Lab diagnosis for Fungal infections

Introduction

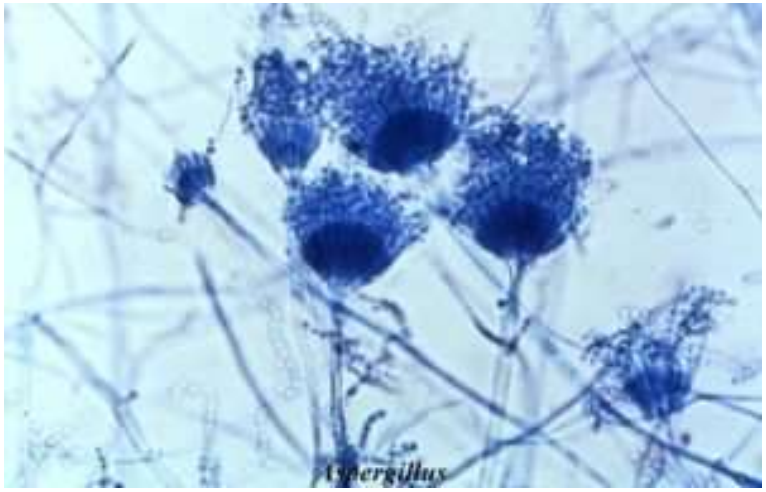
- We need to collect skin, hair or nail tissue to confirm diagnosis of fungal infections
- Then observation under microscope and culturing.

Fungal stains

Wet Preparation	**Differential stains**
KOH preparation	Gram stains
Lactophenol Cotton Blue	Geimsa
Indian ink stain	Hematoxylin and eosin (H & E) stain
Nigrosin stain	
Calcofluor white stain	

The Lactophenol Cotton Blue

(LPCB) wet mount is most widely used method for the staining and observation of fungi.



1. **Phenol** : Kills any live organism.

1. **Lactic acid** : Preserves fungal structures.

2. **Cotton blue** : Stains the chitin of the fungal cell wall intensely blue.

Potassium Hydroxide (KOH) wet preparation

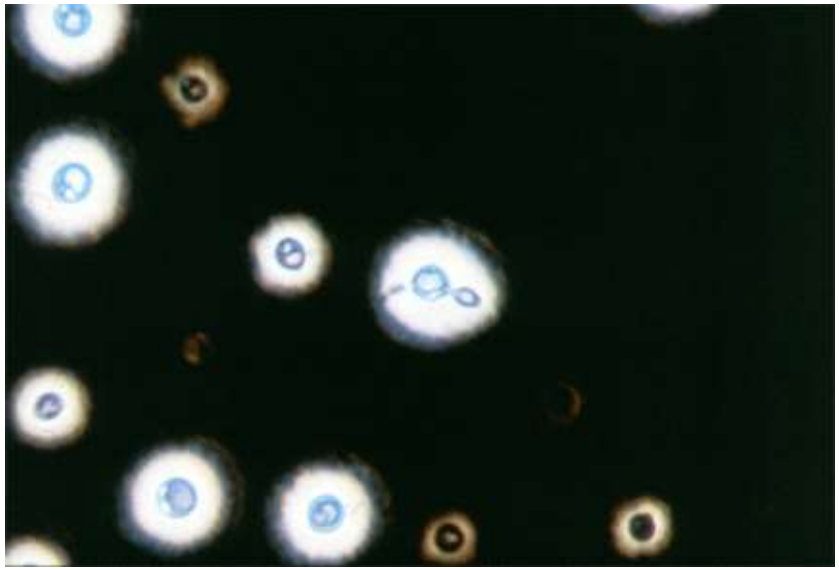
- **Samples**: Skin scrapings, hair or nail clippings, tissue, vaginal swab, body fluids, sputum
- Chemical solution dissolves non-fungal elements; reveals yeast cells and fungal hyphae (branching filaments) on a microscope slide.
- Rapid detection of fungal elements in clinical specimen

KOH wet preparation

KOH dissolve keratin found in specimens and free hyphea from cells

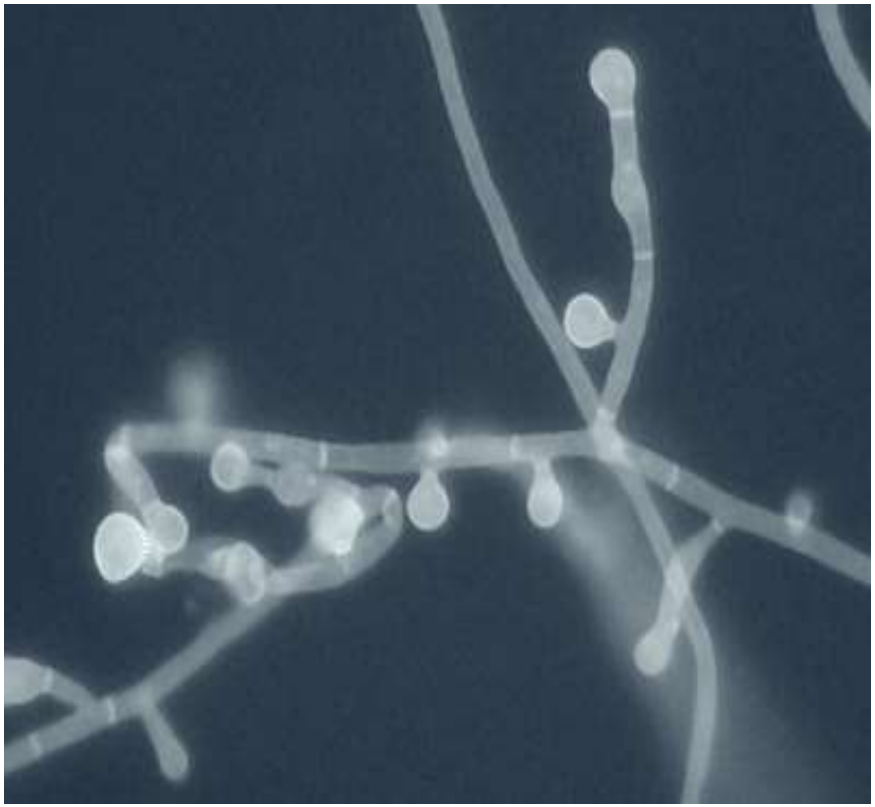
Indian Ink

- It is negative staining, why?
- Primarily used for CSF for detection of Cryptococcus neoformans and C. gattii.
- The appearance of encapsulated yeast cells in CFS is diagnostic for Cryptococcal meningitis.



Calcofluor white stain

- Yeast chitin contained mycelium bind Calcofluor white stain
- Then fluorescence microscope will be used



Media used for fungal culture

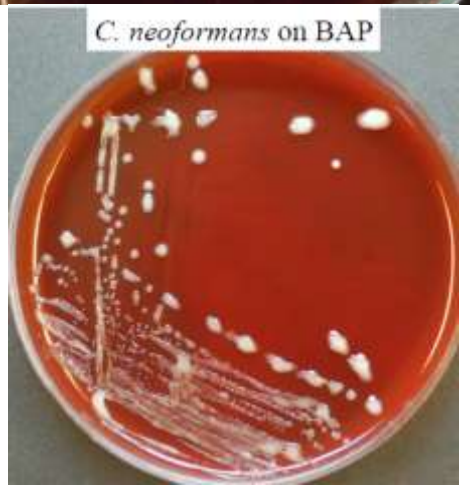
- Fungal culture takes weeks at 30°C.
- Primary tool to diagnose a fungal infection; grows fungi for identification tests
- Sabouraud Dextrose Agar SDA (pH 5-6) or neutral pH (6.5-7) or +

SDA+ antibiotics such as cycloheximide (500mg), gentamycin(20 mg) or chloramphenicol (50mg)

Media used for fungi

Blood agar (BA),

- Histoplasma capsulatum,
- Cryptococcus neofprmans
- Candida moist opaque colonies



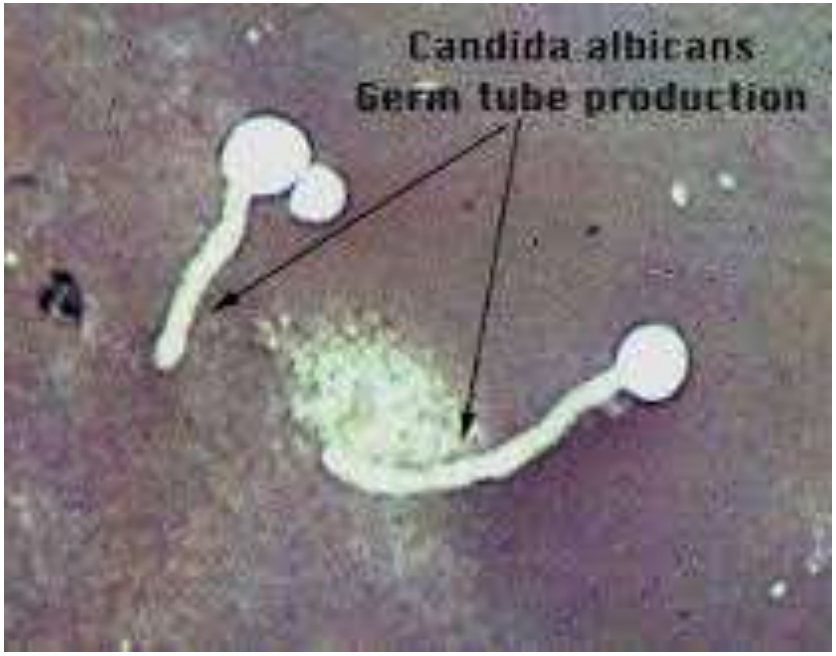
Heart brain infusion (HBI

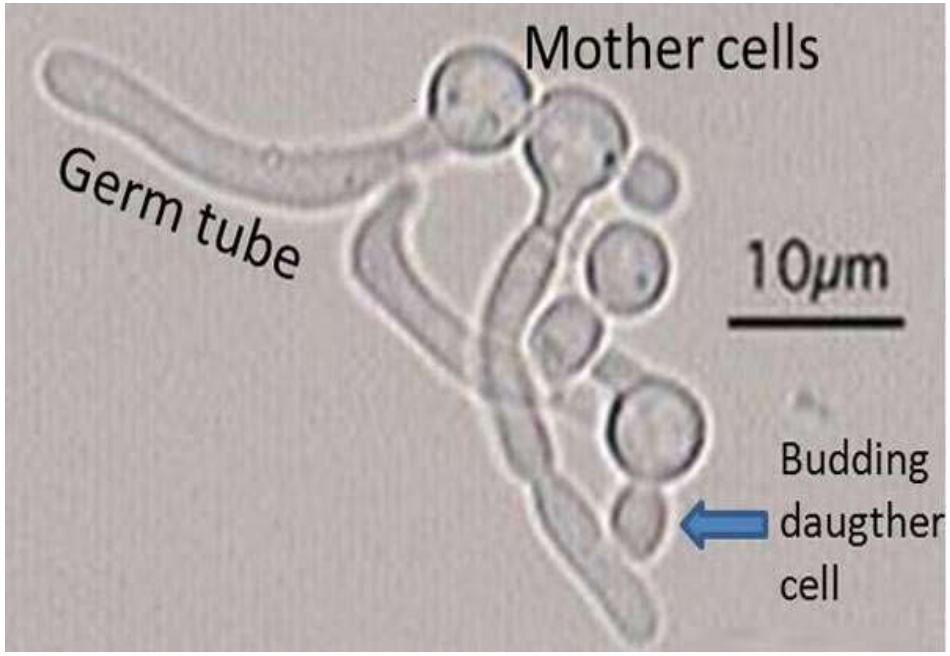
Chocolate agar:
Candida (yellow white colonies)

Germ tube test

- It is a rapid test for a presumptive identification of C. albican
- It is a screening test which is used to differentiate Candida albicans from other yeast.
- When Candida is grown in human or sheep serum at 37°C for 3 hours, they forms a germ tubes, which can be detected with a wet KOH films as filamentous outgrowth extending from yeast cells.

Germ tube test





A negative culture may arise, Why?

1. It is not due to fungal infection.
2. The specimen was not collected properly.
3. Antifungal treatment had been used prior to collection of the specimen.
4. There was a delay before the specimen reached the laboratory.
5. The laboratory procedures were incorrect.
6. The organism grows very slowly.

Serological test

- Blood samples are used fro systemic infection and subcutaneous infection.
- Antibody or antigen detection
- Mab is developed against mycelium cell wall proteins
- Reaction specifically in gel immune diffusion test
- H and M specific antigens for H. capsulatum
- Day(s); rapid tests are available for some fungi (e.g., *Cryptococcus*, *Histoplasma* species)
- ELISA method

Skin test

- Fungal antigen injection leads to hypersensitivity reaction

Molecular techniques

- Sample of fungus isolated in culture, blood, CSF, body fluids
- Detects genetic material of a specific fungus
- By PCR for DNA amplification or DNA probe
- Detects some fungi; not yet widely available, some in research settings only
- Takes days to several weeks

Thank you