MSS Module
Practical
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Laboratory Diagnosis of Staphylococial Infection

Diagnosis

- Sample collection and Transportation
- Direct smear Microscopy
- Culture
- Biochemicals
- Typing of Staphylococcus aureus
- Antibiotic Sensitivity Testing (AST)

Laboratory Diagnosis of Staphylococial Infection

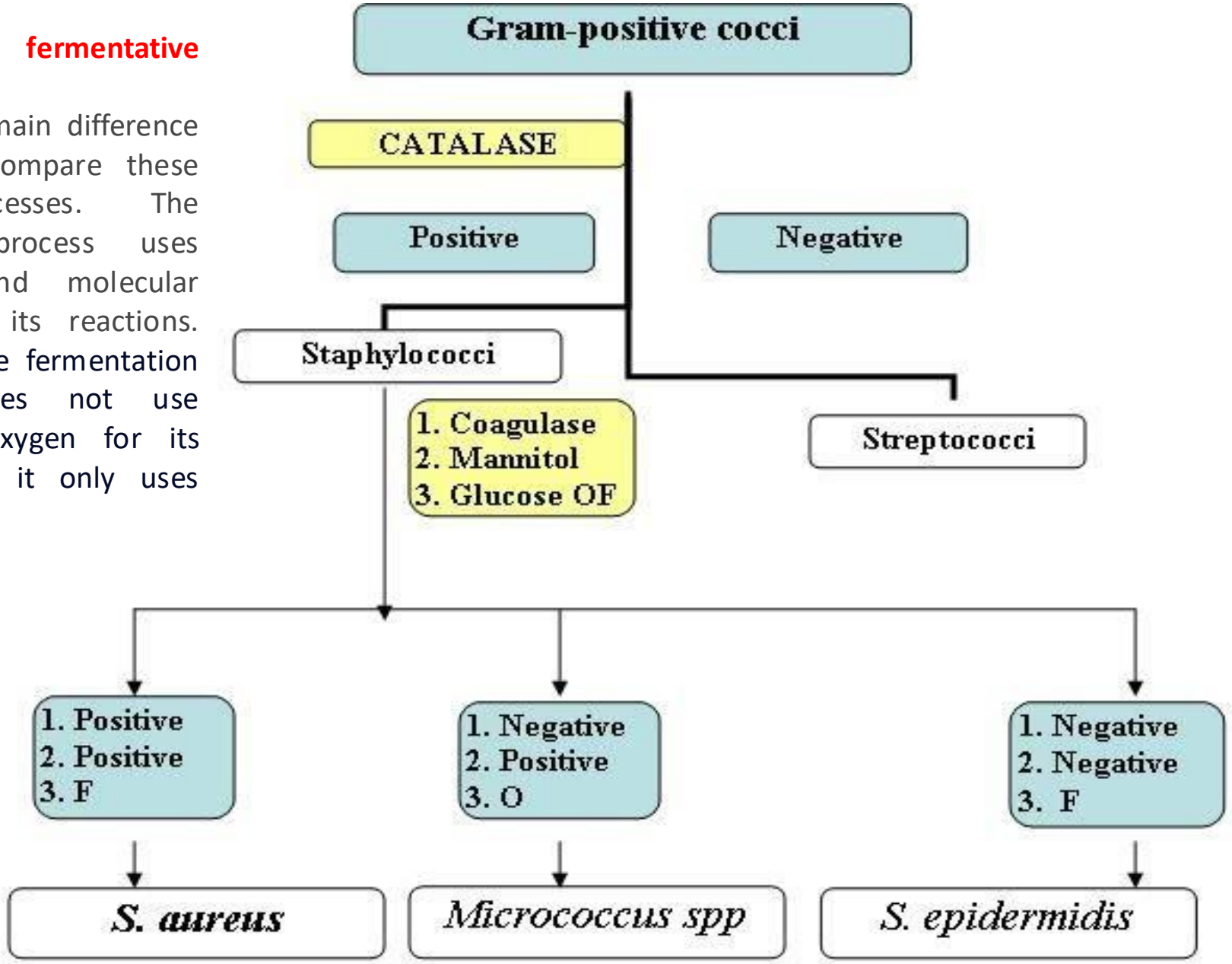
Sample Collection

Type of sample depends on the site of infection.

Infection	Specimen
Suppurative lesion	Pus, wound swab
Respiratory infection	Sputum
UTI	Mid stream urine
PUO, Bacteremia	Blood
Food poisoning	Feces, Vomitus, food
Carriers	Nasal and perianal swab

Oxidative fermentative (OF)

This is the main difference when we compare these two processes. The oxidation process uses enzymes and molecular oxygen for its reactions. However, the fermentation process does not use molecular oxygen for its reactions & it only uses enzymes.



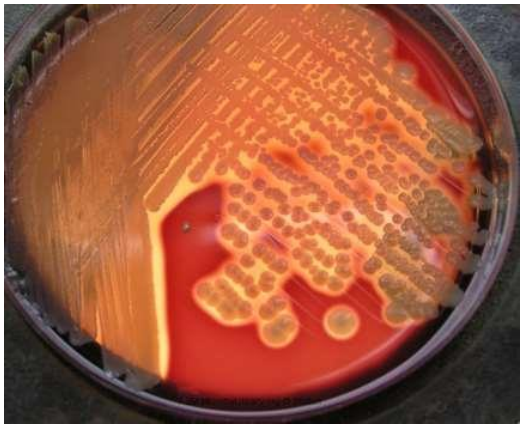
Laboratory Diagnosis of Staphylococial Infection

Direct Smear Microscopy

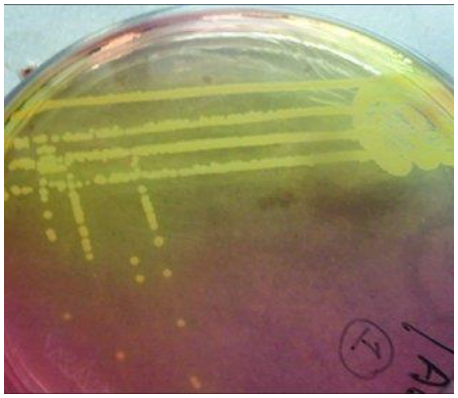
- Staphylococcus measuring 0.5-1.5 microns
- Present within and outside PMNs

Culture

- Blood agar
  - Colonies are beta-hemolytic



- MSA (Selective media)
  - 1% Mannitol + 7.5% NaCl + phenol red



Laboratory Diagnosis of Staphylococial Infection

Biochemical Reactions

- Catalase : positive
- Coagulase test : positive
- Oxidase : negative
- Ferment glucose, lactose, maltose, sucrose and mannitol, with production of acid but no gas
- Indole : negative
- MR test : positive
- VP test : positive
- Gelatin liquefaction : positive
- Phosphatase : positive

Laboratory Diagnosis of Staphylococial Infection

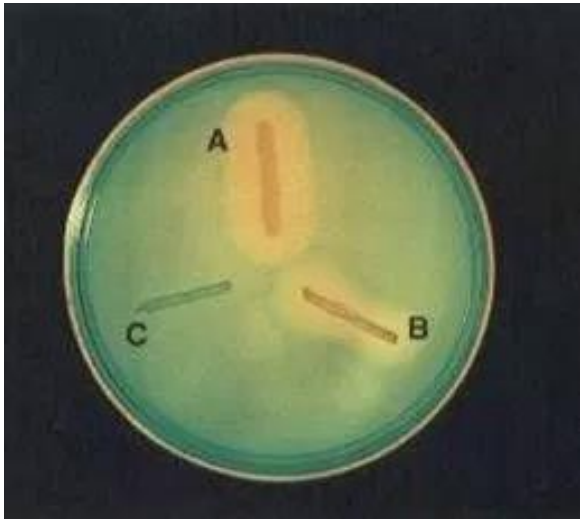
DNA Hydrolysis

- Used to determine the **ability of an organism to hydrolyze DNA (produce deoxyribonuclease or Dnase).**
- Agar medium: pale green in color because of DNA-methyl green (indicator) complex (Note: Methyl green binds to the negatively-charged DNA).
- Organism that **hydrolyze DNA fade to colorless zone** on the blue/green agar.
- Quality control for DNA Hydrolysis
- -**Positive:** ***Staphylococcus aureus***
  -**Negative:** ***Staphylococcus epidermidis***

DNA Hydrolysis test

Positive: *Staphylococcus aureus*

Negative: *Staphylococcus epidermidis*



Laboratory Diagnosis of Staphylococial Infection

Antibiotic Sensitivity Testing

- This is important as staphylococci develop
- resistance to drugs readily.

MRSA

- Methicillin-resistant S. aureus.
- First reported in 1960s.
- May colonize mucosal or epithelial surfaces, (common : anterior nares)
- Nosocomial pathogen.
- Shows Resistant to penicillins, cephalosporins, carbapenems and monobactams.
- Hospital-acquired (HA MRSA)
- Community-acquired cases now (CA MRSA

Laboratory Diagnosis of Streptococcal Infection

Lab Diagnosis

1- Gram staining (Microscopy): Gram-positive cocci

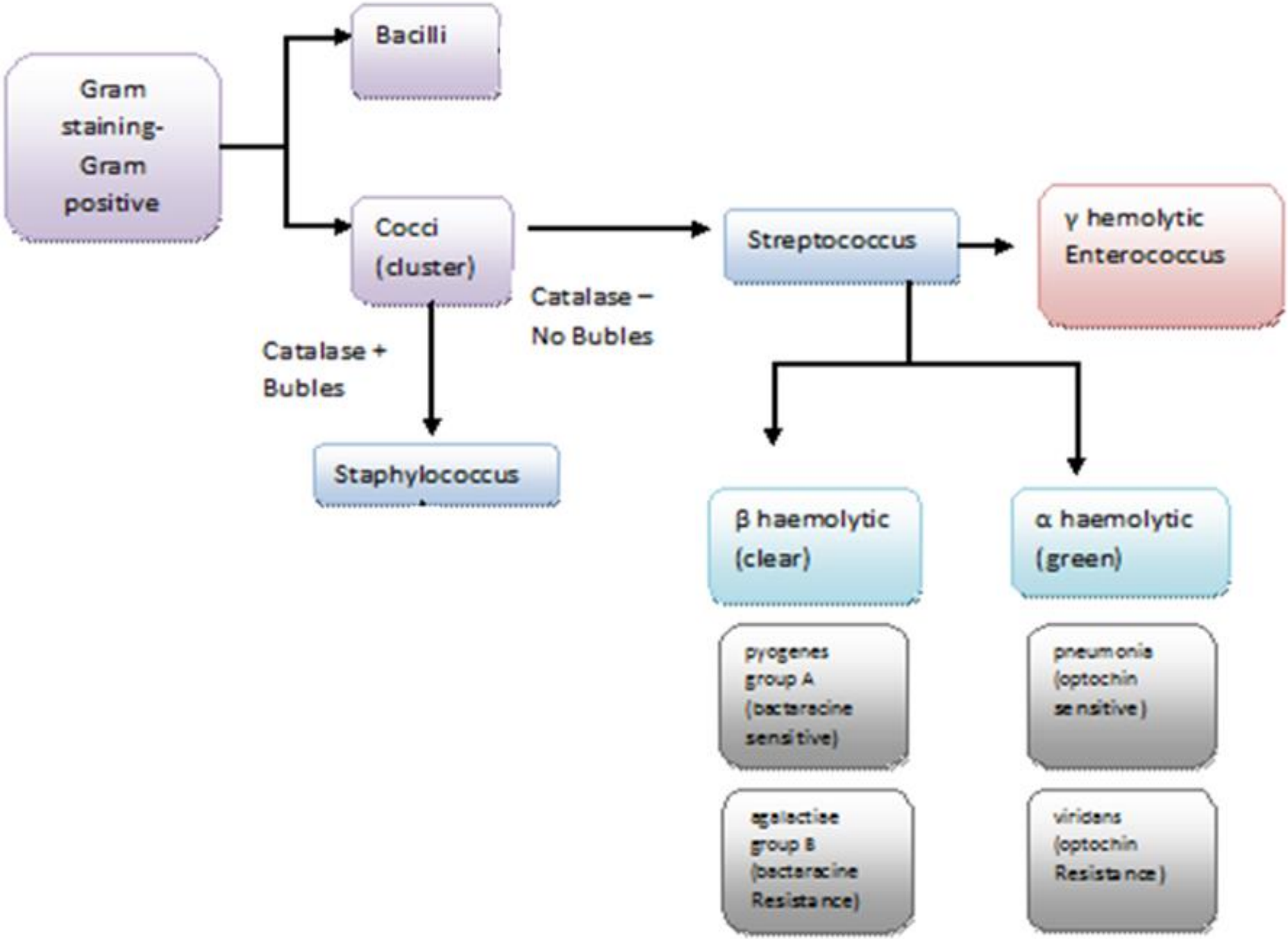
2- Blood Agar

- Small (0.5-1mm), circular, semi-transparent colonies.
- Produce wide zone of β- hemolysis.
- Catalase negative.

3- Biochemical reactions

- Bile insoluble
- Ferments sugars producing acid but no gas.

4- antiDNAse & antihyaluronidase – for skin infections (Titres higher than 300 are taken)


Gram staining- Gram positive
Bacilli
Cocci (cluster)
Catalase + Bubles
Staphylococcus
Catalase – No Bubles
Streptococcus
γ hemolytic Enterococcus
β haemolytic (clear)
α haemolytic (green)
pyogenes group A (bacteracine sensitive)
agalactiae group B (bacteracine Resistance)
pneumonie (optochin sensitive)
viridans (optochin Resistance)

Laboratory Diagnosis of *Propionibacterium acnes* Infection

Laboratory Examination

- No laboratory examinations required.
- If there is suspicion of an endocrine disorder, free testosterone, follicle-stimulating hormone, luteinizing hormone should be determined to exclude hormonal imbalance.
- Transaminases (ALT, AST), triglycerides, and cholesterol levels may be required if systemic treatment is planned

Propionibacterium acnes

Pleomorphic, gram-positive rods

Onchocercus volvolus (River Blindness worm)

Morphological characters

I. Adults:

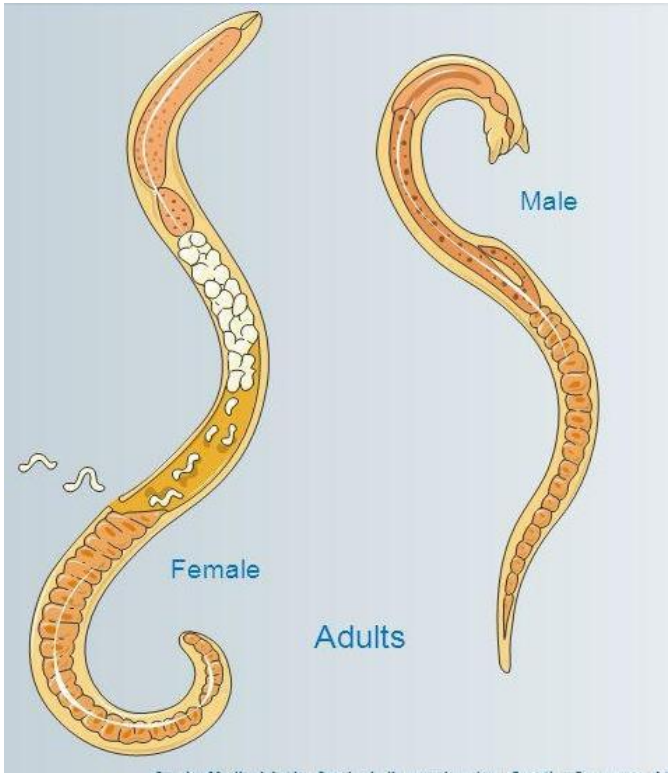
- **Male: shorter than female.**
- **Female: Layes micrifilaria in subcutaneous nodules.**

II. Microfilaria:

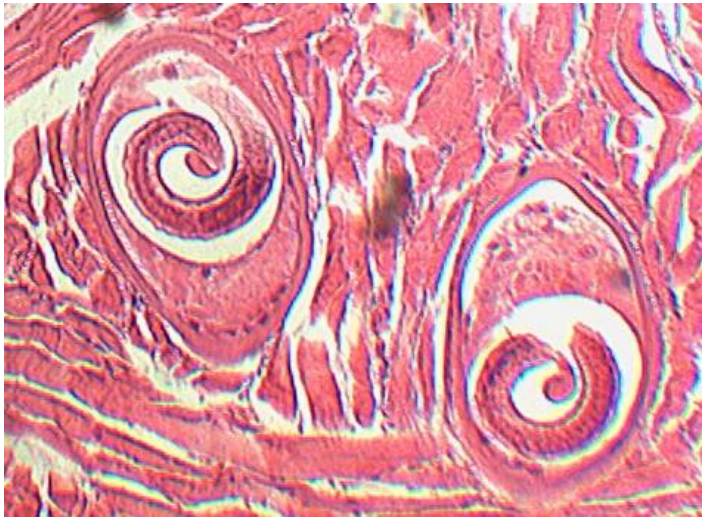
- **Smooth curves.**
- **Non sheathed.**
- **Anterior end & tail free of nuclei.**
- **Not found in blood.**

Trichinella spiralis

1. Muscle biopsy – encysted larva
2. Blood – eosinophilia between 2nd & 4th week
3. Serology – to detect specific Abs



- Male: up to 1.5mm
- Female: up to 4 mm, viviparous



Encysted larva in muscle: lies along the muscle fibers
Shape: Usually seen coiled inside a lemon shaped cyst.

Laboratory Diagnosis of Fungal Infections

(a) Superficial Mycosis

- Dermatophytic lesion – spreads outward in a concentric fashion with healing in the center
  - ➢ scrape outwards from the edge of the lesion with a scalpel blade or use Cellophane tape

Laboratory Diagnosis of Fungal Infections

(b) Subcutaneous Mycosis

- Scrapings or crusts from the superficial parts of lesions
- Pus aspirates
- Biopsy

Laboratory Diagnosis of Fungal Infections

Collection & Transport of specimen

- Skin, Hair & Nail
  - Taken for dermatophytic infections
    - Hair – plucked with forceps
  - Tissue & Body fluids
    - Tissues – grind or mince before culturing
    - Body fluids – centrifuge & use sediment for culture
    - Urine – centrifuge & use sediment for culture

Laboratory Diagnosis of Fungal Infections

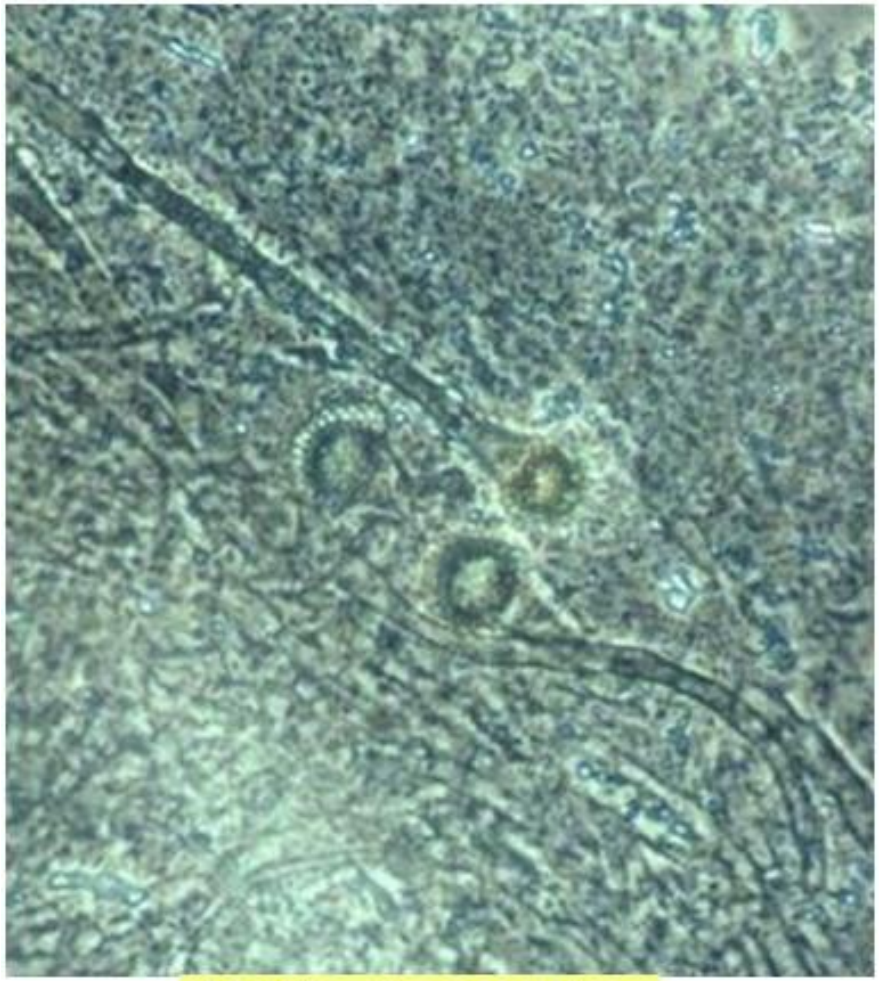
Diagnosis

- Direct examination
- Fungal culture
- Serological tests
- Skin tests
- PCR & other molecular methods

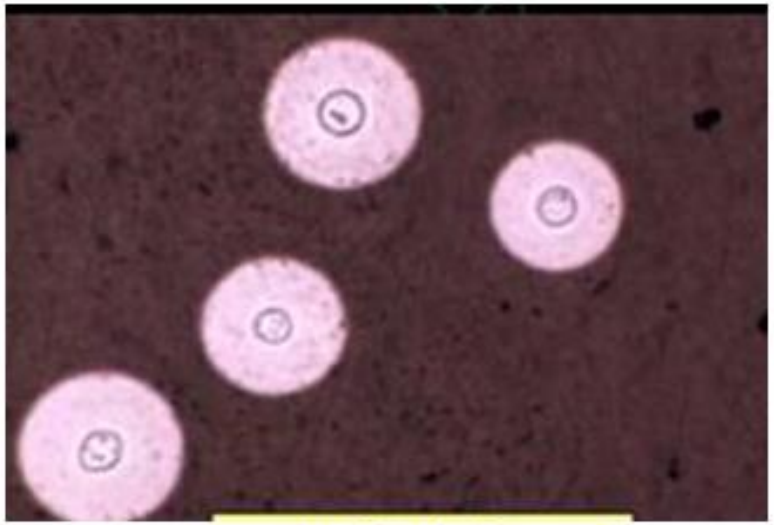
Laboratory Diagnosis of Fungal Infections

Direct Examination

- Very conclusive in the diagnosis of fungal infections
- Wet mounts
- Slide & tube KOH mounts – 10 to 20% KOH – digests protein debris, dissolves keratin.



KOH - Aspergillus



India ink -
Cryptococcus

Laboratory Diagnosis of Fungal Infections

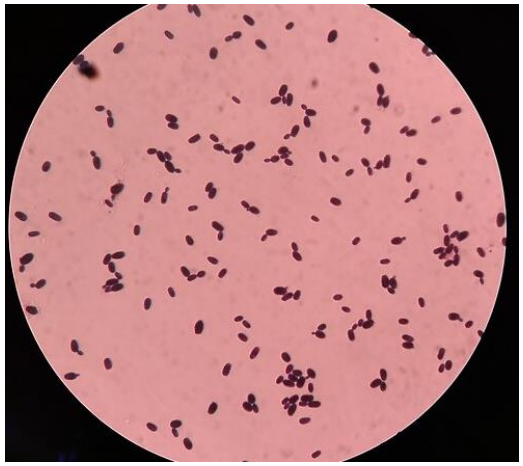
Direct Examination

- Gram stain – fungi are gram positive
- Histopathology

Fungal Culture: Sabouraud Dextrose Agar (SDA)

Colonies with white to cream colored, smooth, and yeast-like in appearance.

Candida albicans in SDA



Unlike bacterial cells, yeast cells are much larger in size, usually ranging from 3 to 8 micrometers in diameter.

Pseudomonas aeruginosa

Pseudomonas aeruginosa on nutrient agar.

Pseudomonas aeruginosa on Triple Sugar Iron agar:

Growth; red slant, red butt, no gas, no H_2S produced