalitis and neurosyphilis.

Gram-stained smears

As the causative agent of bacterial meningitis may often be observed in a Gram-stained smear, this examination is extremely important.

Acid-fast stain (Ziehl–Neelsen)

Examination of an acid-fast-stained preparation of the sediment or of the fibrin web is indicated when tuberculous meningitis is suspected by the physician.

Measure protein (Lower limit 15 mg/dL and Upper limit 40–45 mg/dL)

Measure glucose (Lower limit 50 mg/dL and Upper limit 80 mg/dL)

Glucose in bacterial infections become very low while in viral infections still normal

Protein in bacterial infections were elevated while in viral infections were slightly elevated in early stage of infections

Culture

- The CSF cultures are performed by streaking a loopful on Blood agar, chocolate agar and MacConkey agar, then incubated at 35 -37 C in an atmosphere enriched with carbon dioxide. All media should be incubated for 3 days, with daily inspections.

- When tuberculous meningitis is suspected by *Mycobacterium tuberculosis*, at least three tubes of Löwenstein– Jensen medium should be inoculated with a drop of the sediment and incubated for 6 weeks.
- When *Cryptococcus neoformans* is suspected, either from the India ink preparation or on clinical grounds, the sediment should be inoculated on two tubes of Sabouraud dextrose agar, and incubated at 35 C for up to 1 month. *C. neoformans* also grows on the blood agar plate, which should be incubated at 35C for 1 week.

Urinary Tract Infection (UTI)

Urinary Tract Infection (UTI) is a bacterial infection that affects any part of the urinary tract. The main causal agent is *Escherichia coli*. The most common type of UTI is acute cystitis often referred to as a bladder infection. An infection of the upper urinary tract or kidney is known as pyelonephritis, and is potentially more serious. Women are more prone to UTIs than men.

Factors that increase female susceptibility to UTI :

- Short length of the urethra
- Urethral contamination by rectal pathogens
- Introital & vestibular colonization by pathogenic bacteria
- Decreased urethral resistance after menopause

Urine culture

The urine samples routinely culture on Blood agar and MacConkey agar and now culture on Cystine Lactose electrolyte-deficient (CLED) agar.

Incubate the plate aerobically at 35 – 37Cº overnight.

CLED agar is now used by most laboratories to isolate urinary pathogens because it gives consistent results and allows the growth of both Gram negative and Gram positive pathogens. (the indicator in CLED agar is bromothymol blue and therefore lactose fermenting colonies appear yellow)..

Possible pathogens:

- **Bacteria:**
  - **Gram positive:**
    - *Staphylococcus saprophyticus*
    - *Staphylococcus aureus*
    - *Haemolytic streptococci*
  - **Gram Negative:**
    - *E.coli (commonest about 60 – 90 % of UTI)*
    - *Proteus species(usually in hospitalized patient & with renal stones)*
    - *Pseudomonas aeruginosa*

- *Klebsiella strains*
- *Neisseria gonorrhoeae(not pathogen to urinary tract)*
- *Mycobacterium tuberculosis (not pathogen to urinary tract)*

❑ **Fungi:**

- *Candida species (usually in hospitalized patient, in diabetic patient& immunosuppression*

❑ **Parasite:** *Schistosoma haematobium*

Gastrointestinal Tract Infections (GTI)

Enteric bacterial infections, causing diarrhoea, dysentery, and enteric fevers, are important health problems throughout the world. Diarrhoeal infections are second only to cardiovascular diseases as a cause of death, and they are the leading cause of childhood death.

Etiological agents

The etiological agents which causing gastrointestinal tract infections divided in to:

1- Bacterial infections :

- The genus *Salmonella* cause gastroenteritis and typhoid fever
- *Shigella* spp. are the main cause of bacterial bacillary dysentery
- diarrhoea-producing *Escherichia coli*
- *Vibrio cholerae* cause Cholera, Cholera is a typical example of a toxigenic infection. All the symptoms can be attributed to the intestinal fluid loss caused by an enterotoxin released by *V. cholerae* in the intestine. The stool is voluminous and watery. The main objective of treatment is fluid replacement and antimicrobials have only a secondary role.
- *Campylobacter jejuni* and *C. coli* have emerged as major enteric pathogens that can be isolated as often as *Salmonella* and *Shigella* spp. in most parts of the world.
- *Clostridium difficile* is the primary cause of enteric disease related to antimicrobial therapy. It produces a broad spectrum of diseases ranging from mild diarrhoea to potentially fatal pseudomembranous colitis.

2- **Viral diarrheas:** Rotavirus is a major cause of diarrheal disease in children.

3- **Parasitic diarrheas:** *Entamoeba histolytica* and *Giardia lamblia* can cause of diarrheal disease.

Collection and transport of stool specimens

Faecal (stool) specimens should be collected in the early stages of the diarrhoeal disease, when pathogens are present in the highest number, and preferably before antimicrobial treatment is started.

Enrichment of stool specimens

Enrichment is commonly used for the isolation of *Salmonella* spp. and *Vibrio cholerae* from faecal specimens. Selenite or tetrathionate broths are recommended for the enrichment of *Salmonella* spp., and alkaline peptone water (APW) for the enrichment of *V. cholerae*. Enrichment is not required for *Shigella* spp., *Campylobacter* spp., *Yersinia enterocolitica* and *Clostridium difficile.*

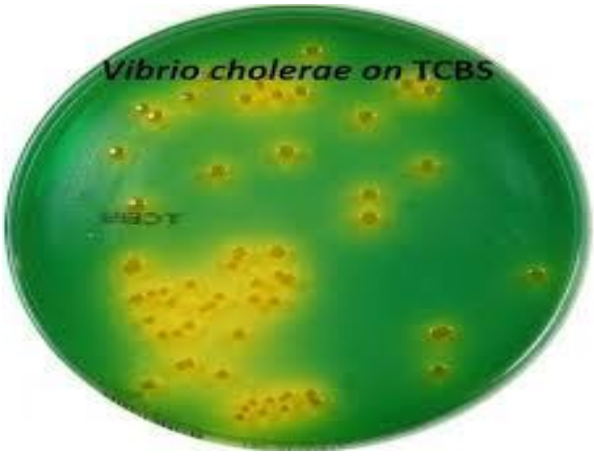
Media for enteric pathogens

For *Shigella* spp., *Salmonella* spp. and *Y. enterocolitica,* MacConkey agar with crystal violet is recommended as a general purpose medium.

Xylose–lysine–deoxycholate (XLD) agar is recommended for the isolation of *Shigella* and *Salmonella.* Hektoen enteric agar (HEA) or *Salmonella–Shigella* (SS) agar are suitable alternatives.

For *Campylobacter* spp. there are several selective media (Blaser, Butzler, Skirrow) containing different antimicrobial supplements used.

Thiosulfate citrate bile salts sucrose (TCBS) agar is selective for *V. cholerae* cefoxitin–cycloserine–fructose agar (CCFA) is selective for *Clostridium difficile*.



After inoculation of these media with one loopful of the faecal suspension, incubate the agar plates. Incubate the plates for the isolation of *Salmonella, Shigella* and *Yersinia* spp. and *V. cholerae* at 35 C in an aerobic incubator (without CO2), the plates for *Campylobacter* spp. at 42 C in an microaerophilic atmosphere with 10% CO2, and the plates for *Clostridium difficile* at 35 ∞C in an anaerobic atmosphere.

Respiratory Tract Infections(RTI)

Respiratory tract infections divided in to:

1- Upper Respiratory tract infections

2- Lower Respiratory tract infections

Upper respiratory tract infections

The upper respiratory tract extends from the larynx to the nostrils and comprises the oropharynx and the nasopharynx together with the communicating cavities, the sinuses and the middle ear. The upper respiratory tract can be the site of several types of infection:

— pharyngitis, sometimes involving tonsillitis, and giving rise to a "sore throat"

— nasopharyngitis

— otitis media

— sinusitis

— epiglottitis

Normal flora of the pharynx

The normal flora of the pharynx includes a large number of species that should be neither fully identified nor reported when observed in throat cultures:

• viridans (a-haemolytic) streptococci and pneumococci

• nonpathogenic *Neisseria* spp.

• *Moraxella* (formerly *Branhamella*) *catarrhalis* (this can also be a respiratory pathogen)

• staphylococci (*S. aureus*, *S. epidermidis*)

• diphtheroids (with the exception of *C. diphtheriae*)

• *Haemophilus* spp.

• yeasts (*Candida* spp.) in limited quantity

Bacterial infections:

Pharyngitis

Streptococcus pyogenes is by far the most frequent cause of bacterial pharyngitis and tonsillitis. This infection is particularly prevalent in young children (5–12 years). When streptococcal pharyngitis is associated with a characteristic skin rash, the patient is said to have scarlet fever. In infants, a streptococcal throat infection may often involve the nasopharynx and be accompanied by a purulent nasal discharge.

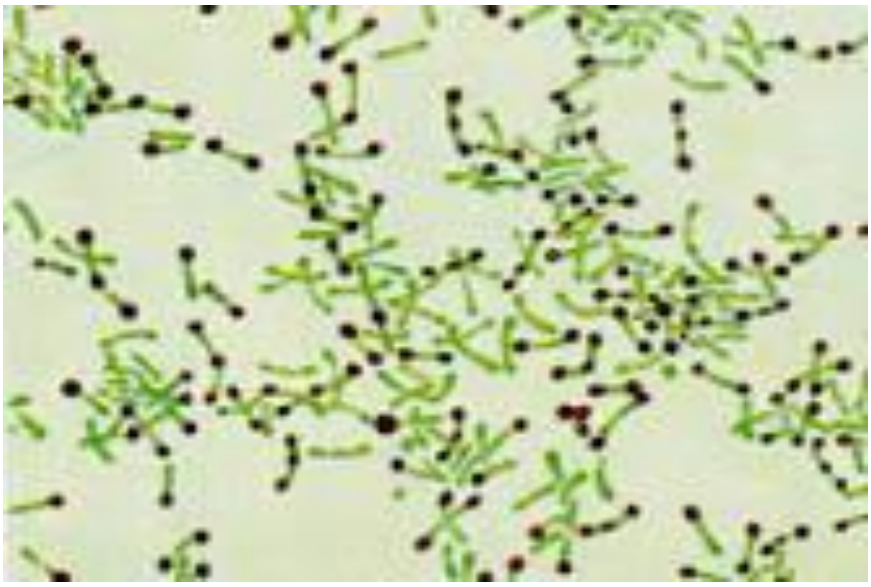
Diphtheria

Corynebacterium diphtheriae is the cause of diphtheria, Diphtheria is a serious disease. *C. diphtheriae* causes a typical form of infection, characterized by a greyish-white membrane at the site of infection (pharynx, tonsils, or larynx).

Culture for *Corynebacterium diphtheriae*

Although the diphtheria bacillus grows well on ordinary blood agar, growth is improved by inoculating one or two special media:

Löffler coagulated serum or Dorset egg medium. Both of these media give abundant growth of the diphtheria bacillus after overnight incubation. Moreover, the cellular morphology of the bacilli is more "typical": irregularly stained, short to long, slightly curved rods, showing metachromatic granules, and this bacteria arranged like Chinese letters. Metachromatic granules are more apparent after staining with **Albert stain** by which the bacillus bacteria stained green while the metachromatic granules stained brown.



Corynebacterium diphtheriae

Gonococcal pharyngitis

Culture of throat swabs for gonococci should be done on specific request from the clinician, using the appropriate selective medium (Thayer–Martin medium).

Whooping Cough (Pertussis)

Whooping cough is the common name for **pertussis,** The disease is caused by *Bordetella pertussis* **,** a tiny, encapsulated, strictly aerobic, Gram-negative rod. These organisms do not tolerate drying or sunlight and die quickly outside the host. laboratory diagnosis include culturing of nasopharyngeal swabs on a selective media (Bordet-Gengou medium).

Lower respiratory tract infections

Lower respiratory tract infections (LRTI) are infections occurring below the level of the larynx, i.e. in the trachea, the bronchi, or in the lung tissue (tracheitis, bronchitis, lung abscess, pneumonia).

A special form of LRTI is pulmonary tuberculosis, which is common in many countries. The patient may cough up aerosols containing tubercle bacilli (*Mycobacterium tuberculosis*).

Many patients with LRTI cough up purulent (pus-containing) sputum that is generally green or yellowish in colour; this sputum may be cultured and examined grossly and microscopically.

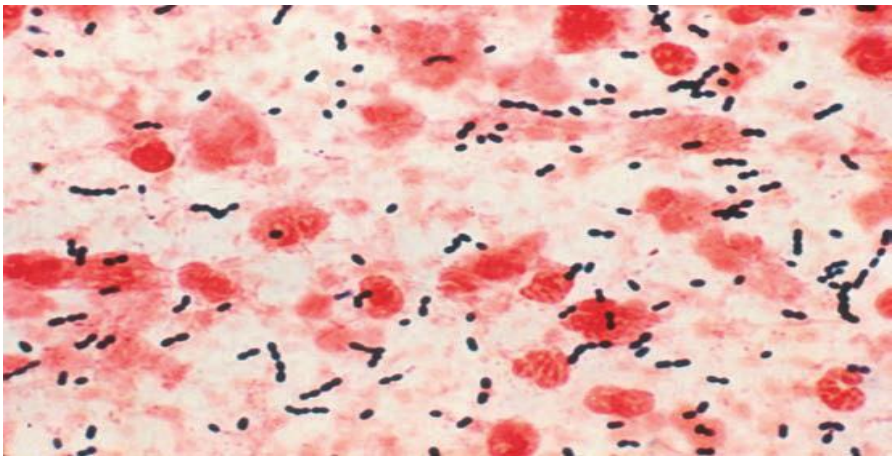
The most common infections

- **Pneumonia**

Causative agent of Pneumonia is *Streptococcus pneumoniae*, the pneumococcus, is a Gram- positive diplococcus. The most striking characteristic of *S. pneumoniae* is its thick polysaccharide capsule, which is responsible for the organism's virulence. This infection is nearly always caused by *S. pneumoniae*.

A rare cause of a rather similar form of pneumonia is *Klebsiella pneumoniae.*

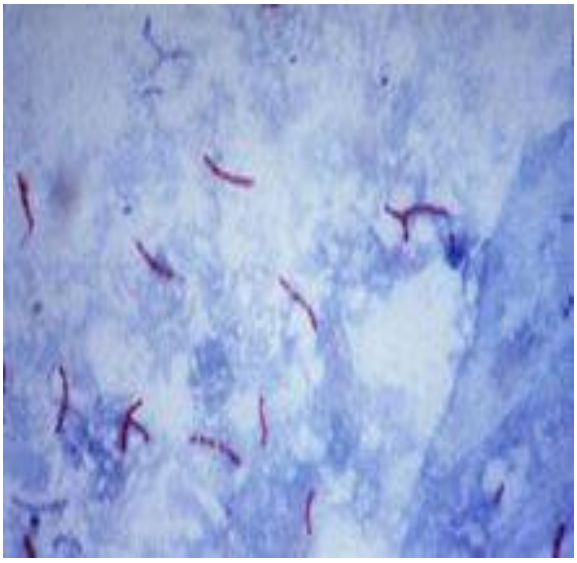
Other Gram-negative rods also can cause pneumonia, especially if host defenses are impaired.



Streptococcus pneumonia

- **Pulmonary tuberculosis**

The sputum of patients with pulmonary tuberculosis should be examined microscopically for acid-fast stained smear (Ziehl–Neelsen) to detect immediately any patients who have acid-fast bacteria in their sputum1. After the smear has been stained, the sputum should be treated by a decontamination procedure in order to kill as many of the non-mycobacterial organisms as possible and to leave the tubercle bacilli viable and thus suitable for culture on Löwenstein–Jensen medium.



(Ziehl–Neelsen)

(Löwenstein–Jensen medium)

Collection of sputum specimens

The sputum should be collected in a sterile wide-mouthed container with a secure, tight-fitting cover and sent to the laboratory without delay.

Microscopic examination

A portion of the purulent or mucopurulent sputum should be used for the preparation of a Gram-stained smear. Gram-stained smear may
provide guidance to the clinician in the choice of antimicrobial chemotherapy.
Possible results include:

• Gram-positive diplococci surrounded by an empty space from the unstained capsules (suggestive of *S. pneumoniae*).

• small Gram-negative coccobacilli (probably *H. influenzae*);

• Gram-negative diplococci, intracellular and extracellular (suggestive of *Movaxella catarrhalis*.

• Gram-positive cocci in grape-like clusters (suggestive of *S. aureus*).

• Gram-negative rods (suggestive of the presence of Enterobacteriaceae or *Pseudomonas* spp.).

• large Gram-positive yeast-like cells, often with mycelia (suggestive of the presence of *Candida* spp.).

Also can see fungi for example; *Histoplasma capsulatum* and *Coccidioides immitis*.

Culture

By using a sterile loop sputum inoculate on to the various culture plates. A suggested routine set of culture media is as follows:

• blood agar, with a streak of *S. aureus* to facilitate satellite growth of *H. influenzae*, and with an optochin disc placed in the middle of the secondary streaking,

• chocolate agar.

• MacConkey agar.

The blood agar and chocolate agar plates are incubated at 36–37 C in an atmosphere containing CO2 (e.g. in a candle jar) and the MacConkey plate is incubated in air.

- Sabouraud dextrose agar used for the isolation of fungi.

Sexually Transmitted Diseases (STDs)

- The laboratory diagnosis of STDs is related to the sex of the patient, although some infections are common to both sexes like gonorrhea, syphilis and chlamydial infection but there are differences in the symptoms, the sites and methods of specimens collection in these infections.

Genital infections and STDs in women

These include:

1- <u>Vaginitis :</u>

Is caused by a limited number of infectious agents include:

- *Trichomons vaginalis*

Trichomoniasis: is an infection of urogenital tract and the most common site of infection is the urethra and vagina in women, it is caused by the single-celled protozoan parasite *Trichomons vaginalis* which classically produce a copious, frothy yellow or yellow-green discharge.

- *Candida albicans*

Vulvovaginal *Candidia*sis: is caused by *Candida albicans, squamous epithelial cells of vaginal is invaded and inflamed causing vaginal discharges and pain.* Discharge is typically more thick than trichomoniasis and curd like.

2- <u>Bacterial Vaginosis:</u> is caused by a number of infectious agents include:

- *Gardnerella vaginalis*
- *Peptococcoccus*
- Mycoplasma

3- <u>Cervisitis with or without Urethritis:</u> is caused by gonococci or *Chlamidia trachomatis*

4- **Uterine sepsis:** is caused by **S.** *pyogenes, S. aureus, Clostridium* and *Mycoplasma*
5- **Genital ulceration**: is caused by *T. pallidum, Haemophilus ducreyi* and *Chlamidia*
6- **Tuberculosis of uterus:** is caused by *Mycobacterium tuberculosis*
7- **Viruses:** is caused by viruses like Cytomegalo virus, Herpes

Genital infections and STDs in men

The infections in men are mostly caused by the same organisms as in women, include:

1- Urethritis:

In men *C. trachomatis* causes urethritis lead to epididymitis and prostatitis.

2- Prostatitis: caused by gonococci or Chlamydia

3*- Ulceration:** caused by Herpes simplex virus, *T. pallidum, Haemophilus ducreyi* and *Chlamydia.**

Collection of specimen in men:

1. Cleanse around the urethral opening using a swab moistened with sterile physiological saline.
2. When culture is indicated collect a sample of pus on a sterile cotton-wool swab. If possible, before inserting the swab in a container of transport medium.
3. Inoculated culture plates must be incubated within 30 minutes.

Collection of Sample in women:

Endocervical canal for isolation *N.gonorrhoeae:*

- Use a sterile vaginal speculum to examine the cervix and collected the specimen:

1. Pass a sterile cotton wool swab 20-30 mm into the endocervical canal and gently rotate the swab against the endocervical wall to obtain a specimen
2. When gonorrhea is suspected , before inserting the swab in transport medium, if possible inoculated a plate of culture medium.
3. Inoculated culture plates must be incubated within 30 minutes.

Collection of vaginal discharge to detect *T. vginalis, C. albicans, G.vaginalis*:

Two preparations are required:

1. Wet preparation to detect motile *T.vaginalis*. Use a sterile swab to collect a specimen from the vagina.
2. Dry smear for Gram staining to detect *Candida* and examine for clue cells

Gram positive cells and pseudohyphae of *C.albicans*

Collection of specimen to detect *T. pallidum*:

Must be collected before antibiotic treatment

1. Wearing protective rubber gloves, cleanse around ulcer(chancre) using a swab moistened with physiological saline
2. Gently squeeze the lesion to obtain serous fluid collect a drop on a cover glass
3. Immediately deliver the preparation to laboratory for examination by dark-field microscopy

Collection of specimen to detect C*hlamydiagranulomatis*:

Most Chlamydia infections are diagnosed clinically or immunologically by detecting specific Chlamydial antigen.

1. Cleanse around the ulcerated area using a swab moistened with physiological saline
2. Pinch off a small piece of tissue from the edge or base of a lesion. Crush this between two microscope slids.

Immunological detection of Chlamydial antigen Monoclonal Ab to detect by:

1. Direct fluorescence techniques (FAT)
2. Enzyme immunoassays (EIA)
3. Rapid immunochromatographic (IC)

Culture the specimen

a) Thayer Martin medium. For isolation of *N. gonorrhoeae* and incubate in moist carbon dioxide enriched atmosphere at 35 – 37 C for up to 48 hr. Thayer Martin medium contains the antibiotics (Vancomycin, Colistin, Nastatin).
b) Blood agar (aerobic and anaerobic)
c) MacConkey agar
d) Cooked meat medium. When puerperal sepsis or septic abortion is suspected. Incubated specimen in cooked meat medium and incubate at 35 -37 C and then sub culturing as indicated 24 hr,48 hr, 72 hr
e) Chocolate agar
f) Sabaroued Agar. For *Candida* isolation

PH of discharge.

The normal reaction of vaginal discharge (puberty to menopause) is PH 3-3.5

- This to indicate the following:
- *T.vaginalis*: yellow-green purulent discharge with PH over 5
- *C.albicans*: White odorless discharge with PH below 5
- *G.vaginalis*: Grey offensive smelling (fishy ammoniacal smell) thin discharge with PH over 5

- ***Gram stain to examine:***

  Pus cells containing Gram negative diplococci or pus cells have been damaged and the organism seen lying outside the pus cells that could be *N.gonorrhoeae*

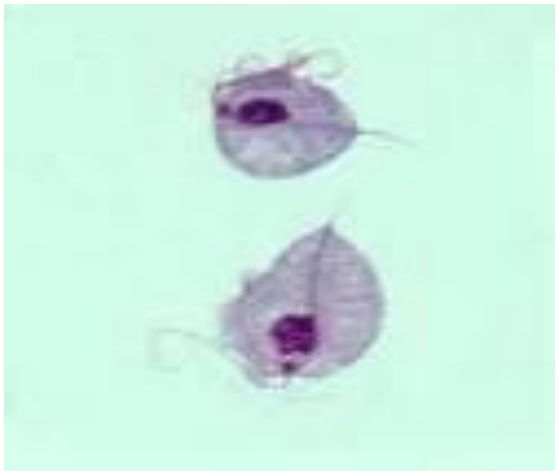
- ***Vaginal smear from a patient with suspected bacterial vaginosis or candidiasis, looked especially for:***

- *Large G+ve yeast cells and Pseudohyphae that could be C.albicans*
- *Clue cells i.e epith.cells with adhering G-ve short bacilli and gram variable coccobacilli could be G.vaginalis*

- **Smear from a patient with suspected puerperal sepsis or septic abortion, looked especially among pus cells for:**

- *Large G+ve rods with straight ends –C.perfringens*
- *G+ve Streptococci –S.pyogenes*
- *G+ve cocci- Staph.aureus*
- *G-ve rods –Bacteroides or coliforms*

- **Wet (saline) preparation to detect *T.vaginalis* To detect motile *T.vaginalis trophozoites***.



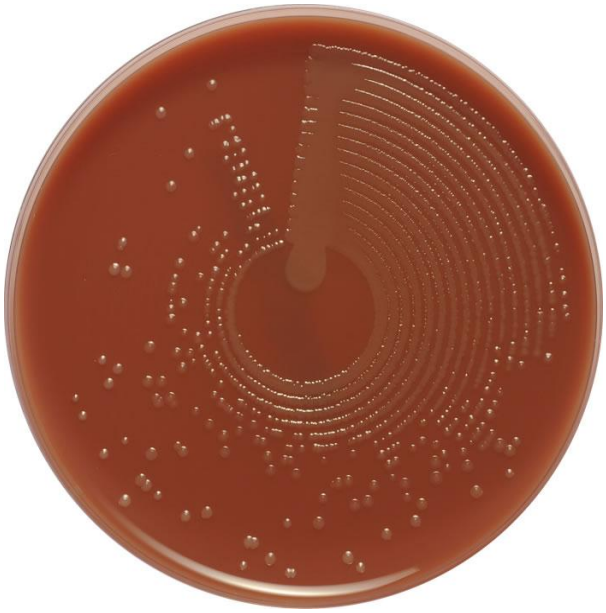
- Dark field preparation to detect motile *T.pallidium*

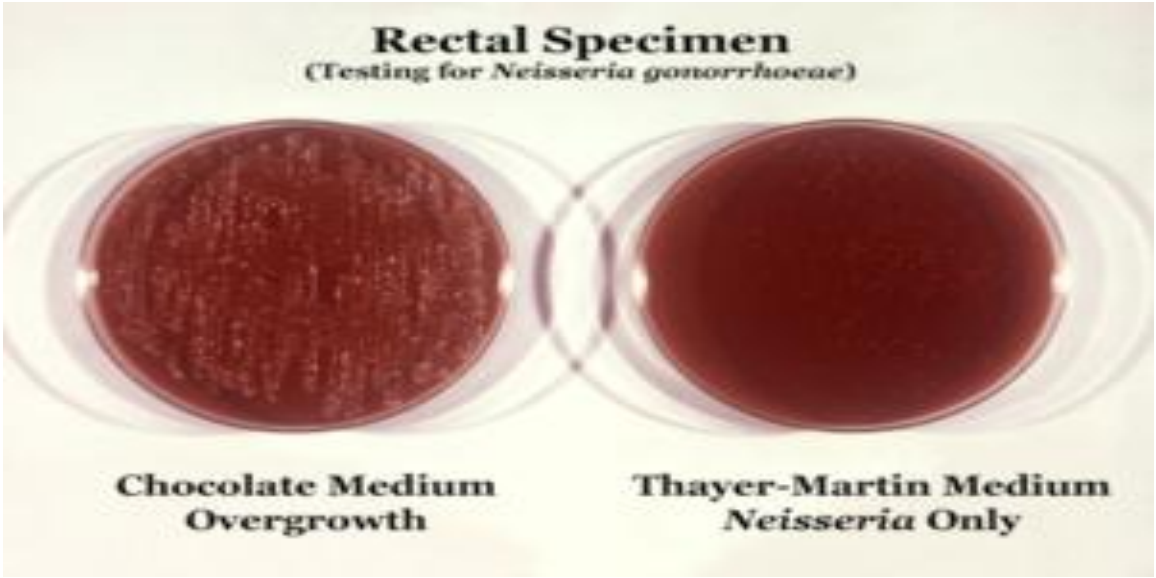
Examine & report the results on Thayer Martin cultures:

- *N.gonorrhoeae* produces small raised . Grey shiny colonies on MNYC medium and Thayer Martin medium after overnight incubation
- Oxidase test strongly positive in *N.gonorrhoeae*
- *Gram stain the colonies N.gonorrhoeae appears as a Gram negative cocci*
- *Test the colonies for beta-lactamase production*

1. Blood agar and MacConkey agar cultures. Look for colonies that could be:

- Streptococcus pyogenes or other beta-haemolytic streptococcus
- Staphylococcus aureus
- Clostridium perfringens
- Proteus species
- Enterococcus
- Escherichia coli





Purulent exudates, burns, wounds and abscesses

One of the most commonly observed infectious disease processes is the production of a purulent (sometimes seropurulent) exudate as the result of bacterial invasion of a cavity, tissue, or organ of the body.

A smear for Gram-staining and examination should be made for every specimen

Culture

all specimens of wounds, burns, pus or exudate should preferably be inoculated onto a minimum of two culture media:

— a blood agar plate for the isolation of staphylococci, streptococci and *Clostridium*

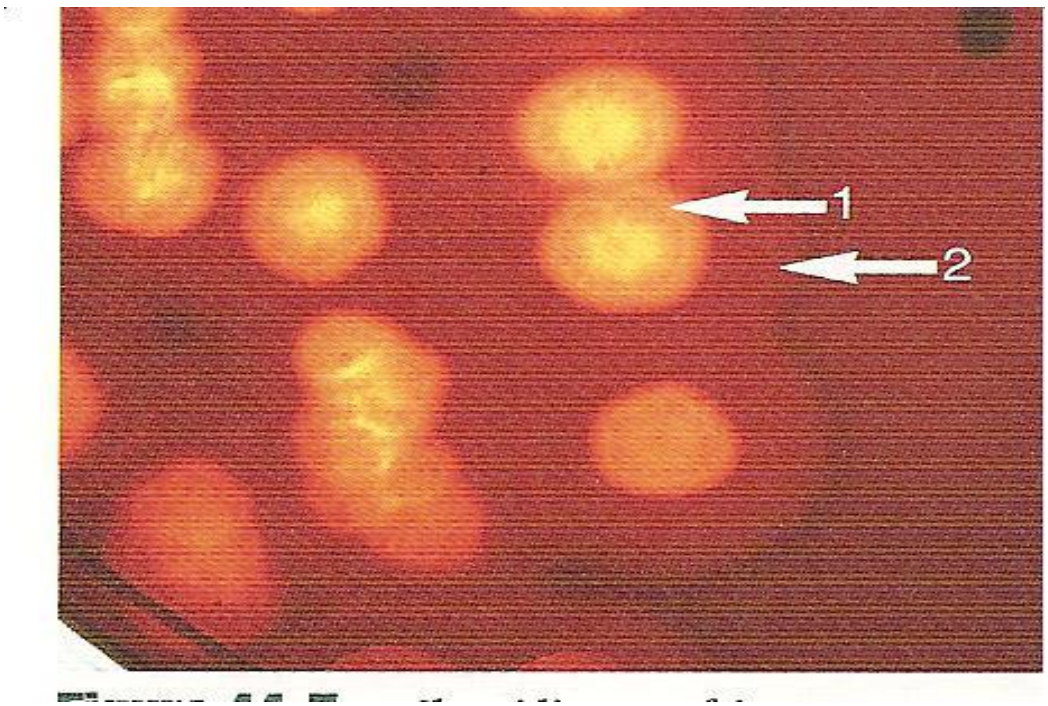
— a MacConkey agar plate for the isolation of Gram-negative rods;

all organisms isolated from wounds, pus, or exudates should be considered significant and efforts made to identify them.

Wound swabs

The clinically most significant species is *Clostridium perfringens*. It is commonly associated with gas gangrene.

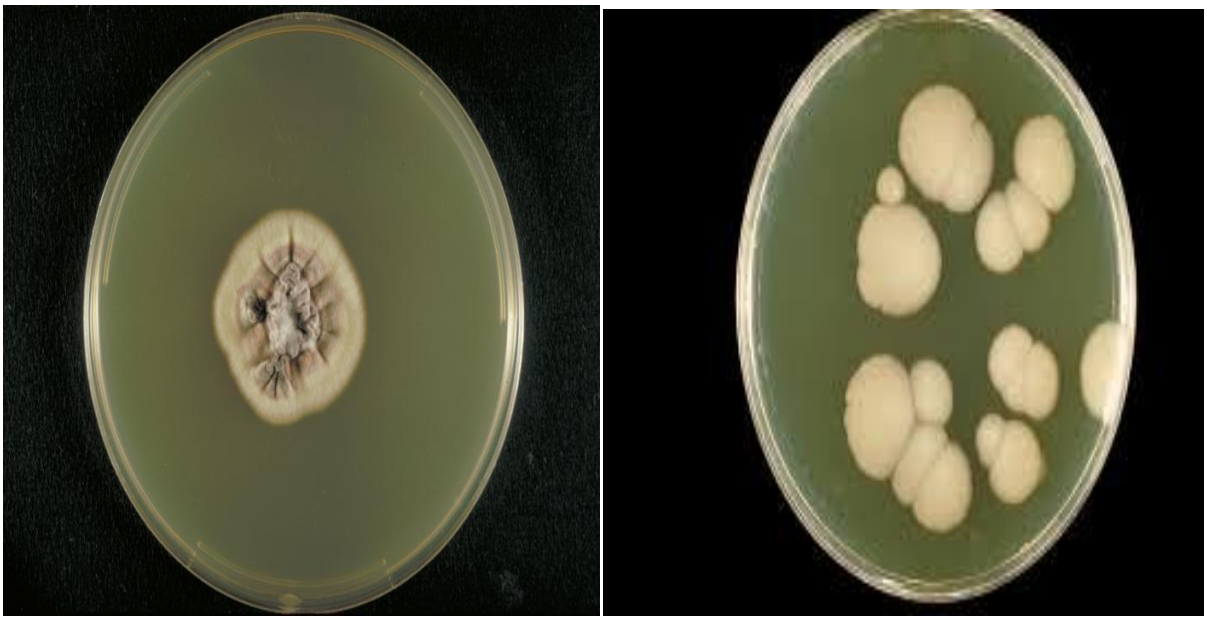
C. perfringens grows rapidly in anaerobic broth with the production of abundant gas. On anaerobic blood agar, colonies of moderate size (2–3 mm) are seen after 48 hours. **Most strains produce a double zone of haemolysis**: an inner zone of complete clear haemolysis, and an outer zone of partial haemolysis.



Clostridium perfringens

Eye and Ear infections

Specimens of eye and ear should be inoculated onto a minimum culture media:
— Blood agar plate for the isolation of staphylococci and streptococci.
— MacConkey agar plate for the isolation of Gram-negative bacteria.
— Chocolate agar plate for the isolation of *Neisseria*.
— Sabouraud dextrose agar plate for the isolation of fungi.



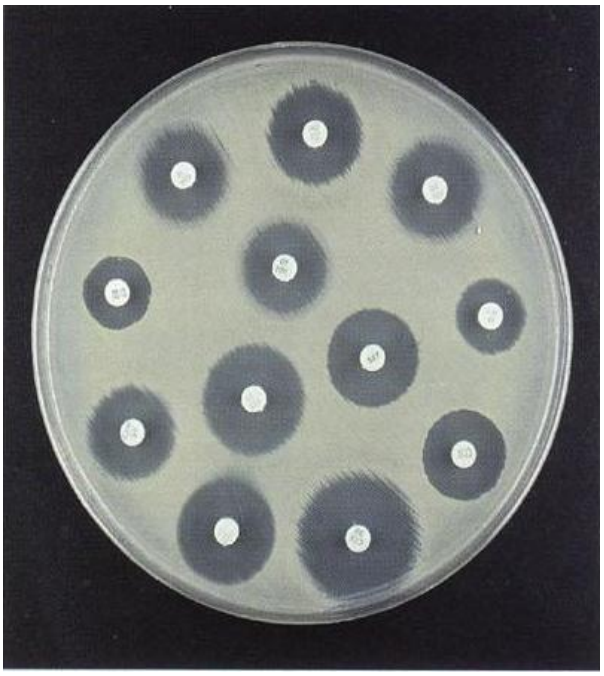
Sabouraud dextrose agar plate

Antibiotic susceptibility tests

Sensitivity (susceptibility) testing is used to select effective antimicrobial drugs. The standardized disc-diffusion method (Kirby–Bauer) is used.
Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial sensitivity. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of sensitivity testing agar (Mueller–Hinton agar for most bacteria and blood agar for some bacteria) which uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited. Strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc.

- All strains of streptococci (such as *S. pneumoniae*) should be tested on blood agar for susceptibility.
- All Gram-negative rods and staphylococci were tested on mueller hinton for susceptibility.
- Strains of *H. influenzae* and Neisseria should be tested for susceptibility using chocolate agar.

Blood agar plate



Mueller–Hinton agar plate