

Introduction to Diagnostic Mycology

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Mycology

- Mycology is the study of (fungi, molds and yeasts) which are eukaryotic organisms.
- Unlike animals, most fungi are nonmotile and have a rigid cell wall. Unlike plant, fungi are nonphotosynthetic.
- Most fungi are obligate or facultative aerobes.

- Fungi are heterotrophic, unicellular to filamentous, spore bearing organisms. Usually reproduce by both sexual and asexual means.
- Fungi are ubiquitous in nature, being found in the air, in soil, on plants, and in water, including the oceans.

A heterotroph is an organism that eats other plants or animals for energy and nutrients.

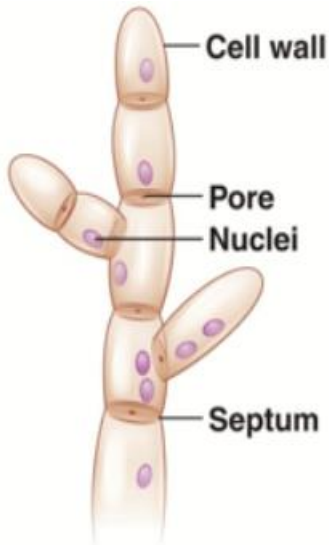
- Fungi are dependent upon enzymes system to derive energy from organic substrates.
- Like animals and most bacteria, fungi require organic nutrients as a source of energy. They are absorptive organisms rather than either saprophytes or parasites.

Saprophytes are organisms that can't make their own food. In order to survive, they feed on dead and decaying matter.

What is the different between saprophytes and heterotrophs ??

- Most species of fungi are beneficial to humankind (recycling organic matter, production of food and spirits).
- Some fungi have served medicine by providing useful bioactive secondary metabolites such as antibiotics (Penicillin) and immunosuppressive drugs (cyclosporine).
- 100,000 fungal species, only about 100 have pathogenic potential for humans

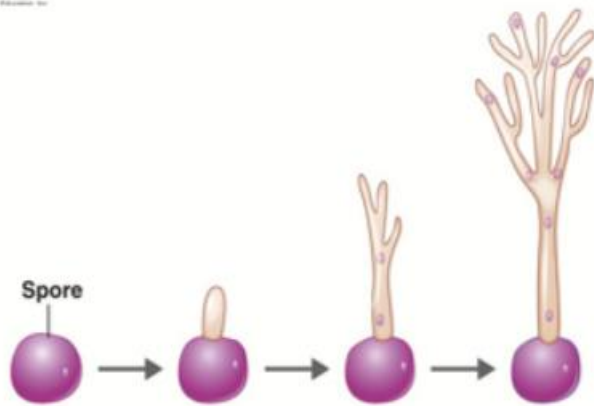
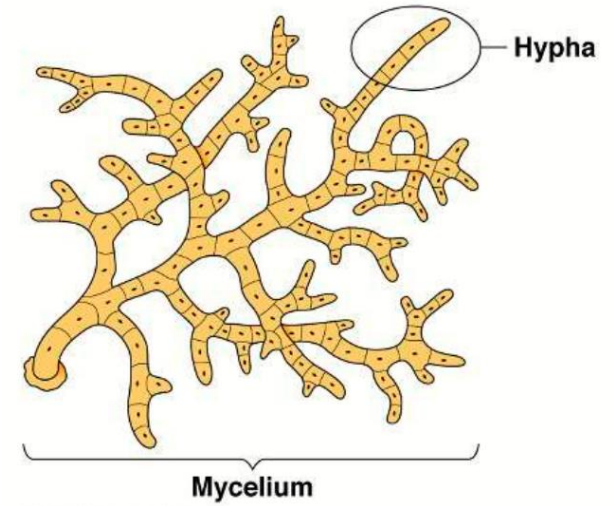
Figure: Structure of fungi



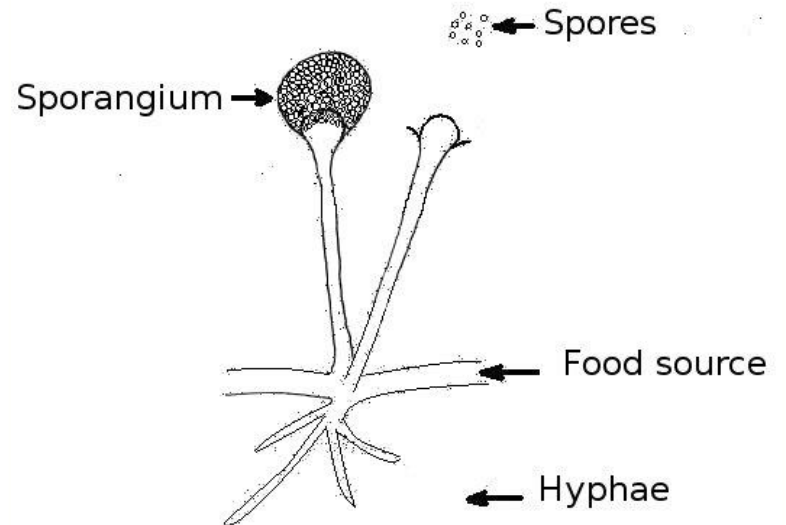
(a) Septate hypha



(b) Coenocytic hypha



(c) Growth of a hypha from a spore



General Properties and Classification of Fungi

- Fungi grow in two basic form, as **yeasts** and **molds** (moulds).
- Growth in the molds forms occurs by the production of multicellular filamentous colonies, which consisting of branching cylinder tubules called **hyphae**.
- The mass of hyphae that accumulates during active growth is a **mycelium**.
- Asexual as well as sexual reproduction can result in the production of **spores**, which enhance fungal survive.

Figure: A- Represents creamy white *Candida albicans* colonies cultured on Sabouraud's Dextrose agar containing Chloramphenicol. B- Represents green colonies of *Candida albicans* subcultured on HiCrome agar. Both were cultured at 37 C° for 24 hours.

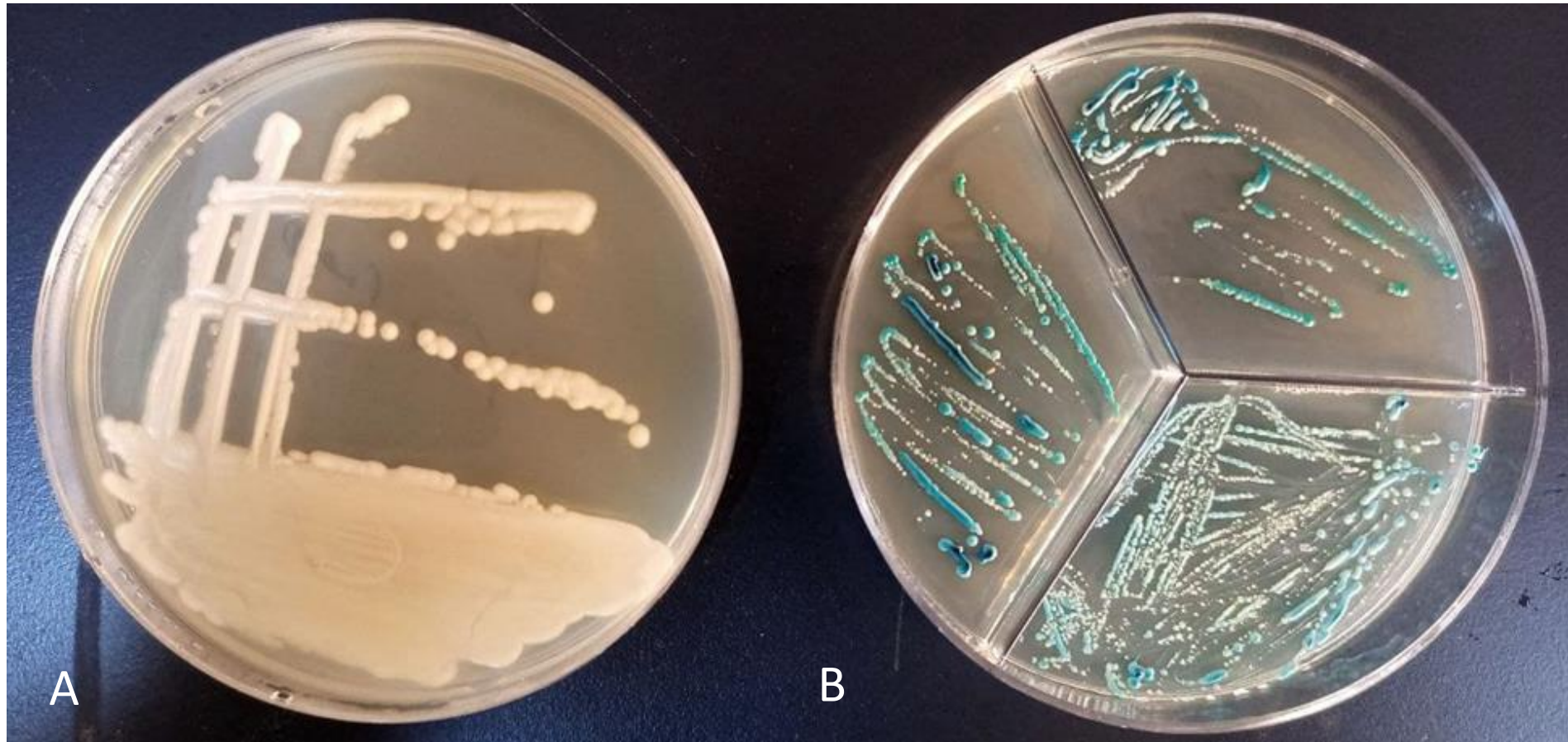


Figure: fungal morphology of filamentous allergenic fungus *Alternaria alternata* and photograph of fungal colonies on Sabouraud Dextrose Agar.



Figure: *Candida albicans* under light microscope 1000x (oil immersion lens) using simple stain (safranin). A- Pseudohyphae, B- Budding yeast cells.

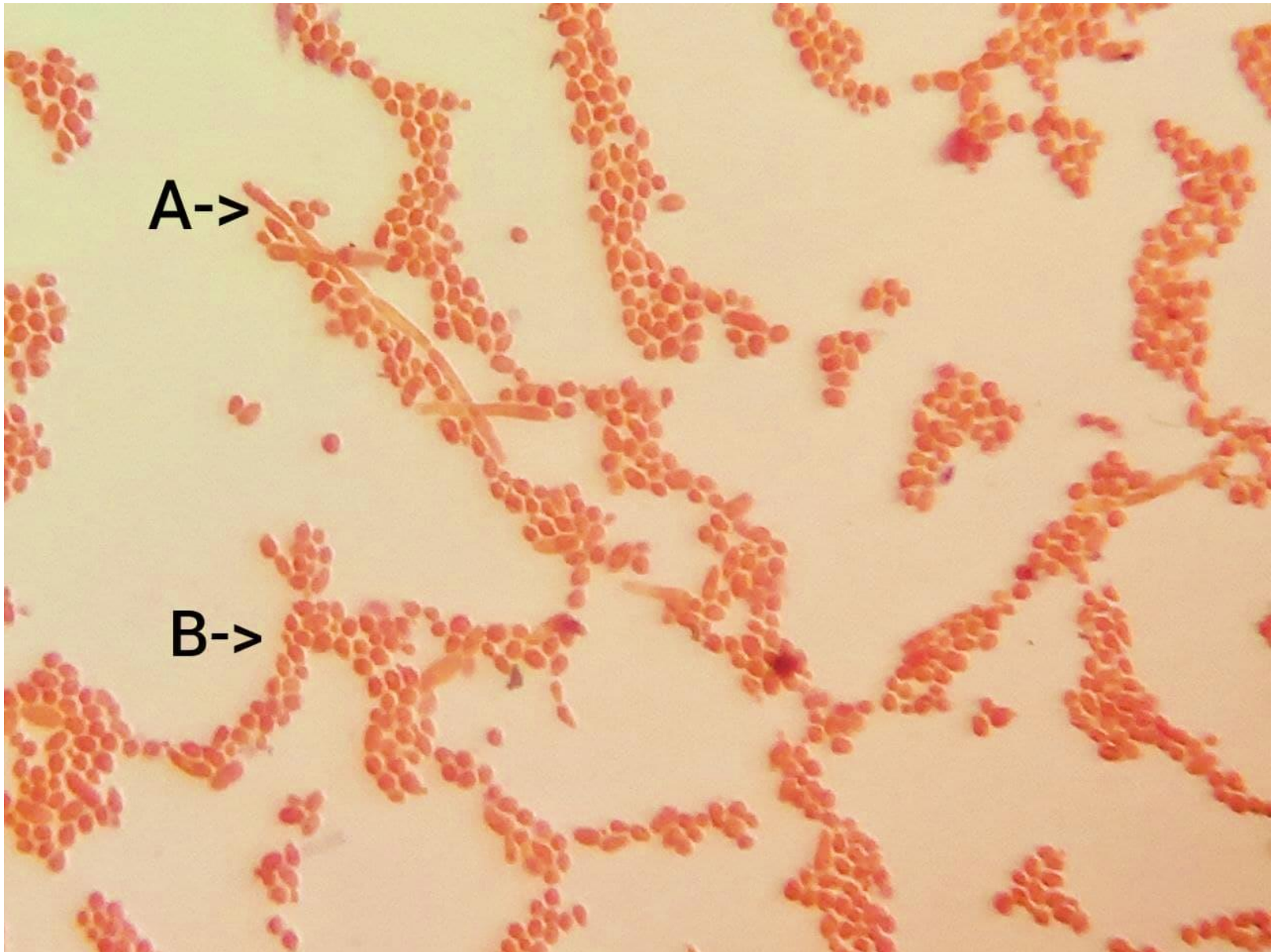


Figure: hyphae, mycelium and spores of fungi

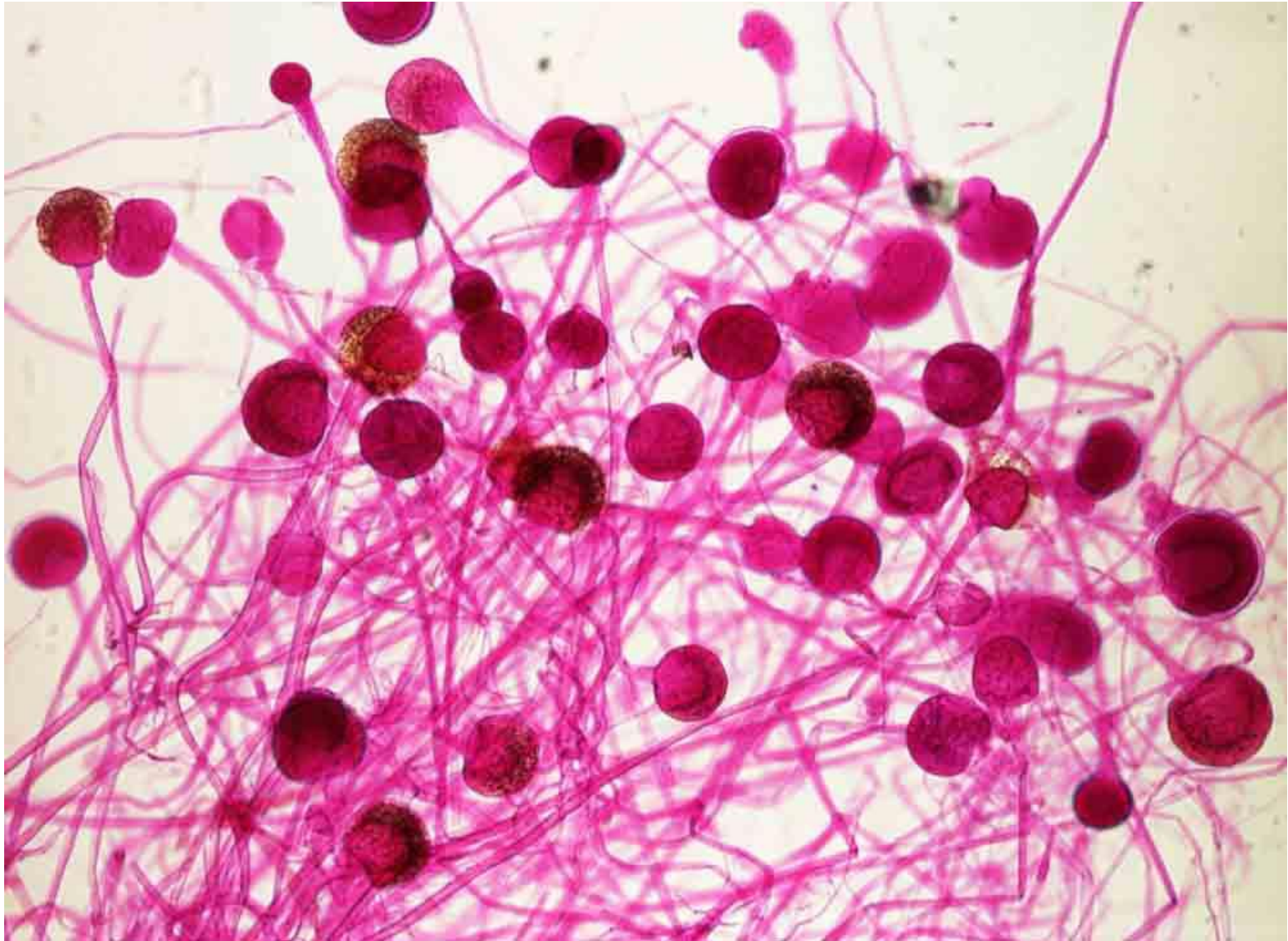
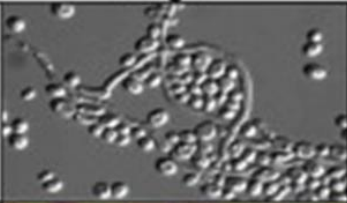
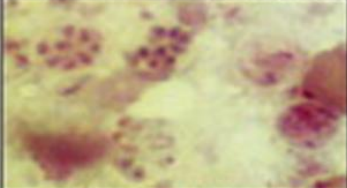
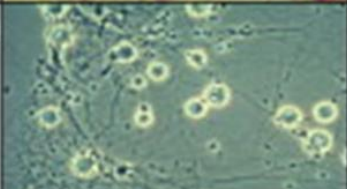

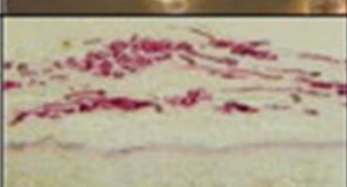
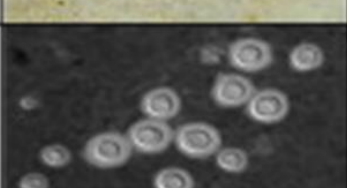


Table: Examples of some medically important Fungi

Organism	Source	Image	Disease Spectrum
<i>Candida spp.</i> ¹	Commensal of the skin, GI tract, and vagina.		<ul style="list-style-type: none"> •Hematogenously disseminated infections •Mucocutaneous infections: <ul style="list-style-type: none"> Oropharyngeal infections (thrush) Skin/nail infections Vaginitis
<i>Pneumocystis spp.</i> ²	Species are host specific, with no known environmental reservoir.		<ul style="list-style-type: none"> •Pneumonia
<i>Histoplasma capsulatum</i> ³	Found in the environment in soil contaminated with bird or bat guano. Humans infected by inhaling conidia (spores).		<ul style="list-style-type: none"> •Self-limited flu-like syndrome •Acute pneumonia •Chronic pulmonary infection •Disseminated disease
<i>Blastomyces dermatitidis</i> ⁴	Found in the environment in soil / decaying wood. Humans infected by inhaling conidia (spores) or direct inoculation of the skin.		<ul style="list-style-type: none"> •Acute/chronic pulmonary infections •Skin lesions/ subcutaneous nodules •Disseminated disease
<i>Malassezia spp.</i> ⁵	Commensal of the skin.		<ul style="list-style-type: none"> •Primarily cutaneous infections <ul style="list-style-type: none"> Pityriasis versicolor Seborrheic dermatitis •Allergic atopic eczema
<i>Cryptococcus spp.</i> ⁶	Found in the environment in association with soil, pigeon guano, and trees. Infection occurs through inhalation of spores or small yeast.		<ul style="list-style-type: none"> •Pneumonia •Meningitis

Specimen collection:

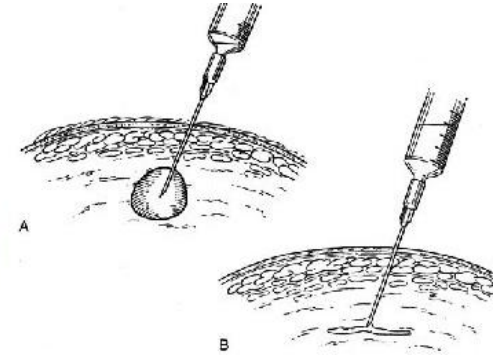
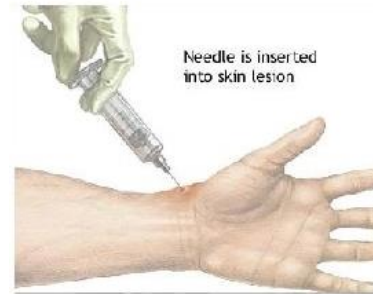
(a) Superficial Mycosis

- **Dermatophytic lesion (Ringworm)** – 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
- **Scalp lesion** – scraping with a blunt scalpel, including hair stubs, scales & contents of plugged follicles.
- **Onychomycosis** – stop antifungals one week prior to collection
- **Mucosal scrapings**



(b) Subcutaneous Mycosis

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



(c) Systemic Mycosis

- CSF
- Blood
- Pus
- Biopsy
- Feces
- Urine
- Sputum
- Scrapings or swabs from the edge of lesions.



Laboratory Diagnosis

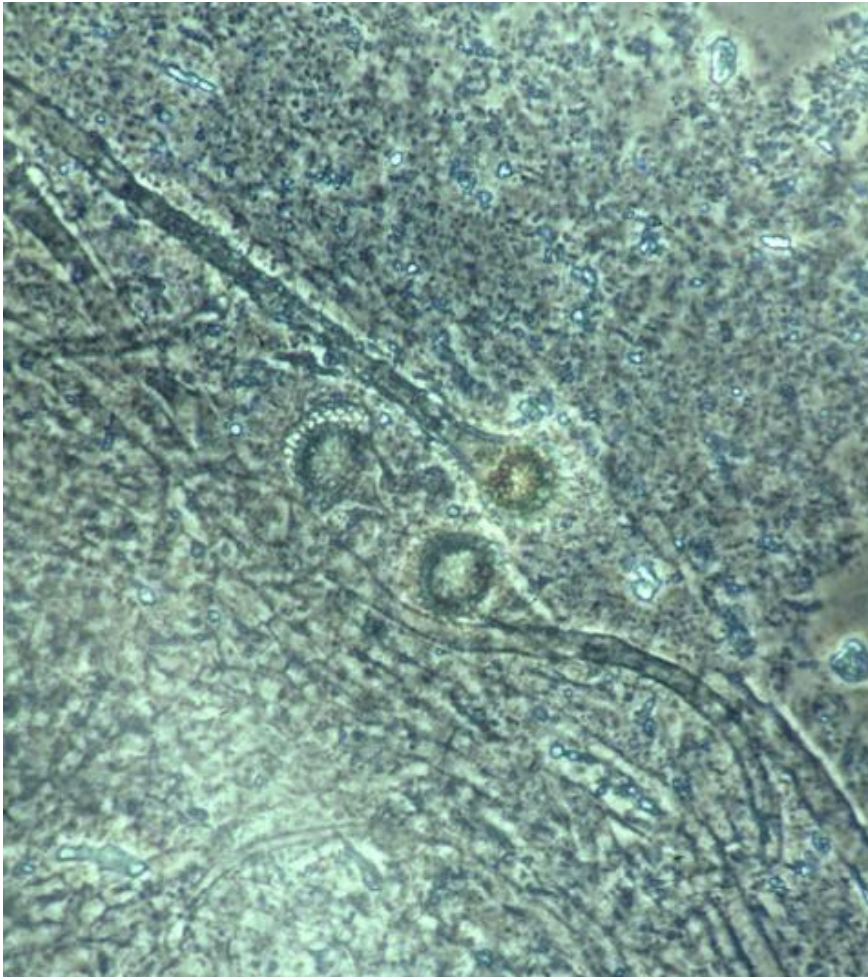
1. Direct examination
2. Fungal culture
3. Serological tests
4. Skin tests
5. PCR & other molecular methods

1. Direct Examination

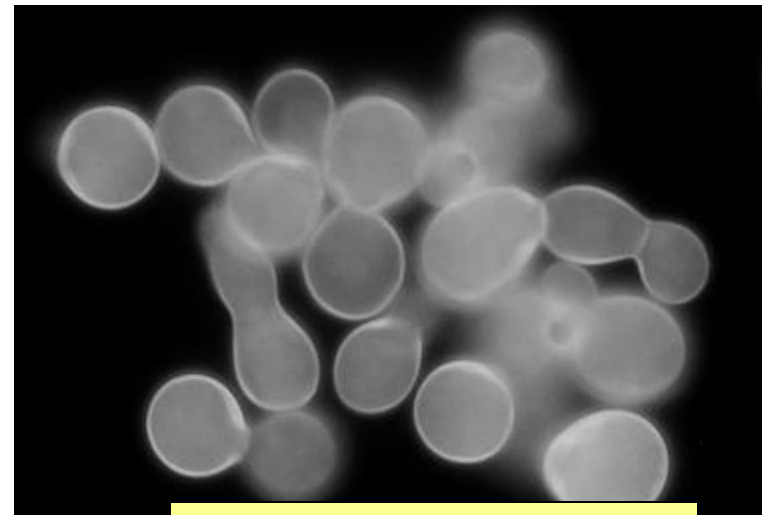
I- Wet mounts

- **KOH mounts** – 10 to 20% KOH – digests protein debris, dissolves keratin. DMSO can be added to KOH to hasten clearing in skin scrapings & nail clippings
- **Calcofluor white** – fluorescent stain – excellent morphology of pathogenic fungi
- **India ink** – capsulated fungi

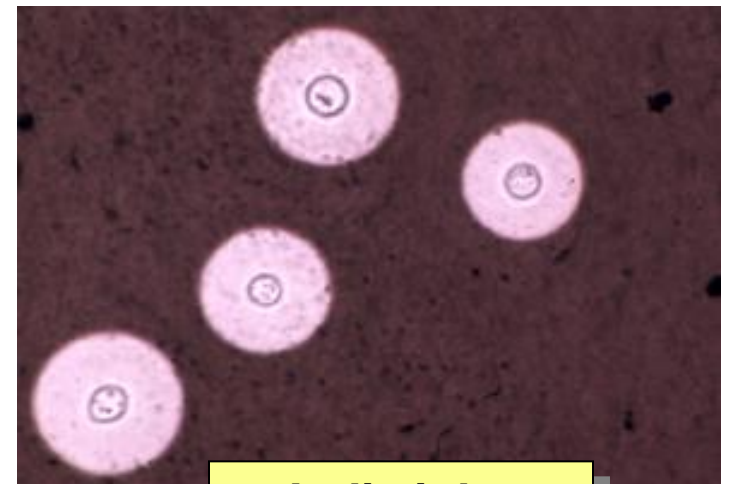
KOH stands for potassium (K), oxygen (O), and hydrogen (H). These elements make up potassium hydroxide.



KOH - Aspergillus



**CFW – yeast form of
Blastomyces**

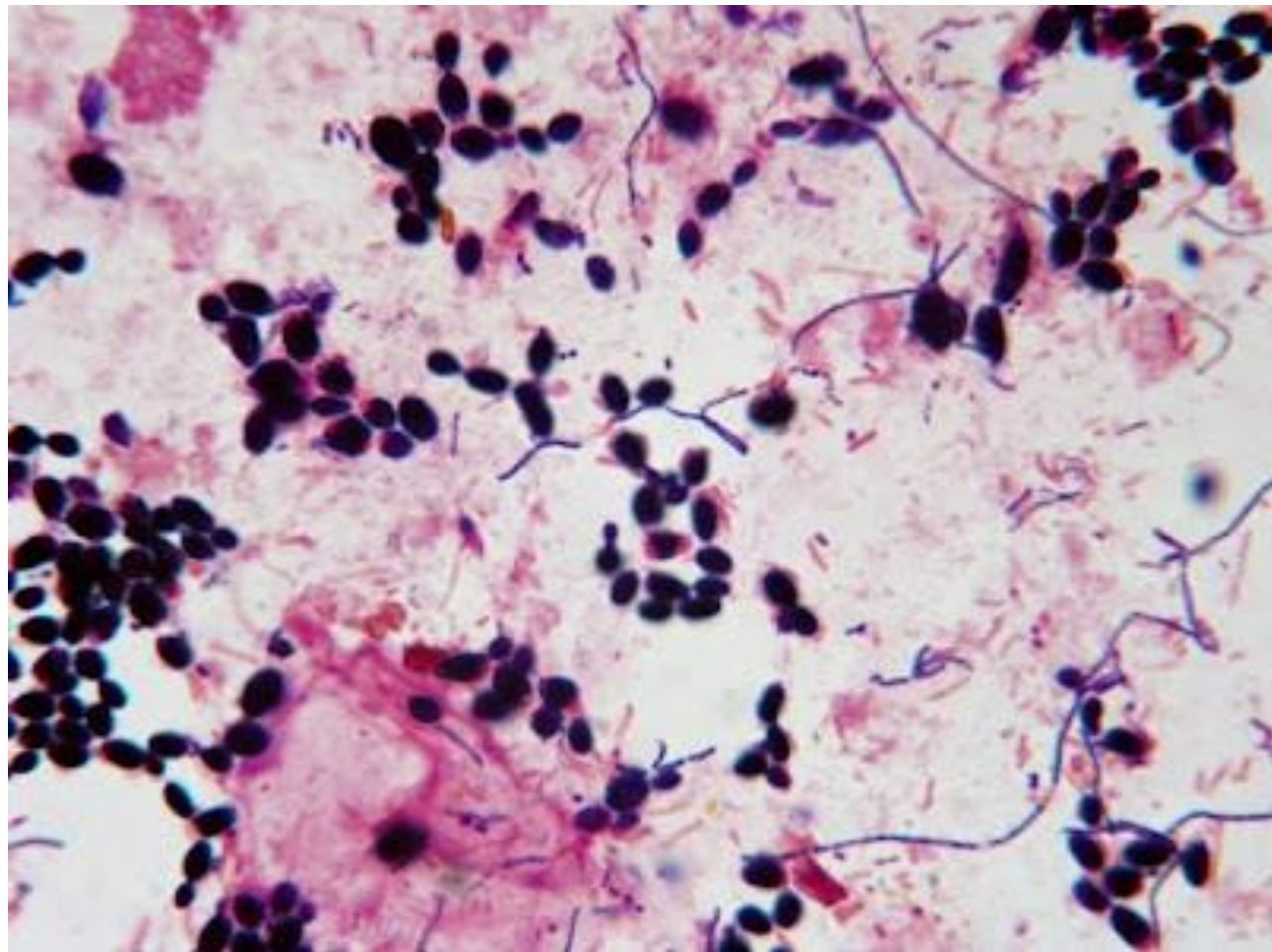


**India ink -
Cryptococcus**

1. Direct Examination

II- Gram stain – fungi are gram positive

Figure: Numerous yeast cells, many budding, along with other bacteria in this sputum sample. A large epithelial cell is seen in the lower left.

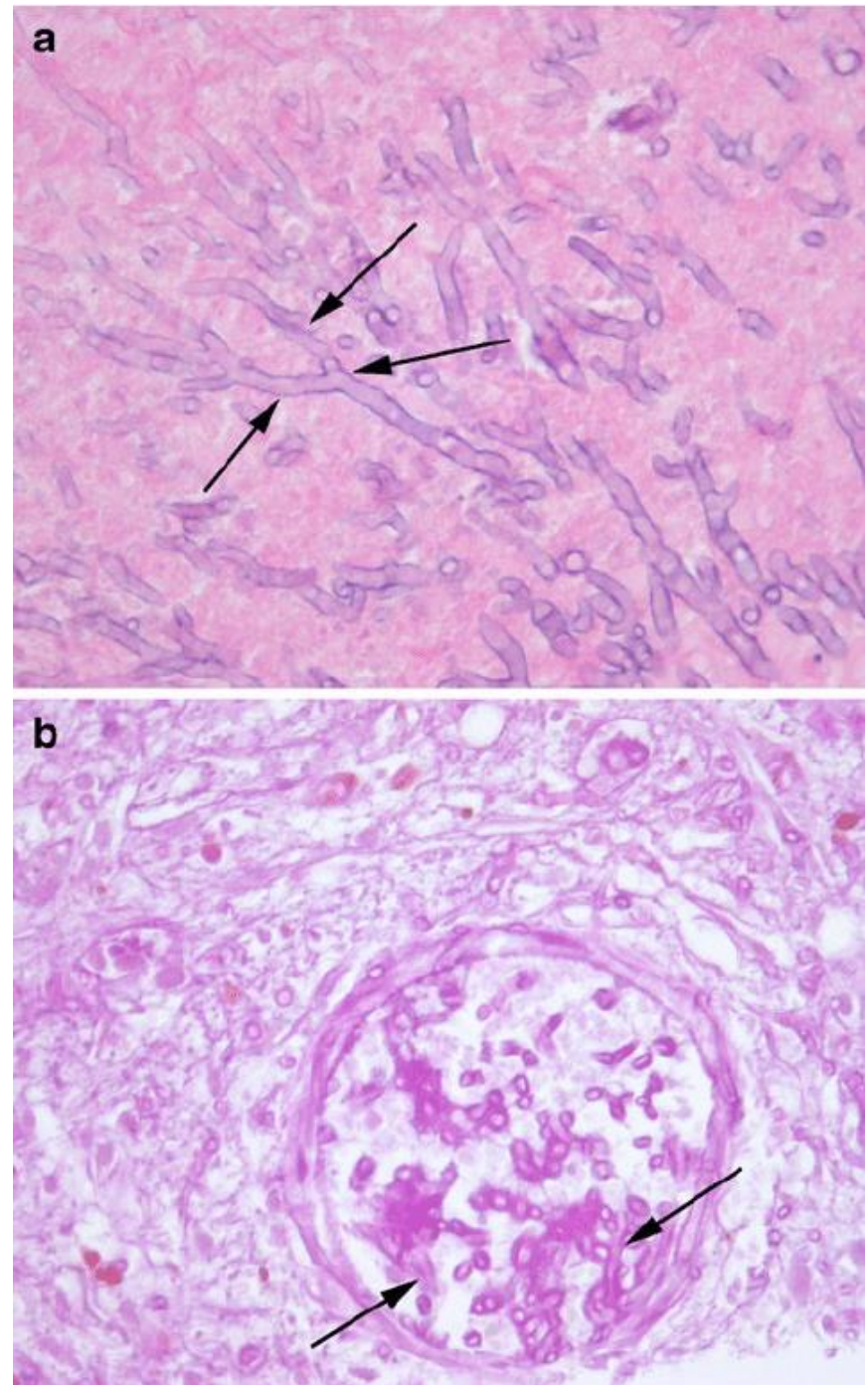


1. Direct Examination

III- Histopathology

- **Superficial infection** – acute, subacute or chronic dermatitis with folliculitis
- **Subcutaneous & systemic infections** – granulomatous reaction with fibrosis or pyogenic inflammation
- **Routine stain** – Hematoxylin & Eosin (HE)

Figure: Histology
(hematoxylin and eosin stain). (a) Fungal hyphae with dichotomous branching diagnostic of aspergillus species are depicted (arrows). (b) Angioinvasion. Fungal hyphae are observed within small veins (arrows)



2. Fungal culture: Growth and Isolation of Fungi

Most fungi occur in nature and grow readily on simple sources of nitrogen and carbohydrate .

- **Sabouraud Dextrose Agar (SDA)** traditional mycological media (glucose, peptone, PH=7) which is not support the growth of bacteria.
- **Potato dextrose agar (PDA)**: is the most widely used medium for growing fungi and bacteria.
- **Brain-heart infusion (BHI) agar**: It is a non-selective fungal culture medium that permits the growth of virtually all clinically relevant fungi. It is used for the primary recovery of saprophytic and dimorphic fungi

- However other media, such as **Inhibitory Mold Agar (IMA)**, have facilitated the recovery of fungi from clinical specimens.



To culture medical fungi from non sterile specimens, antibiotics (gentamicin, chloramphenicol) are added to the media to inhibit bacteria and saprobic molds.

2. Fungal Culture

Temperature requirement

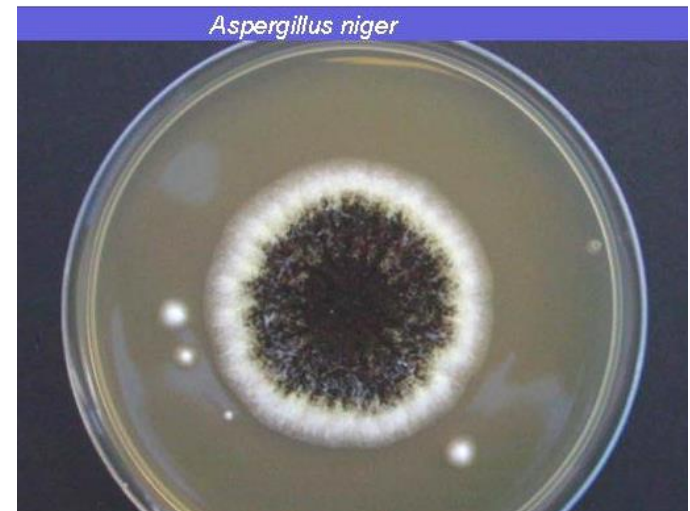
- Majority of fungi – 37°C
- Superficial mycosis – 30°C
- Dimorphic fungi – 25°C & 37°C

Incubation time

- At least 4 weeks
- Usually positive cultures are obtained in 7-10 days
- Candida & Aspergillus - 24 to 72 hrs

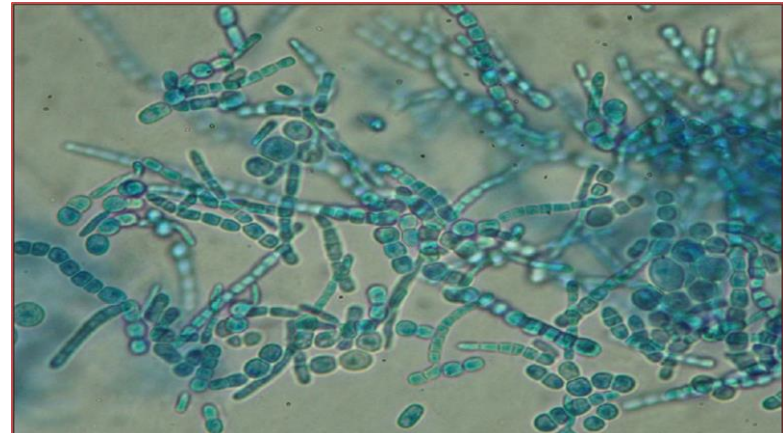
Identification of fungal cultures

- **Colony morphology** – colour, texture, pigment production



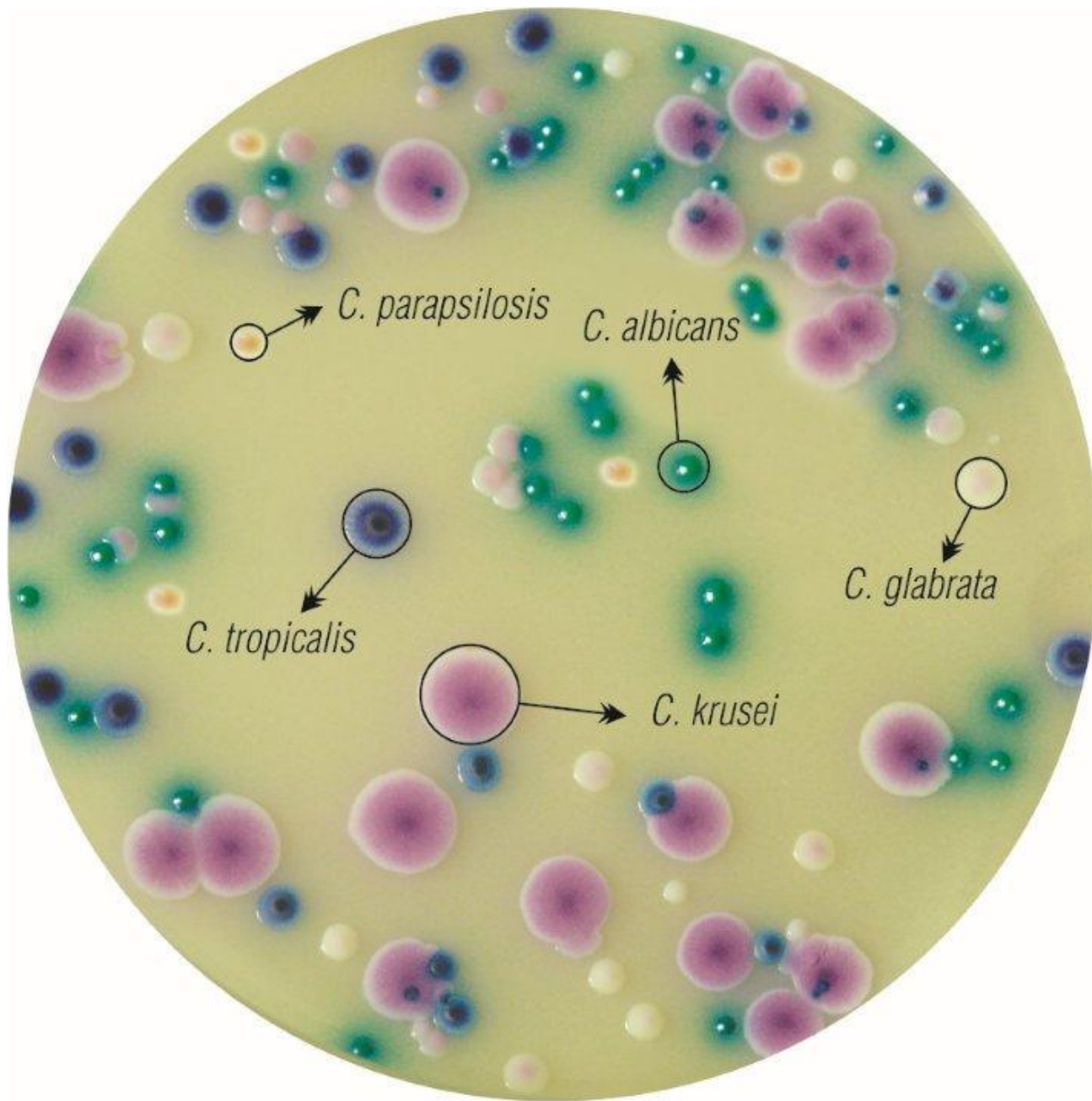
Identification of fungal cultures

- Fungal morphology under microscope – using Lactophenol Cotton Blue (LPCB) stain
- **Composition of LPCB**
 - Lactic acid - preserves fungal structure
 - Phenol – kills any live organism
 - Glycerol – prevents drying
 - Cotton blue – imparts blue color to structures



Identification of fungal cultures

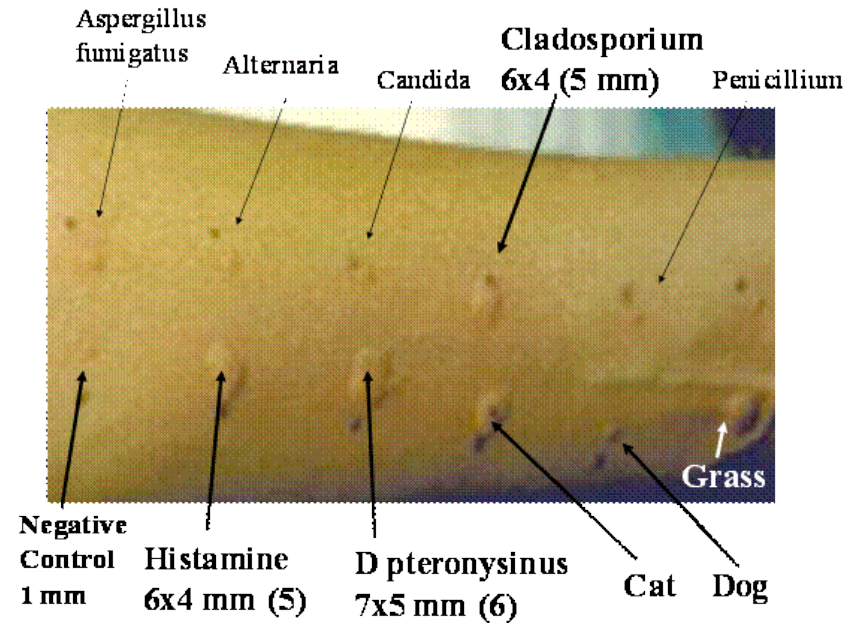
- **Special culture techniques** – to see sporing structures & spore arrangement, CHROM agar for candida sps.
- **Biochemicals** – ability to assimilate carbon & nitrogen, sugar fermentation



M1297AR – HiCrome™ Candida Differential Agar

3. Serological tests - Detection of Ag or Ab in serum or body fluids

4. Skin tests



5. PCR & other molecular methods



T H A N K Y O U