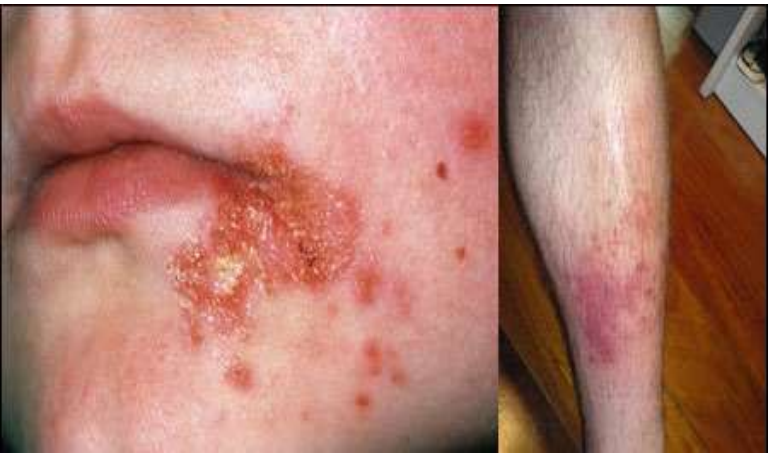
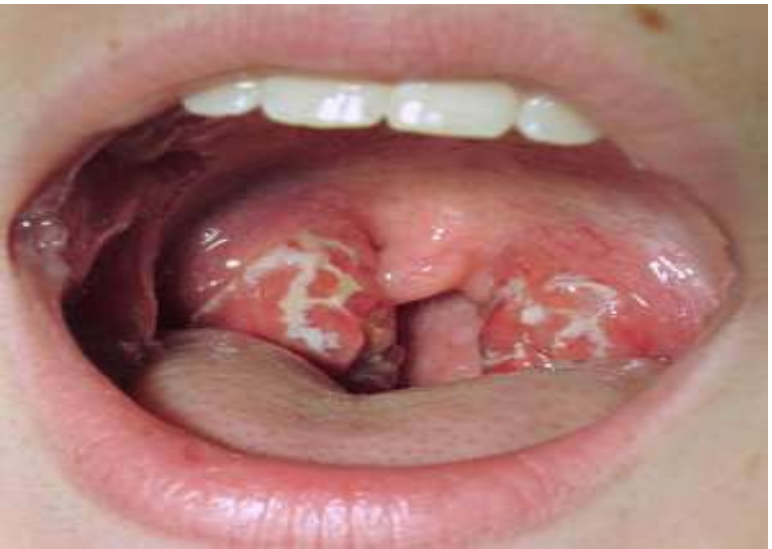
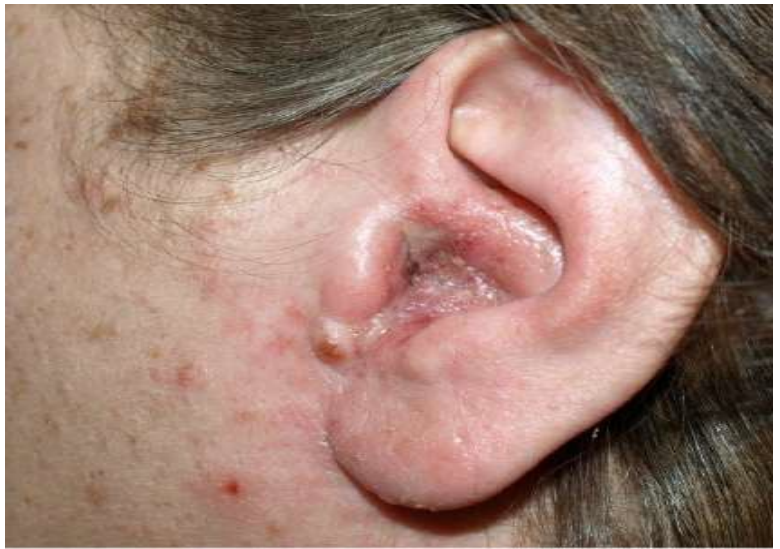
Pyogenic Infection
DR ZAHID KHATTAK







Introduction

- Infection characterized by severe local inflammation, usually with pus formation, generally caused by one of the pyogenic bacteria.
- **Sepsis:The term sepsis covers numerous and diverse pyogenic infections**

  which includes superficial skin infections,wound infections,infection of burns,infection of eyes,peritonitis and abscesses.

Introduction

- **Pus is an exudate typically white yellow or yellow formed at the site of**

  inflammation during infection.
- **Abscesses are localized collection of pus composed of living and dead**

  WBC, components of tissue break down.
- 70% of tissue infection is mainly caused by *Staphylococcus aureus*.

- Pus is a fluid composed of : dead & dying WBC, dead

  & dying bacteria (in bacterial cause of pus),tissue

  debris, edema, fibrin, lipid and nucleic acid.
- Pus cells : it is degranulated WBC, neutrophils.

- The body responds to invasion by a wide variety of bacteria by an

  increased blood supply to the area and

  by an outpouring of serous

  fluid and white blood cells.

- This is the typical inflammatory response

- The white cells which pass from the blood into the infected tissues

  attempt to ingest the bacteria (phagocytosis), many cells die and the resultant material consisting of both living and dead white cells

  (leucocytes or pus cells) and bacteria, together with damaged local tissues and blood proteins, constitutes PUS.

- Infections in which pus is produced are known as pyogenic, i.e. pusproducing infections.
- Pus may be present as a localised collection deep in the tissues—an ABSCESS, it may be produced on a surface, e.g. the mucosa of the pharynx, the mucosa of the bladder, the méninges, indeed any body surface, it is then known as a PURULENT EXUDATE.

- Alternatively infection may spread evenly through the tissues causing a diffuse inflammation :CELLULITIS.
- The type of pus production will depend on the organism causing the infection, on the tissue in which the infective process is taking place, and also on the body resistance to the infection.

- Although the pyogenic infections have very similar appearances whatever the causative organism, different sites of the body have a tendency to be infected with particular species of bacteria.

Common organisms causing Pyogenic infections

- **Gram positive**

  Staphylococcus aureus

  – *Streptococcus pyogenes*

  – *Enterococcus species*

  – *Anareobic streptococci*

  – *Clostridium perfrinhes*

  and other clostridia

  – *Actinomycetes*

  – *Actinomyces israaeli*

  – *Mycobacterium tuberculosis*

- **Gram negative(rare)**

  Pseudomonas aeruginosa

  – *Proteus species*

  – *Escherichia coli*

  – *Bacterioids species*

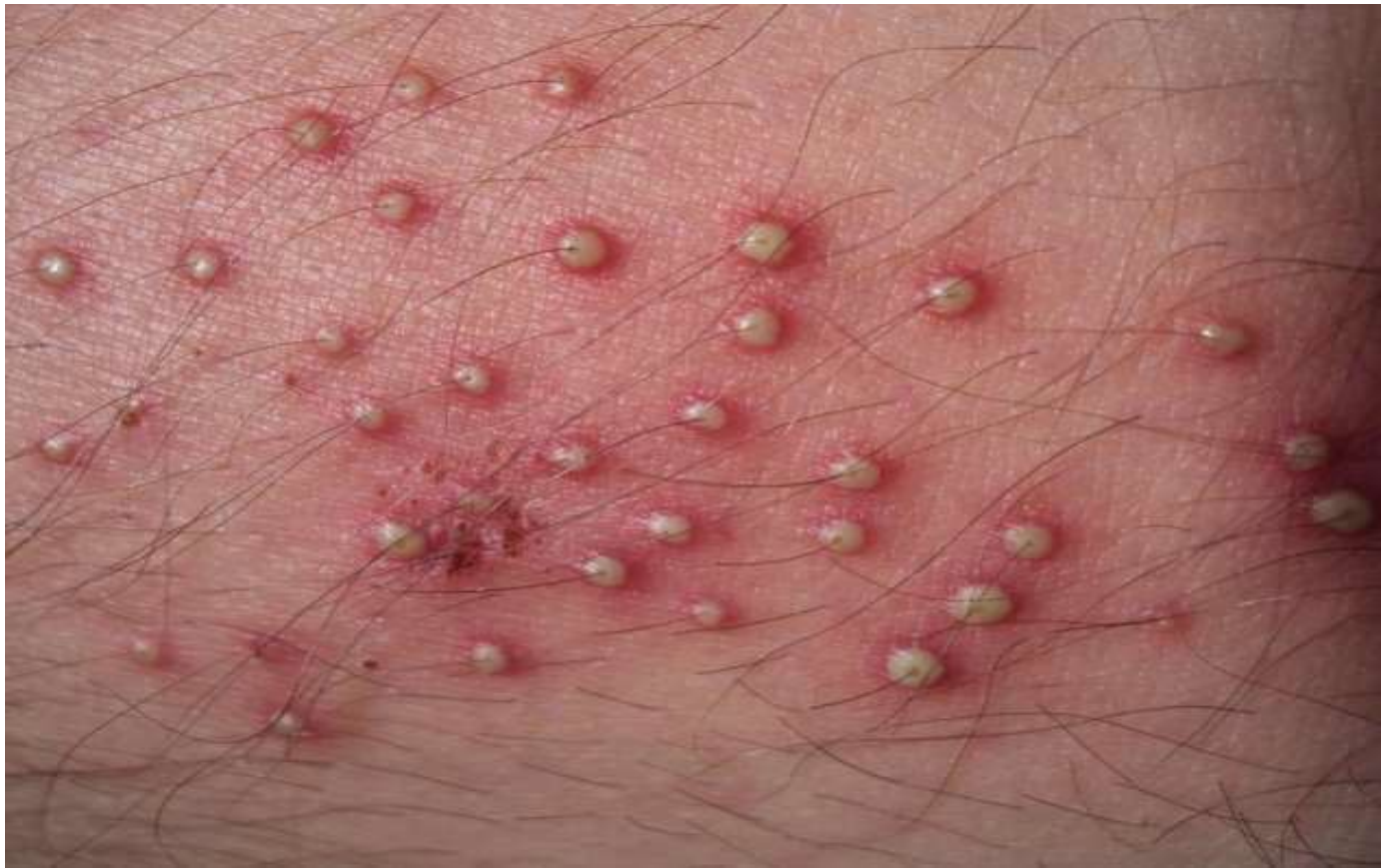
  – *Klebsiella species*

  – *Pasteurella species*

- **Fungi** **Parasites**
- *Mycetoma spp* *Entamoeba histolytica*
- *Histoplasma*
- *Blastomyces*
- *Candida albicans*
- *Cryptocooccus neoformans*

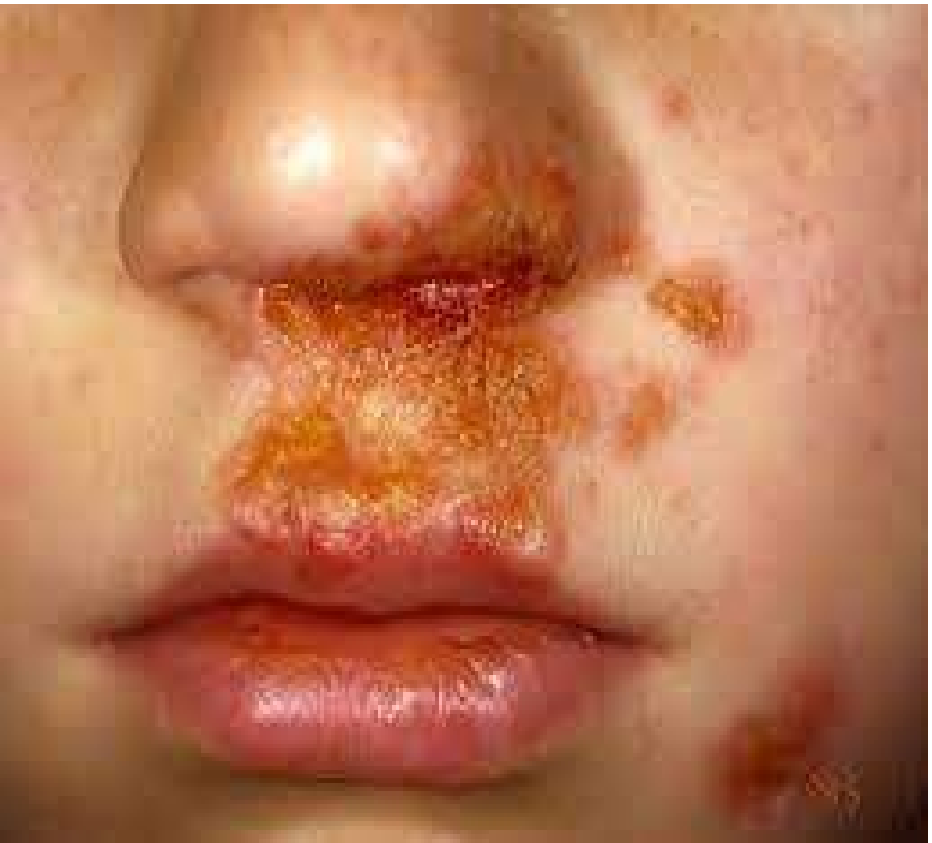
Some of the common infections caused by pyogenic bacteria's are:

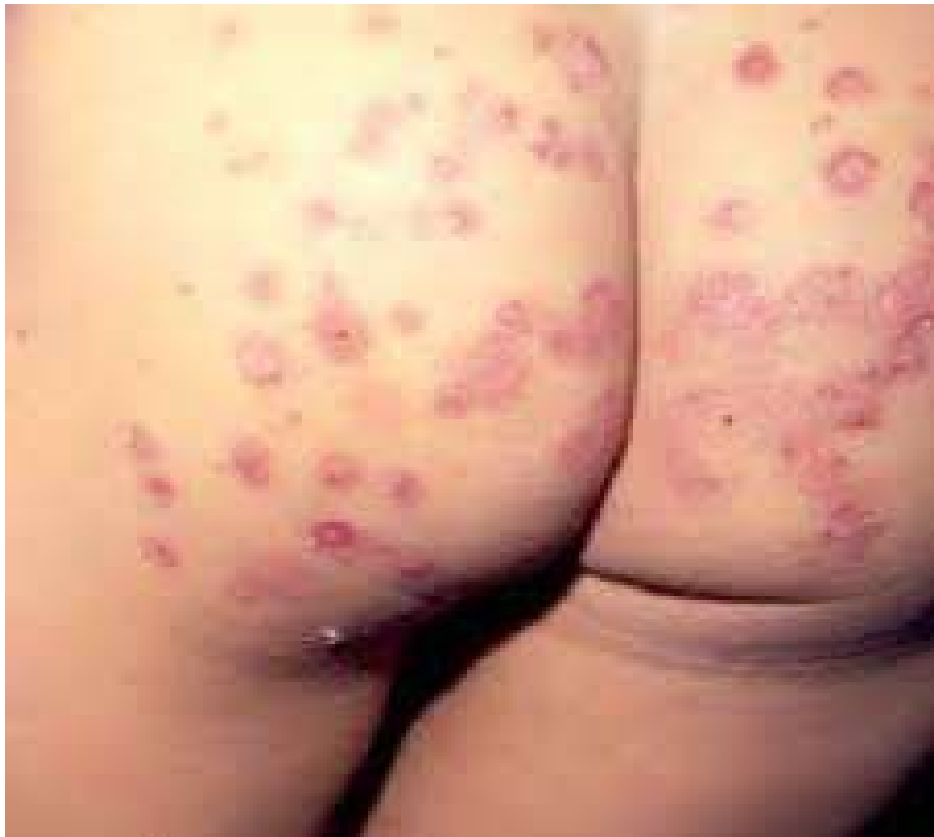
Folliculitis: It is the infection and inflammation of one or more hair follicles.
Potential bacteria:***S.aureus***



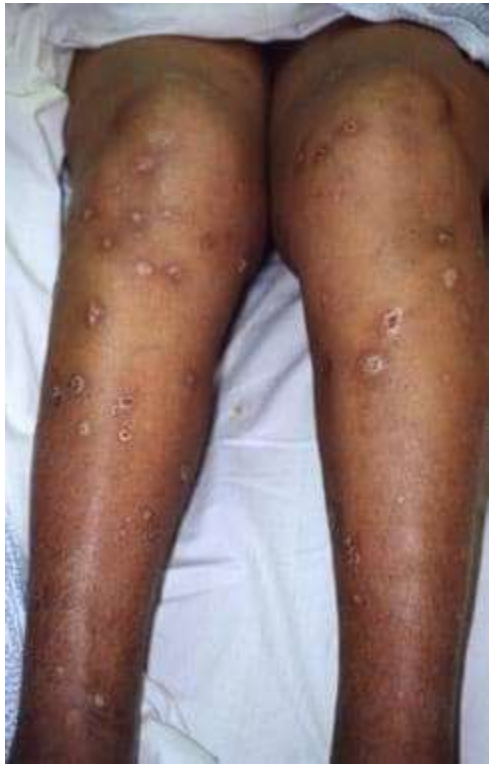
Impetigo: ***It is a contagious skin infection that usually*** produces blisters or sores on the face, neck, hands, and diaper area.

Potential bacteria:***S.aureus, S.pyogenes***

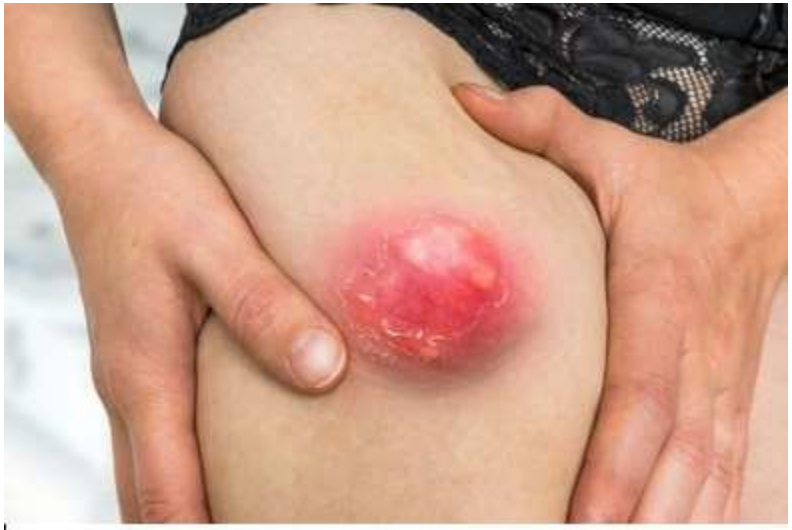


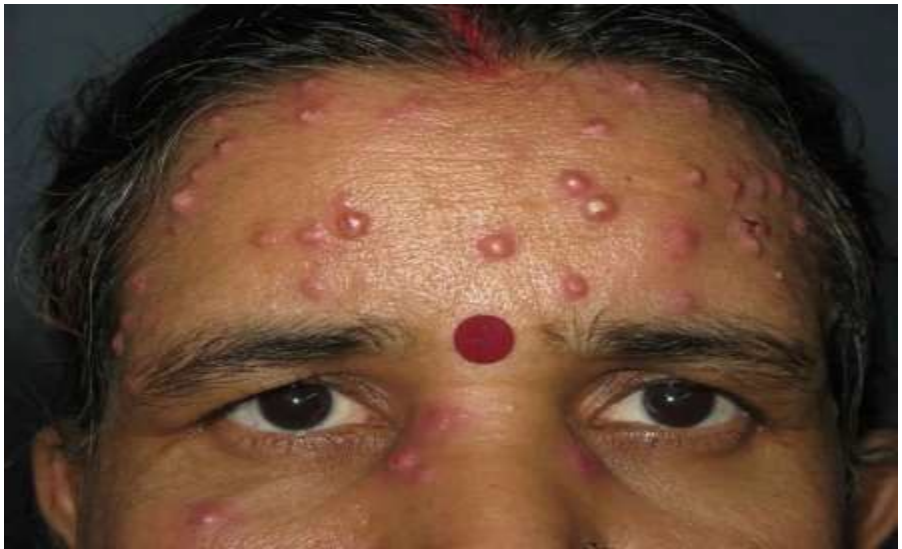


Ecthyma :It is similar to impetigo, but occurs deep inside the skin.

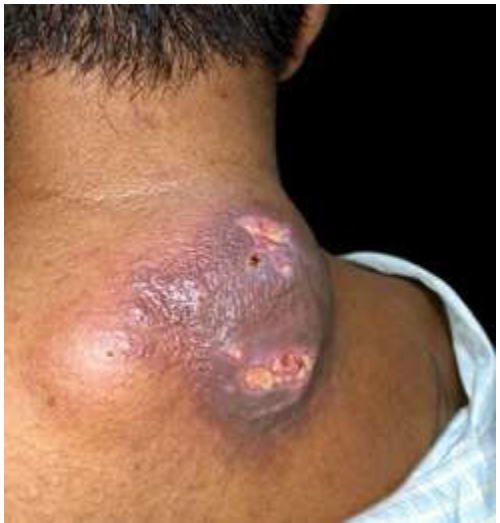


Furncles: It is another word for a boil. Boils are bacterial or
fungal infections of hair follicles. *S.aureus*

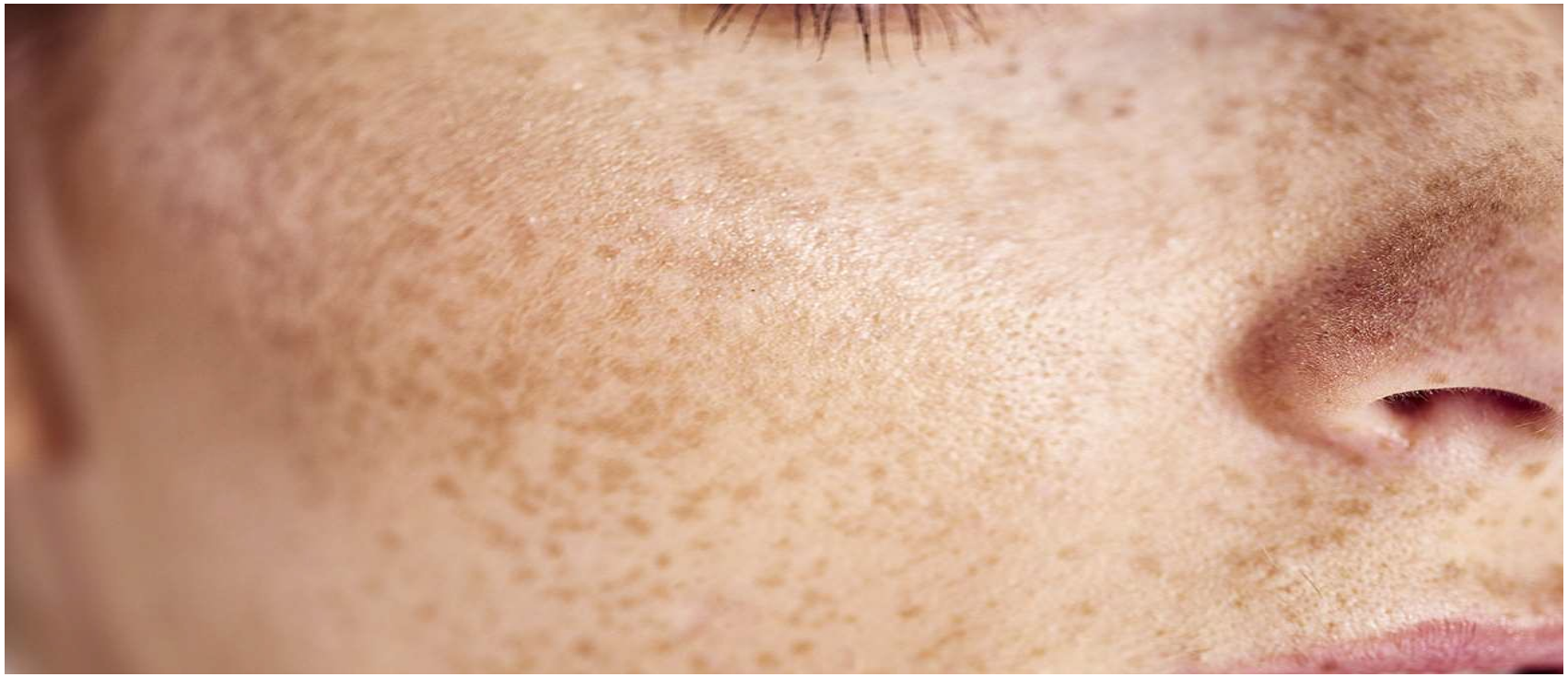




Carbuncle:clustures of furuncle connected subcutaneously causing deeper supperations.A large abscess,usually occurs in thick collagenous tissue such as back of the neck.



Macules: A patch of skin that is altered in color but usually not elevated and that is a characteristic feature of various disease.

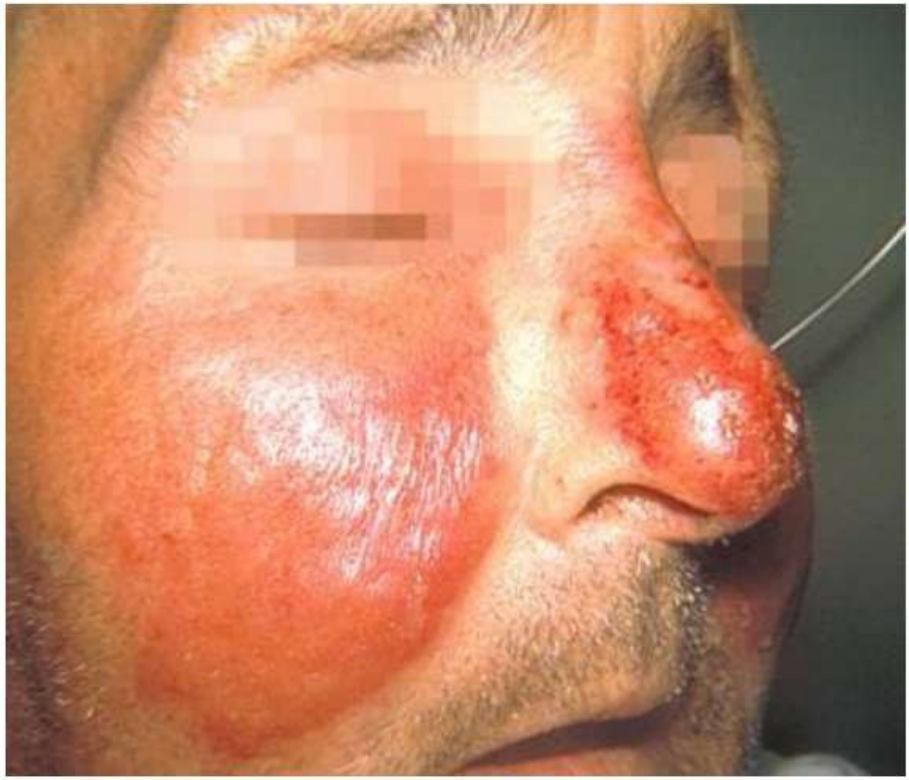


Papules: It is a circumscribed, solid elevation of skin with no visible fluid, varying in size from a pinhead to 1 cm. They can be brown, purple, pink or red in color, and can cluster into a rash. The papules may open when scratched and become infected and crusty.

Stye:It is a small boil or abscess in one of the glands of lash follicles caused by *S.aureus of endogenous* origin(anterior nares or implantation by finger from a septic lesion elsewhere in the body.

Acne: It is a common skin condition where the pores of skin become blocked by hair, sebum (an oily substance), bacteria and dead skin cells. Those blockages produce blackheads, whiteheads, nodules and other types of pimples.

Erysipelas :It is an infection of the upper layers of the skin (superficial). The most common cause is group A streptococcal bacteria, especially *Streptococcus pyogenes*. *Erysipelas results in* a fiery red rash with raised edges that can easily be distinguished from the skin around it. The affected skin may be warm to the touch.



Well-demarcated, erythematous plaque of erysipelas. Courtesy of the US Centers for Disease Control and Prevention.

1.Infection of wounds

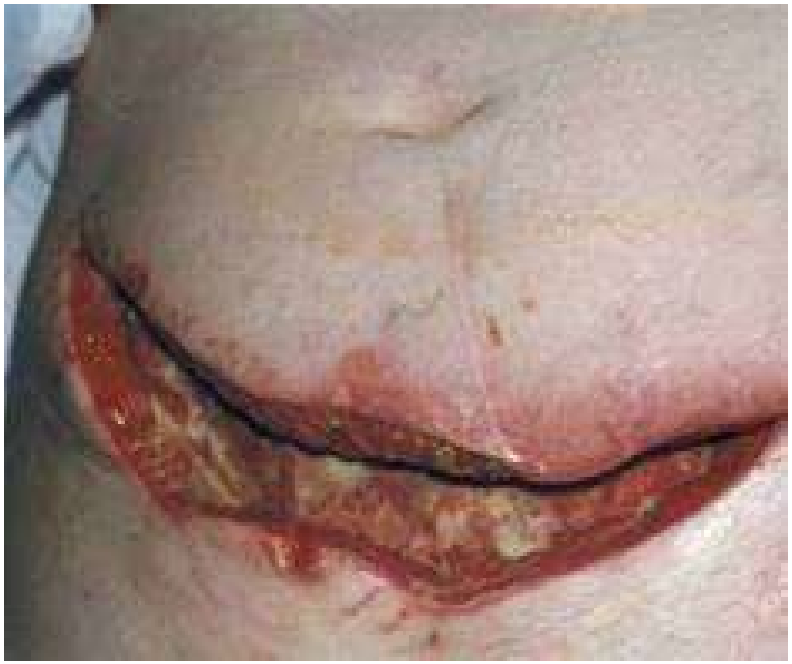
- Infection may occur following accidental trauma and injections, but postoperative wound infections in hospital are most common .Wound infection may be:
- **Endogenous infection:** Infection occurs by patients own bacteria flora such as S.aureus from skin and anterior nares or coliforms

- **Exogeneous infection** Spread of organisms from hospital staff and visitors occur by direct and indirect airborne routes. more than 60% of hospital acquired infections are due to Gram negative enteric bacilli and only in 30% cases Gram positive cocci responsible

SURGICAL WOUND INFECTION

MINOR WOUND INFECTION—NO,SIRS

MAJOR WOUND INFECTION-TOO MUCH PUS +SIRS



2.Infections of skin and subcutaneous Tissue

Clinical types

Acne(pimple):

caused by *Propionibacterium acne*, *anaerobic diphtheroids*, *coagulase negative Staphylococci and micrococci*

Staphylococcal infection

Boils , furuncles and

Carbuncle are commonest leisons caused by *S.aureus*

- **Streptococcal infections** includes cellulitis, erysipelas, ecythema and impetigo and scarlet fever
- **Gram negative infections** are rare on healthy skin except moist area of groin and axilla

Infection of Burns

- The large moist exposed surface of burns become colonised by bacteria within 24 hours
- Bacterial flora of skin, respiratory tract and colon, streptococci and aerobic spore-bearing bacilli and non-fermenting bacilli are often involved
- *S. aureus is the commonest isolate from burns* , followed by *P. aeruginosa and then various GNB* e.g *E. coli*, *Klebsiella spp*, *Acinetobacter spp and S. pyogenes groups A, B, C and G*

Infection of Eyes

- Eyelid infections: Stye- *S.aureus*
- Infection of Lacrimal apparatus
- Conjunctivitis/ Keratitis/ Orbital Cellulites: Causative agents: *S. aureus*, *Hemophilus spp*. *Moraxella Lacunata*, *Chlamydia trachomatis*, *S. pneumoniae* , *Adenovirus*, *HSV 1*

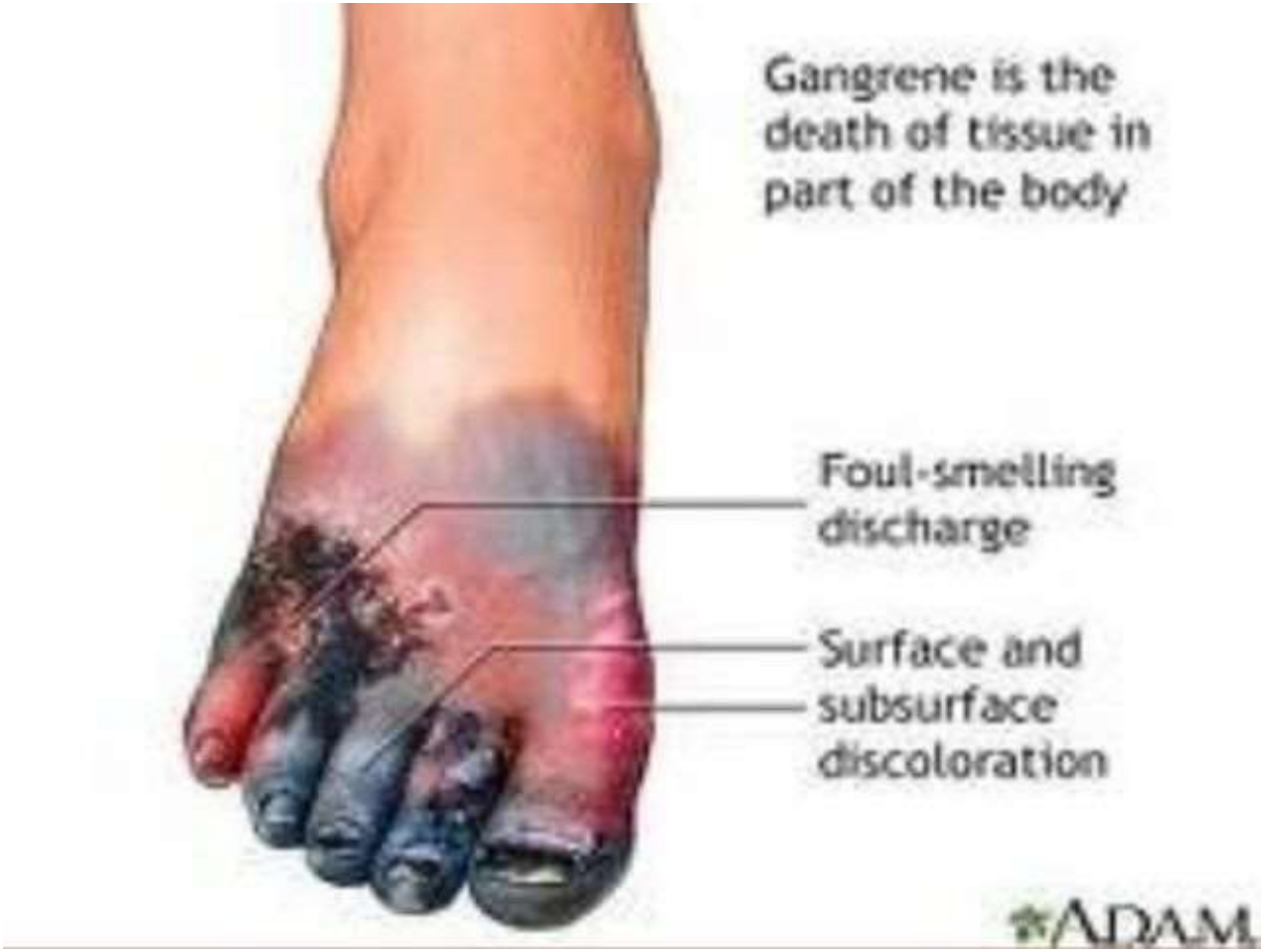
Notes on pathogen

- *Staphylococcus aureus is the commonest* pathogen isolated from subcutaneous abscesses and skin wounds. It also causes impetigo. Penicillin and methicillin resistant strains of *S.aureus are common causes of* hospital acquired wound infections.
- *Pseudomonas aeruginosa is associated with* infected burns and hospital acquired infection

- *Escherichia coli*, *Proteus species*, *Pseudomonas aeruginosa and Bacterioides* species are the pathogens most frequently isolated from abdominal abscesses and wounds. Pus containing *Bacterioides species* has very unpleasant smell.

- *C. perfringens is found mainly in deep wounds* where anaerobic conditions exist. The toxins produced cause putrefactive decay of the infected tissue with gas production. The **death and decay of tissue by *C. perfringens is called* gas gangrene.**

- Chronic leg ulceration is common in those with sickle cell disease. The commonest pathogens isolated are *S. aureus*, *P. aeruginosa*, *S. pyogenes*, and *Bacteroides species*.


Gangrene is the death of tissue in part of the body
Foul-smelling discharge
Surface and subsurface discoloration
ADAM.

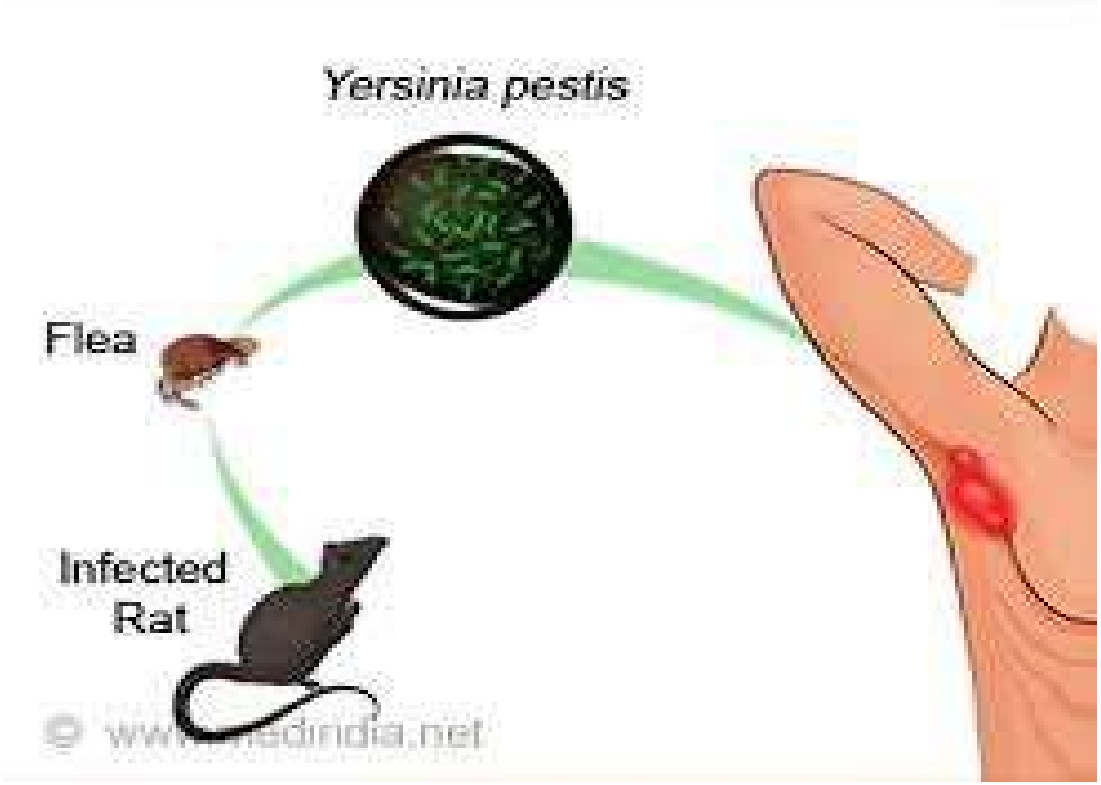
- *Mycobacterium tuberculosis is associated with* cold abscesses. **It is cold because it is not accompanied by the classical signs of inflammation .**
- *Bacillus anthracis causes anthrax, with Cutaneous* form of the disease producing pustule usually on the hand or arm.

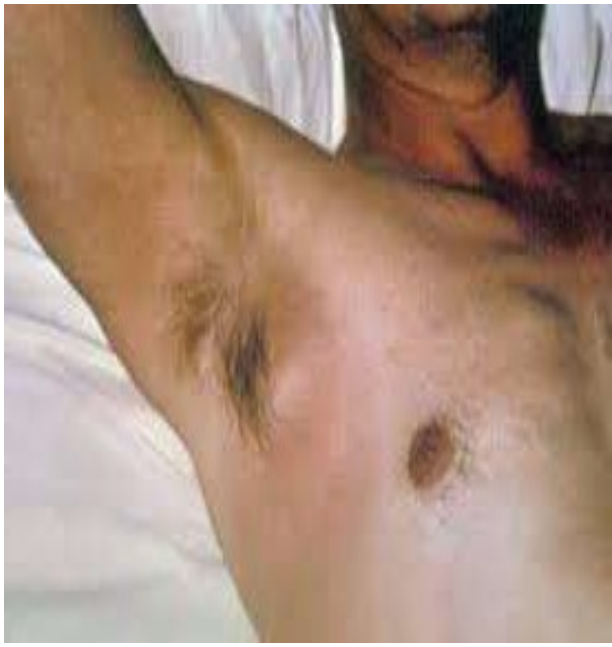
- Vincent’s organisms (*Borrelia vincenti with Gram* negative anaerobic *fusiform bacilli) are associated* with tropical ulcer. The ulcer is commonly found on the leg, often of malnourished persons, especially children. Staphylococci and streptococci are frequently secondary invaders.

- Actinomycetes (filamentous bacteria) and several species of fungi cause mycetom. Specimens of pus from the draining sinuses contain granules, examination of which helps to differentiate whether the mycetoma is bacterial (treatable) or fungal (less easily treated).

- *A. israeli and other species of Actinomyces cause* actinomycosis. Small yellow granules can be found in pus from a draining sinus (often in the neck).

- *Y. pestis causes plague. The disease is referred* to as **bubonic plague when the organism** infects a lymph gland and produces a painful swelling referred to as a bubo. The organism can be found in the fluid aspirated from the bubo and in the surrounding inflamed tissue. The organism is highly infectious.


Yersinia pestis
Flea
Infected
Rat



Laboratory diagnosis

Specimens

- Tissue biopsies
- Aspirates /swabs/pus
- Material obtained by surgical debridement
- Drainage samples
- Exudates
- FNAC

Aspirated material is superior to a swabspecimen because swabs:

1. Commonly yield host of mixed bacterial flora
2. Often don’t reflect true organisms.
3. Easily contaminated
4. Tend to dry out.
5. Likely to expose anaerobes to too much oxygen
6 . Small volume

Sample collection

- Pus from abscess is best collected at the time the abscess is incised and drained, or after it is ruptured naturally
- Care should be taken to avoid contamination with commensal organism from the skin

- As far as possible a specimen from wound should be collected before an antiseptic dressing is applied.
- If a swab must be used, collect two, one for culture and one for Gram stain.

Sampling Deep Wounds:

- 1. Disinfect the surface with 70% alcohol and then with 2% tincture of iodine.
- 2. Aspirate the deepest portion of the lesion or pass a swab deep into the lesion, firmly sampling the lesion's leading edge. Avoid contamination by the wound surface.
- 3. If collection is done at surgery, a portion of the abscess wall should also be sent for culture.

- 4. Transfer material into a sterile container
- 5. Remove tincture of iodine with 70% alcohol to prevent burn.

Sampling Soft Tissue Aspirate:

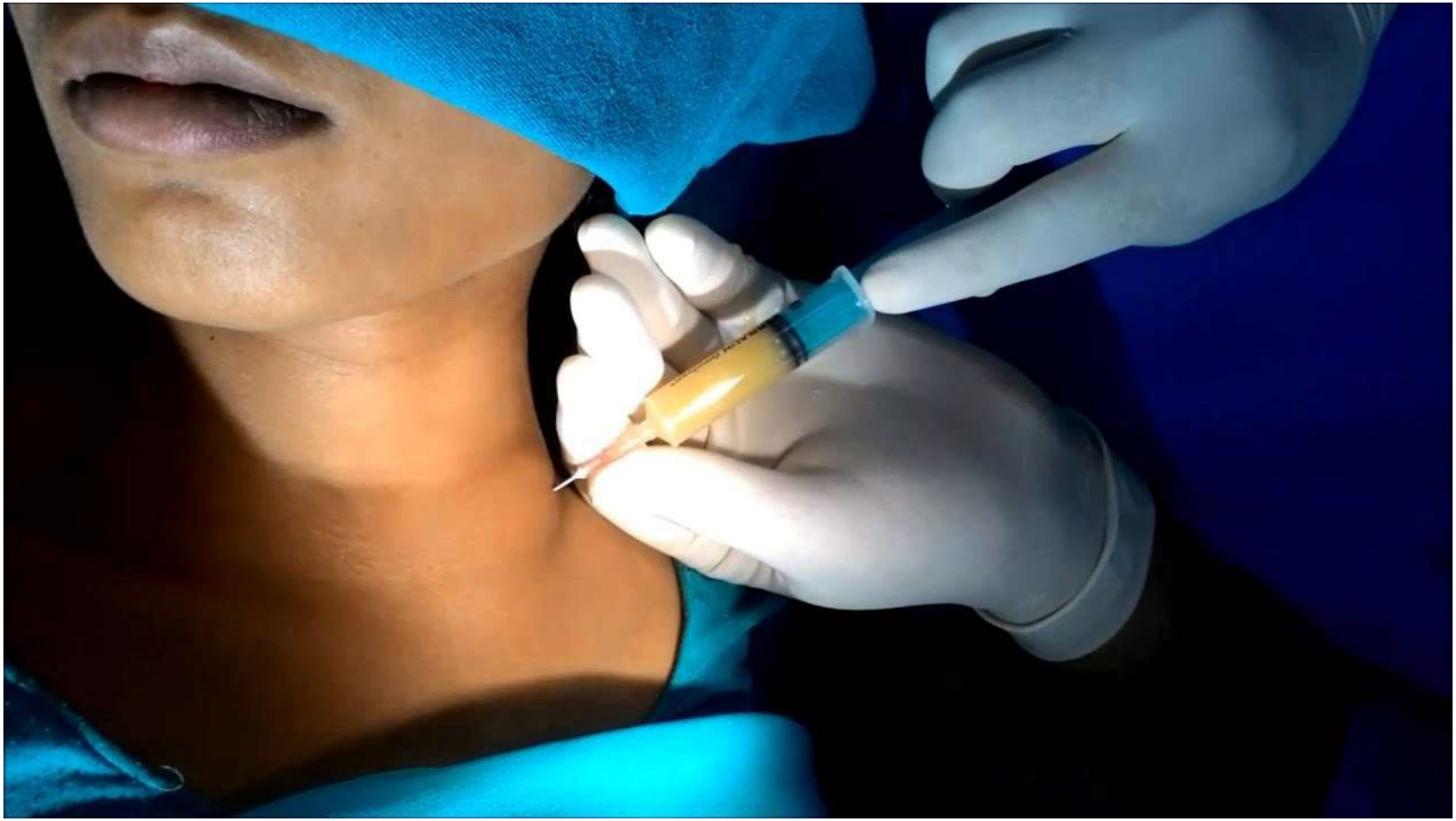
- 1. Disinfect the surface with 70% alcohol and then with 2% tincture of iodine.
- 2. Aspirate the deepest portion of the lesion or sinus tract or pass a swab into the lesion, firmly sampling the lesion's edge. Avoid contamination by the wound surface.
- 3. Transfer material into a sterile container

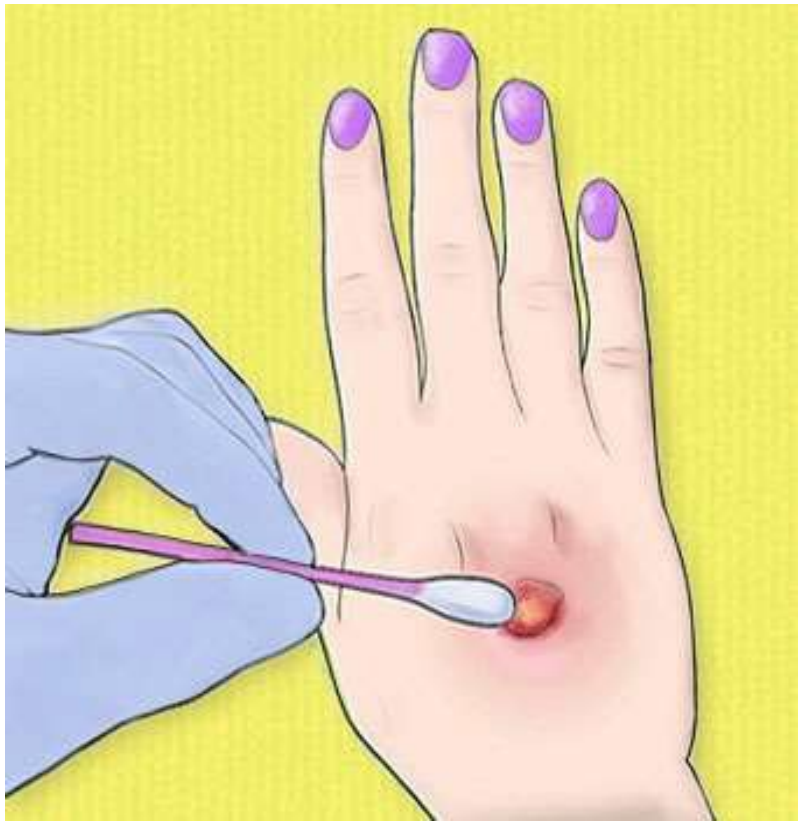
Transport/Storage:

- **Onsite collections** Transport to the Microbiology Laboratory immediately at room temperature. Do not refrigerate.
- **Off site collections :** Specimens must be promptly transported to the laboratory, with the next available courier, not to exceed 24 hours from the time of collection.
- Transport aerobic swab or aspirate refrigerated.

Transportation medium

- Amies transport medium





Swabs

- Swabs suitable for taking specimens of exudates from the throat, nostril, ear, skin, wounds and other accessible lesions consist of a sterile pledget of absorbent material, usually cotton wool or synthetic fiber, mounted on a thin wore or stick.
- Different swabs for special purposes are
  - Baby swabs, Per nasal swab, post nasal swab, laryngeal swab and High vaginal swab.

- Sampling swabs for the safe collection, transport and preservation of all types of microorganisms to the laboratory.
- Synthetic and natural materials are available allowing the user to tailor cost and performance
- Swabs are available dry or with culture media

(Amies Clear, Amies Charcoal, MRD); with wooden, aluminium, polystyrene or polypropylene shafts; and with cotton, viscose or alginate tips.

Different types of swab sticks

- Always submit **two swabs so that Gram stain can be** performed.
- Limit swab sampling to wounds that are clinically infected or those that are chronic and are not healing.
- To minimize contamination, it is important to cleanse the wound to remove superficial debris by thorough irrigation and cleansing with non-bacteriostatic sterile saline.
- If the wound is relatively dry, collect the specimen with two cotton-tipped swabs moistened with sterile nonbacteriostatic saline. Gently roll the swab over the surface of the wound approximately five times, focusing on an area where there is evidence of pus or inflamed tissue.

Criteria of specimen rejection

- Inappropriate specimen transport device;
- mislabeled specimen;
- unlabeled specimen;
- specimen received after prolonged delay (usually more than 72 hours);
- specimen received in expired transport media and dried samples.

In a hospital with a microbiology laboratory

- Using a sterile technique, aspirate or collect from a drainage tube up to 5ml of pus.
- It is then transferred to a sterile leak proof sterile container.
- When pus is not being discharged, a sterile cotton wool swab is used to collect sample from the infected site.Immerse the swab in a container of Amies transport medium.

- Label the specimen and as soon as possible deliver it with a completed request form to the laboratory.
- ***Caution: Specimens from patients with suspected plague or anthrax are*** **highly infectious. Label such specimens HIGH RISK and handle them with care.**

In a health centre for dispatch to a microbiology laboratory

- Collect the specimen using a sterile cotton-wool swab. Insert it in a container of Amies transport medium , breaking off the swab stick to allow the bottle top to be replaced tightly.
- When the material is aspirated fluid from a pustule, transfer the fluid to a sterile, leak-proof container. Stopper, and seal in a leak-proof plastic or metal container
- ***Note: It is not possible to transport exudate from a suspected treponemal* ulcer because the treponemes remain motile for only a short time.**

- Make a smear of the material on a clean slide (for Gram staining) and allow to air-dry in a safe place. Heat-fix the smear
- Send the specimens with a completed request form to reach the microbiology laboratory within 6 hours.

Laboratory examination

Macroscopic examination

- – The appearance of pus and the amount of pus should be noted on initial examination.
- – The pus of staphylococcal lesion is typically creamy and thick.


1
2
3
4
5

- – That of Streptococcus pyogenes infection is generally straw colored and watery.
- – Proteus infection has a fishy smell and the Pseudomonas infection a sweet, musty odour and often a blue pigmentation.
- – Pus containing anaerobic organism often has an offensive putrid smell.
- Examination of wet film may reveal the presence of fungi or motile bacteria

- When mycetoma or actinomycosis is suspected, the appearance of specimen and presence of granules is reported.
- Detection of granules:

– White, yellow, brown, red or black granules may be present in the pus in mycetoma.

– The sample is mixed with distilled water to free the granules.

– A hand lense can be used to view the granules.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Microscopic examination

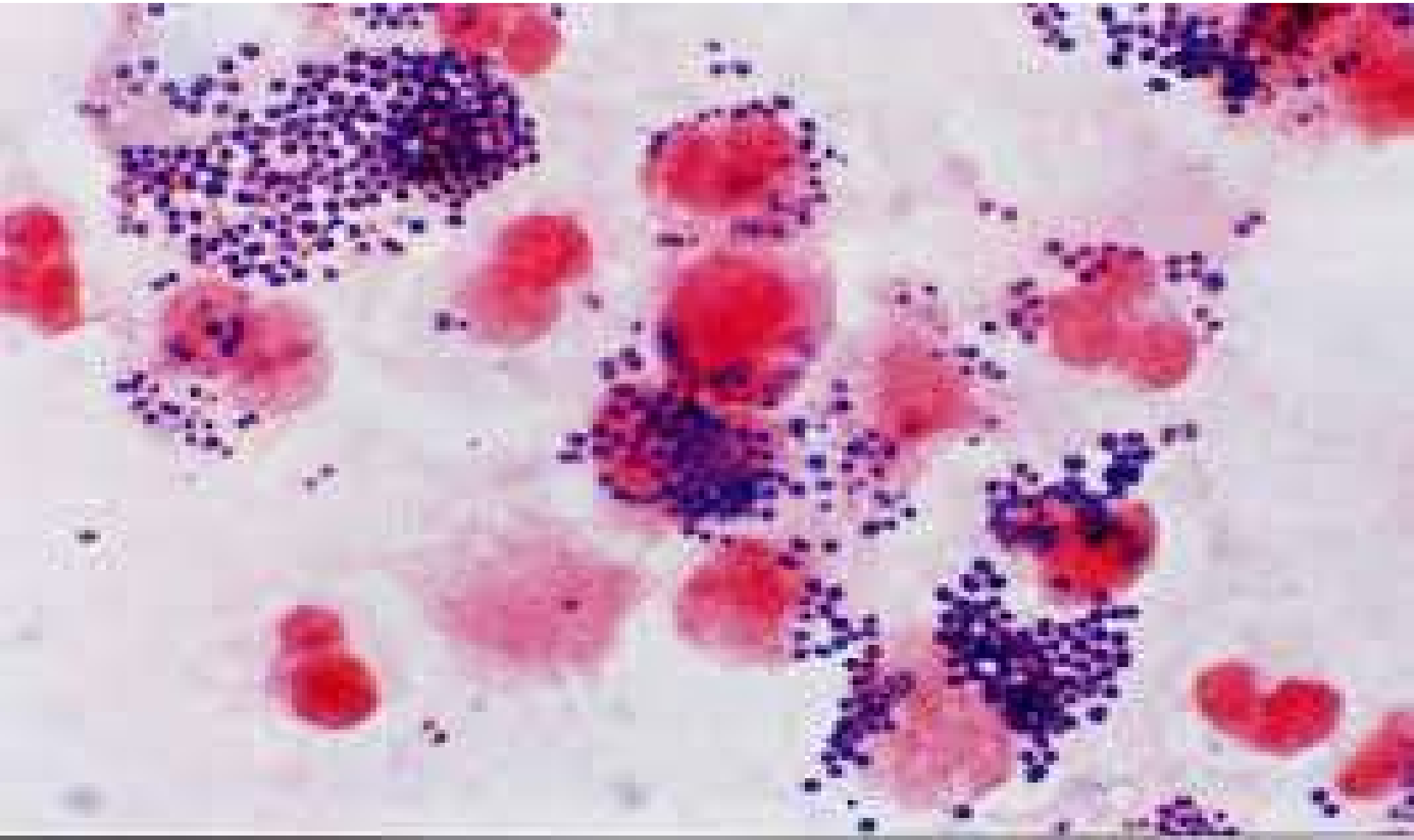
Direct examination

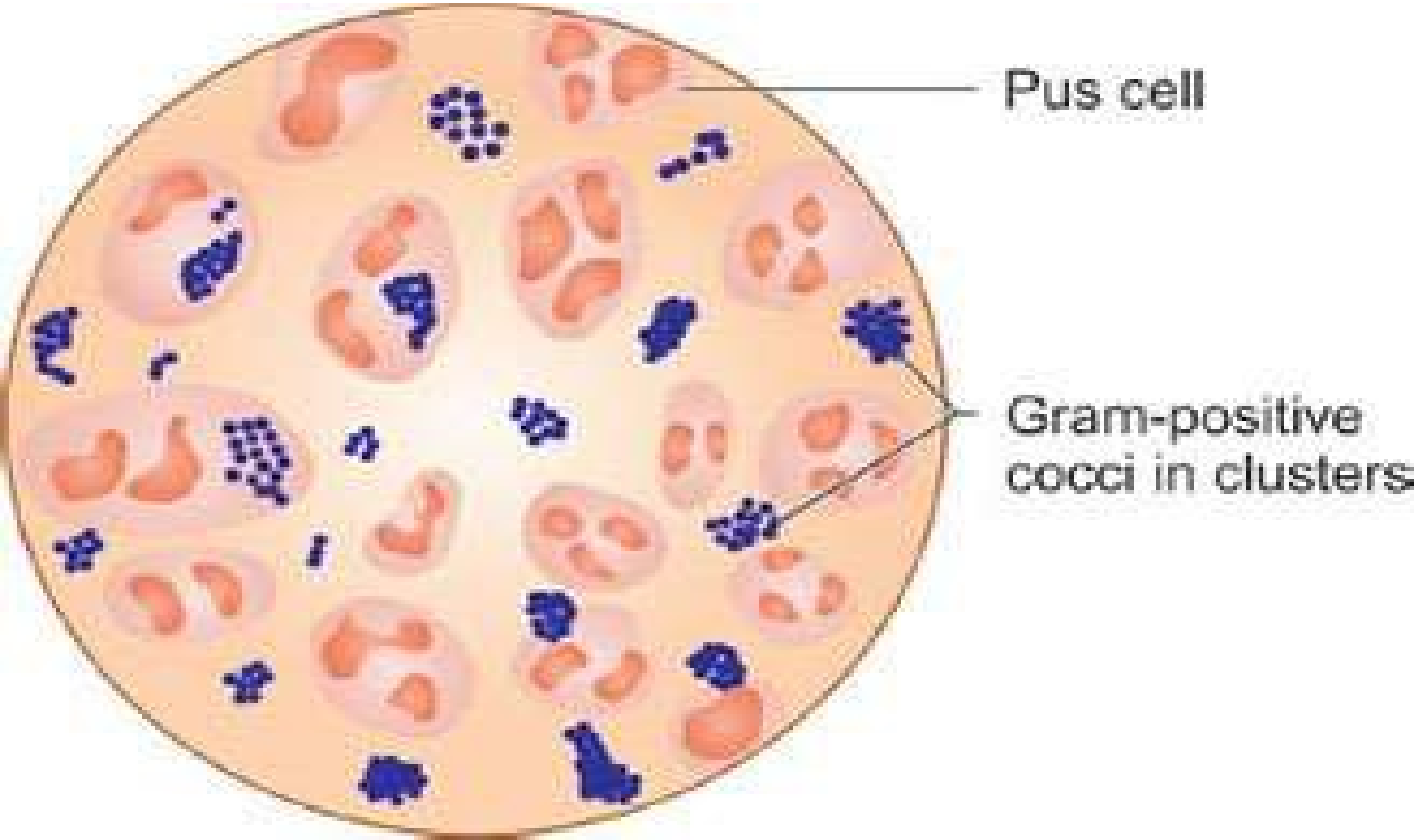
- Staphylococcal lesion shows the evidence of pus cells.
- Streptococcal infection is generally with lysed pus cells.

- Presence of many epithelium cells resembles skin contamination but presence of PMNs suggest good quality of sample.

Gram Smear

- Make an evenly spread smear of the specimen on a clean, grease-free slide
- It is air dried and stained by Gram technique.
- The smear is examined for bacteria among the pus cells using 40x and 100x.
- and stain by **Gram staining technique. Examine the smear** for the presence of bacteria and pus cells (PMNs).

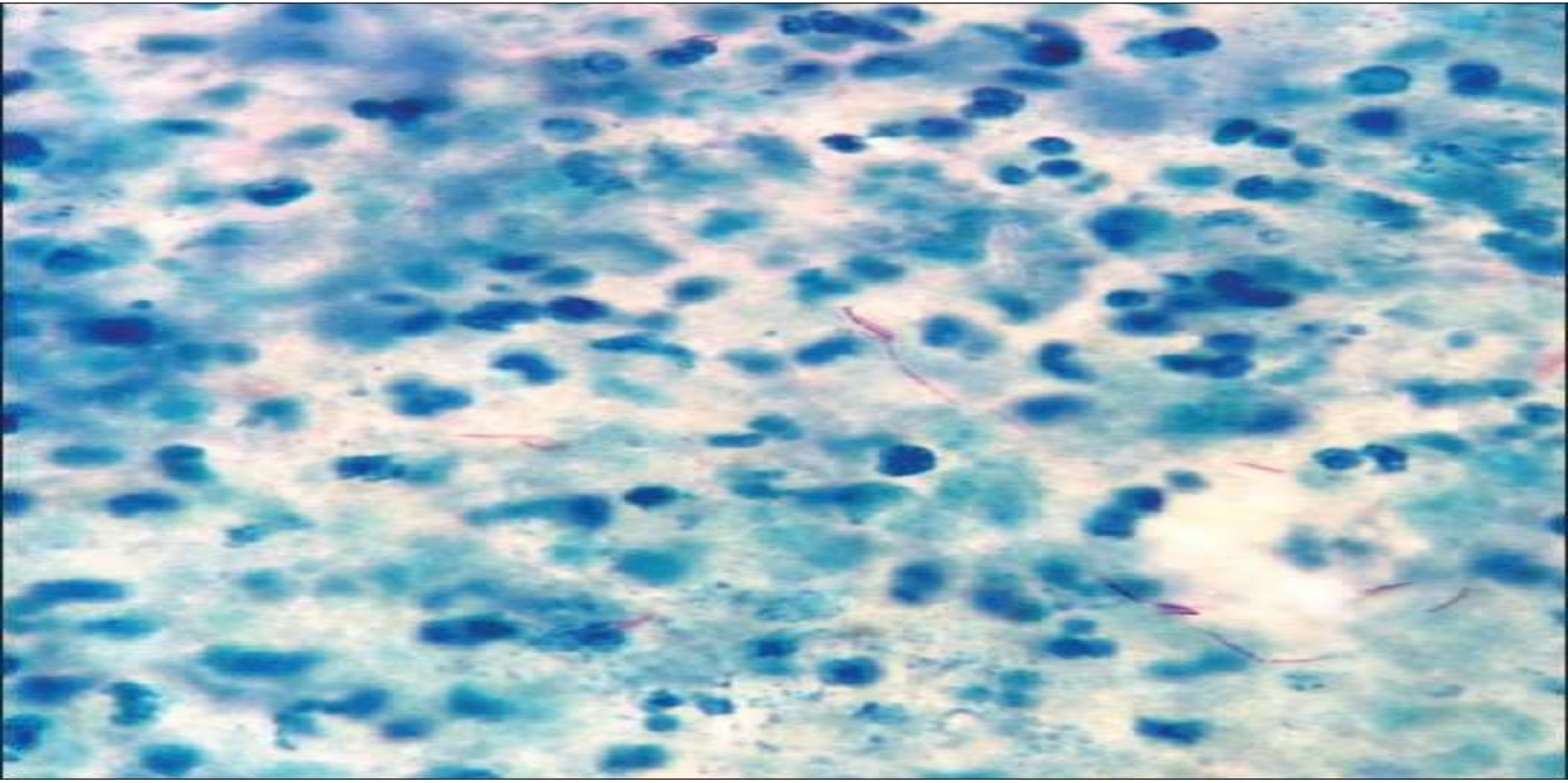



Pus cell
Gram-positive
cocci in clusters

- using 100x objective lens and look especially for:
- – Gram negative rods (Possible pathogens are *E.coli*, ***Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus or* Bacteroides species)**
- – Gram positive cocci in pairs, chains or clusters (possible pathogens are ***Staphylococcus aureu ,Streptococcus pyogenes*, *anaerobic streptococc or*** enterococci).

- – Gram positive large rods with square ends (possible pathogens are ***Clostridium perfringens or Bacillus anthracis***
- – In the case of anaerobic infections large number of pleomorphic bacteria (streptococci, Gram positive and Gram negative rods of various size and fusiform bacteria) may be seen. Sometimes, Gram positive yeast cells with psuedohyphae may be seen, which can be ***Candida albicans***

Ziehl-Neelsen smear is prepared when tuberculosis is suspected



KOH preparation:

When a fungal or actinomycete infection is suspected.

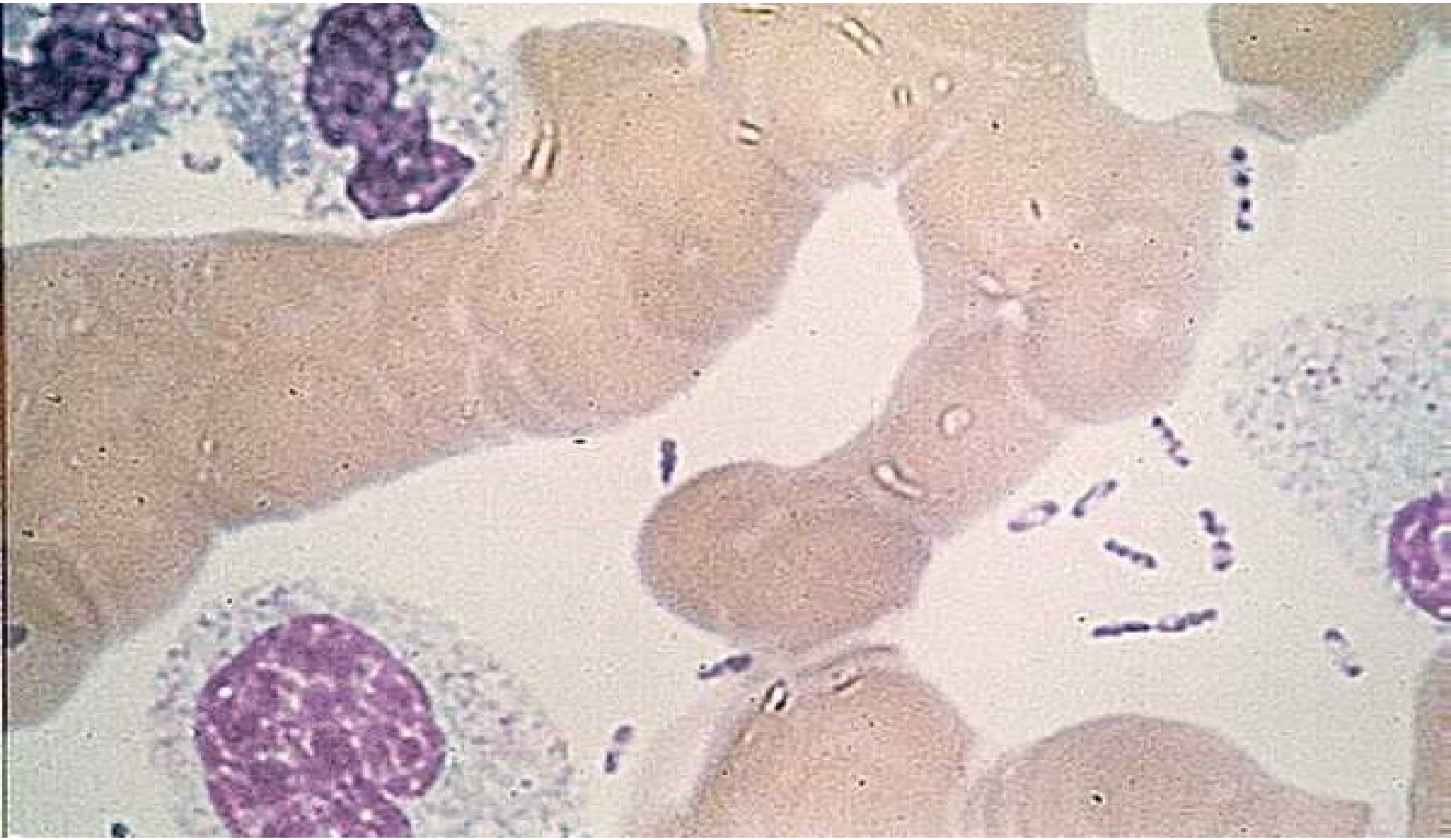
Giemsa or Wayson's smear:

When bubonic plague is suspected

Polychrome methylene blue

When cutaneous anthrax is suspected

Dark-field microscopy To detect treponemes when yaws or pinta is suspected.



Fluorescent Ab stain

- polyclonal or monoclonal ab against specific organisms.

Direct or indirect .

- most commnly used fluorescein isothiocyanate as flurochrome

Culture

The specimen is inoculated in following culture medias to obtain isolated colonies.

1)Blood agar

Incubate aerobically

2)MacConkey agar

Incubate aerobically

3) *Cooked meat medium*

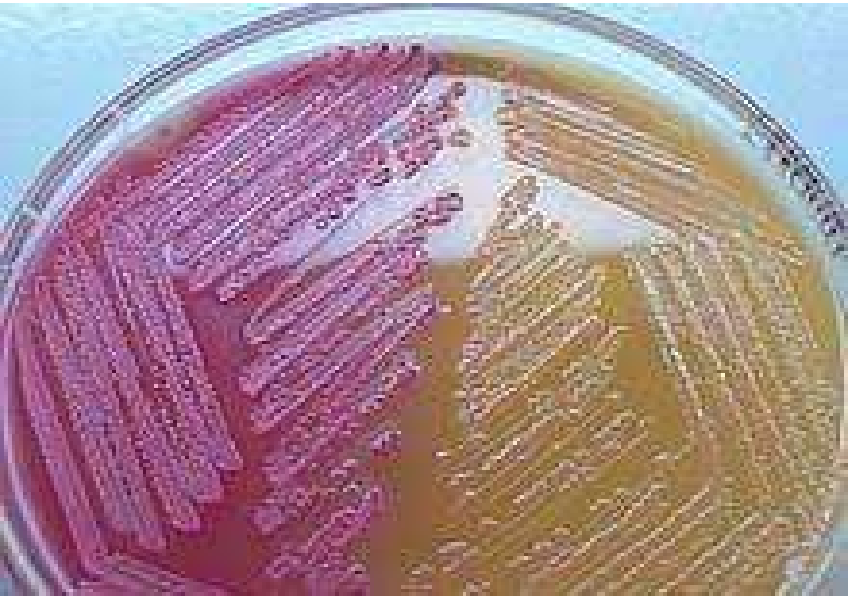
Subculture at 24 h, 48 h, and 72 h as indicated

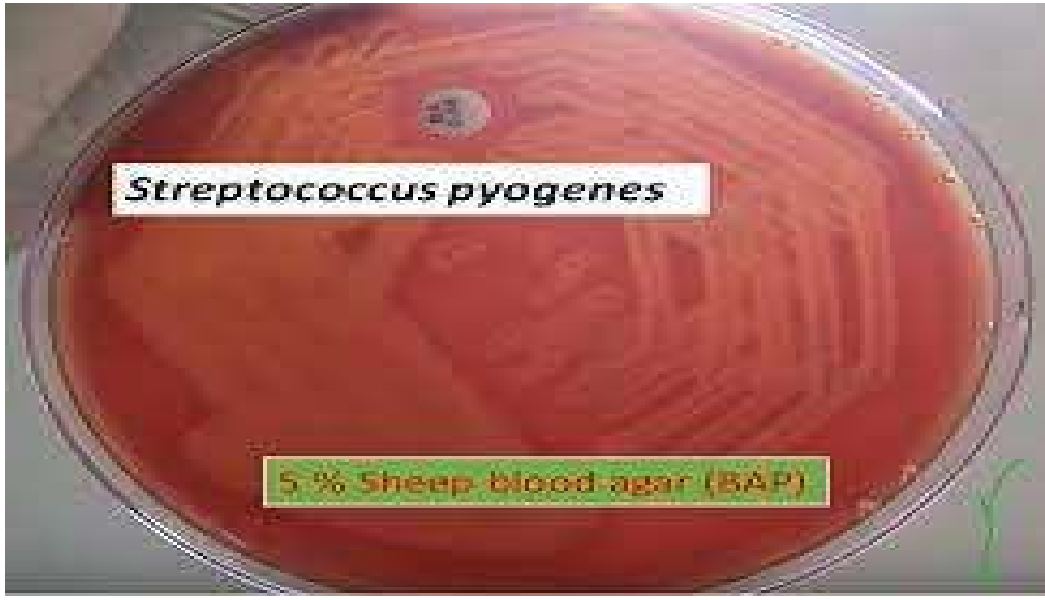
4) ***Neomycin blood agar when anaerobic infection is suspected***

Incubate anaerobically up to 48 h

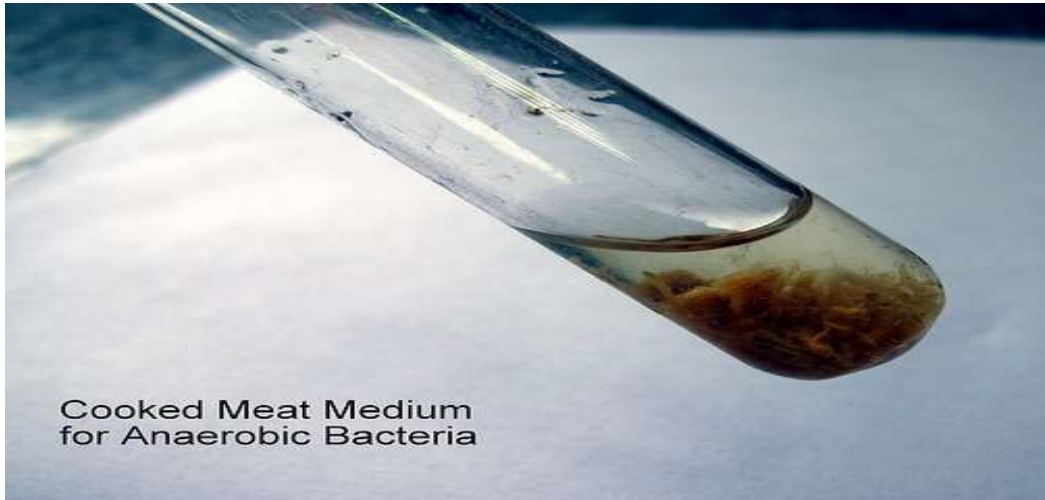
5)Culture for M. tuberculosis or M. ulcerans

Requires facilities of a Tuberculosis Reference Laboratory





Acid-fast stain and growth of Mycobacterium tuberculosis:
M. tuberculosis colonies on Löwenstein-Jensen



- The specimen should be incubated on two plates of blood agar, the one for incubation at 37**°C aerobically and other for incubation** anaerobically in nitrogen/ hydrogen plus 5-10% carbon dioxide.
- It should also be plated for aerobic incubation on MacConkey agar or CLED agar for differentiation of coliforms, staphylococci and enterococci.

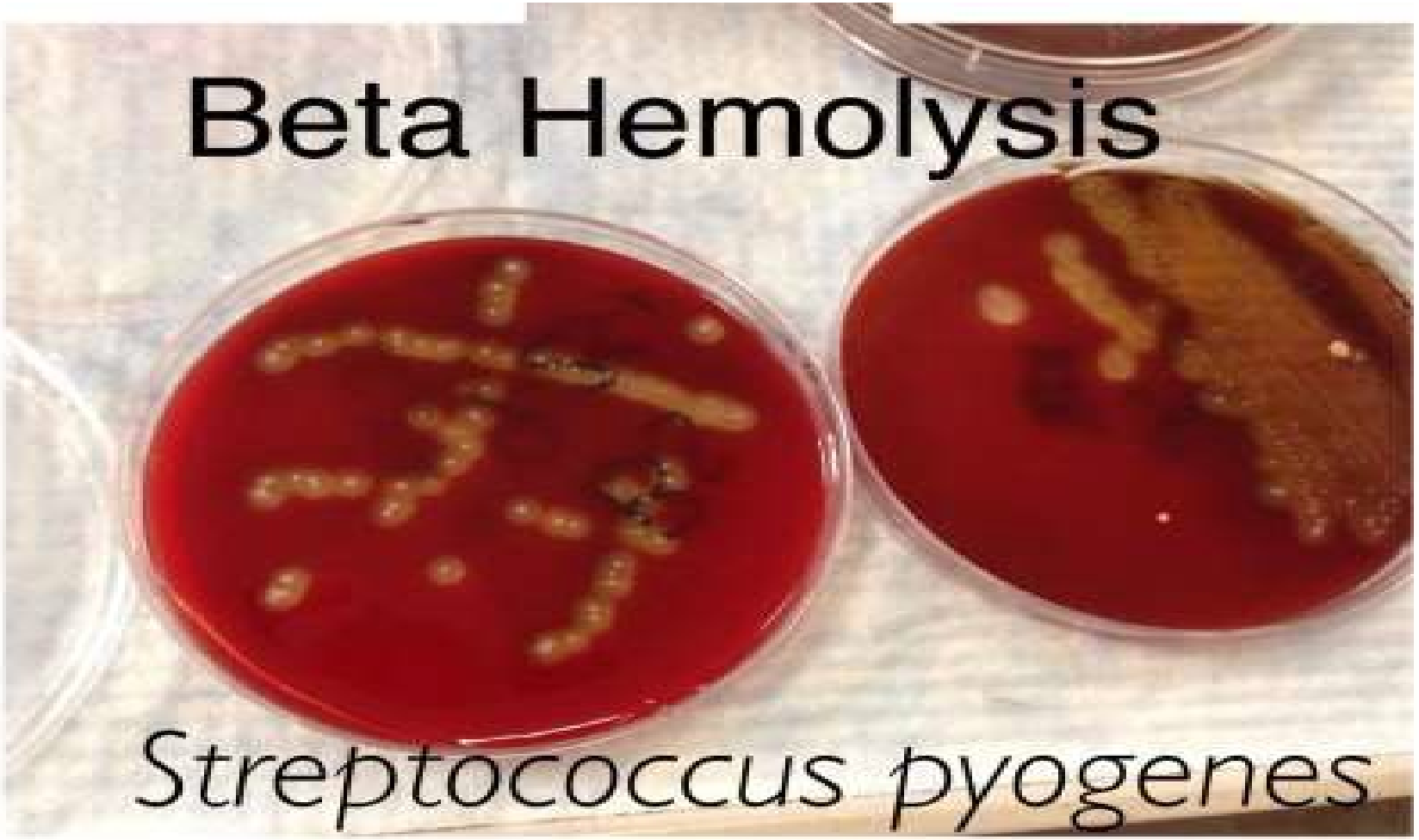
- It should be inoculated into a tube of cooked meat broth for enrichment of extracting aerobes and anaerobes

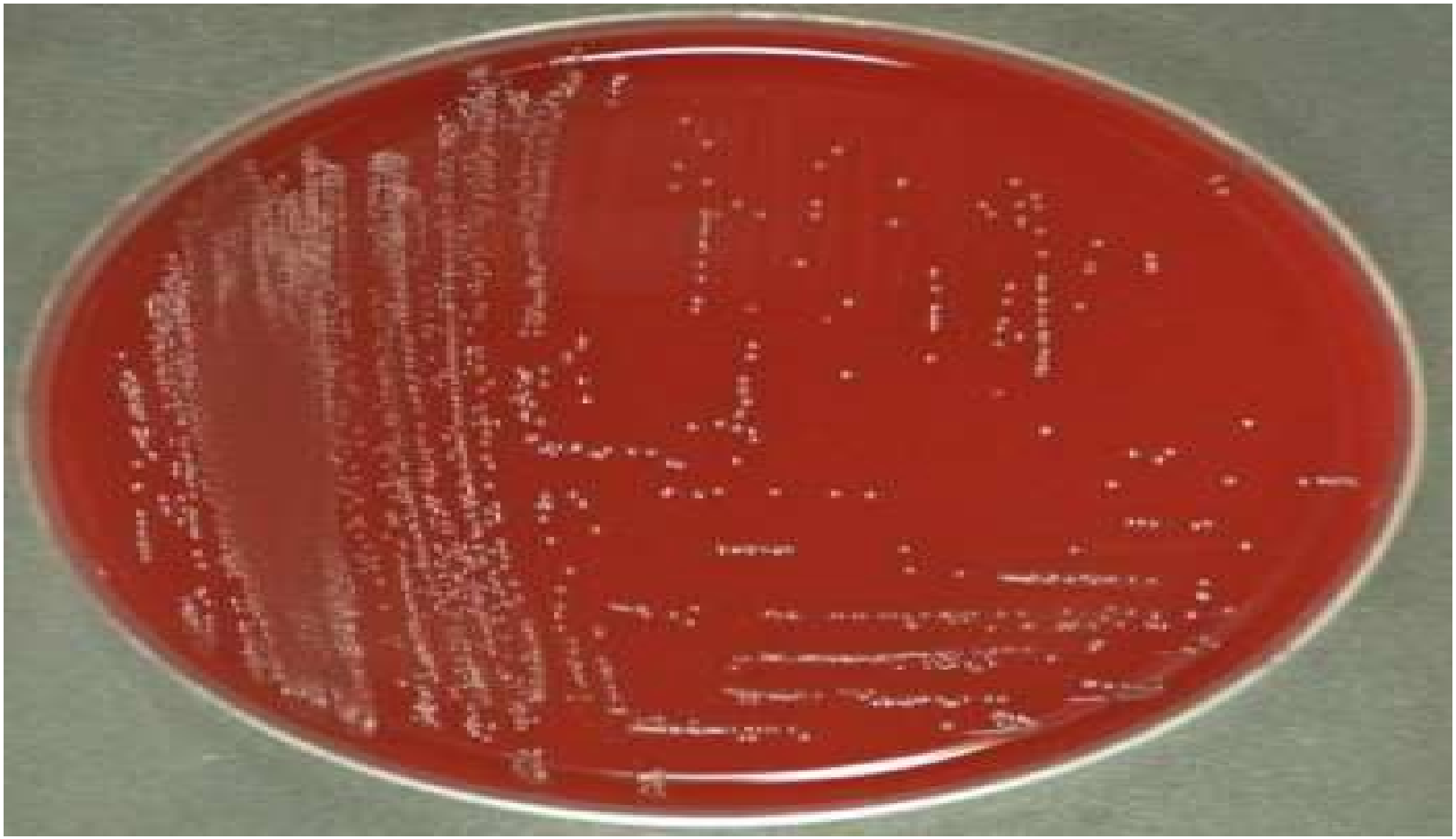
Examination and Reporting the Culture results:

- If the growth is seen after 24/48 hours of culture, examination of the colony morphology and identification of the isolates should be done.
- In the Blood Agar plate look for the hemolysis. Both ***Staphylococus aureus and Streptococcus pyogenes*** gives beta-hemolysis in Blood Agar (Some *S. aureus isolates* may not show hemolysis).

- *S. aureus gives yellow to cream or white colonies. Colonies are* slightly raised and easily emulsified. Beta-hemolytic.
- *S.pyogenes produces beta-hemolytic colonies. Colonies are* usually small, colorless, dry, shiny or mucoid.
- Enterococci gives non-hemolytic colonies in blood agar.

Staphylococcus aureus- An Overview


Beta Hemolysis
Streptococcus pyogenes



Enterococcus faecium

- We can differentiate between streptococci and staphylococci by a very simple and rapid test-**Catalase test**(Staphylococcus-positive, Streptococcus-negative).
- For identification of suspected *S. aureus colonies* perform **coagulase test (**to differentiate coagulase negative Staphylococci from *S. aureus) and for* suspected Group A Streptococci (*S.pyogenes)* perform **bacitracin sensitivity test**(can be added in the blood agar plate with other antibiotics).
- If enterococci is suspected perform **bile esculin test.**

- Look for growth of lactose fermenter colonies (pink)or non-lactose fermenter colonies (pale) in MacConkey Agar plate.
- Lactose fermenter colonies can be of ***Escherichiacoli, Klebsiella spps or Enterobacter spps and nonlactose*** fermenter colonies can be of ***Psuedomonas aeruginosa, Acinetobacter spp, Proteus spps etc.***

- Member of the family of the ***Enterobacteriaceae*** can be differentiated from other Gram-negative bacilli by performing two rapid tests (catalase test +ve, and oxidase test –ve).
- Identifications of the enteric bacteria can be done by using biochemical tests such as **citrate utilization test,Triple Sugar Iron (TSI) Agar test,Sulphite-Indole Motility(SIM) test,** and **urease test.**

- *Pseudomonas aeruginosa gives large, flat,* spreading pale colored colonies in MacConkey Agar. It is oxidase positive and can be identified by its pigments and/or distinctive smell (characteristics fruity smell).
- Depending on the facilities available in the diagnostic laboratories, organisms can be identified using enterotube test or **API-20E test or other newer diagnostics test available for** the identification of isolates.

Anaerobic blood agar culture and cooked meat culture

- The growth could be *Clostridium perfringens*, *Bacteroides fragilis group or Peptostreptococcus species*.
- ***C.perfringens grows rapidly in cooked meat medium with*** hydrogen sulphide gas production and reddening but no decomposition of meat.
- On anaerobic blood agar, colonies are seen after 48hrs of incubation.

- ***B. fragilis grows in cooked meat medium*** producing decomposition blackening of the meat.
- On blood agar non hemolytic grey colonies are seen.
- ***Peptostreptococcus*** grows in cooked meat medium with production of large number of hydrogen sulphide gas.
- Small non hemolytic colonies are seen in anaerobic blood agar after 48hrs of incubation

Robertson's Cooked Meat Medium (RCM) showing saccharolytic property (left), proteolytic property (middle), uninoculated RCM (right)

- **Antigen detection by agglutination or EIA**

latex agglutination

fungal antigen(C. neoformans)

bacterial antigen(CSF specimen)

EIA

- bacterial Ag- group A Streptococci
- bacterial toxin

Molecular based assay

THANK YOU