

Mycology Lab

CLS 417: Clinical Practice in Microbiology

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Topics to be covered in this lecture:

- Fungi overview
- Mycosis: diseases caused by Fungi
- Specimens for Fungal Infection
- Laboratory Tests for Fungal Infection
- Direct Microscopic examination of Fungi
- Culture of specimens in Mycology

<http://dermnetnz.org>

<http://labtestsonline.org>

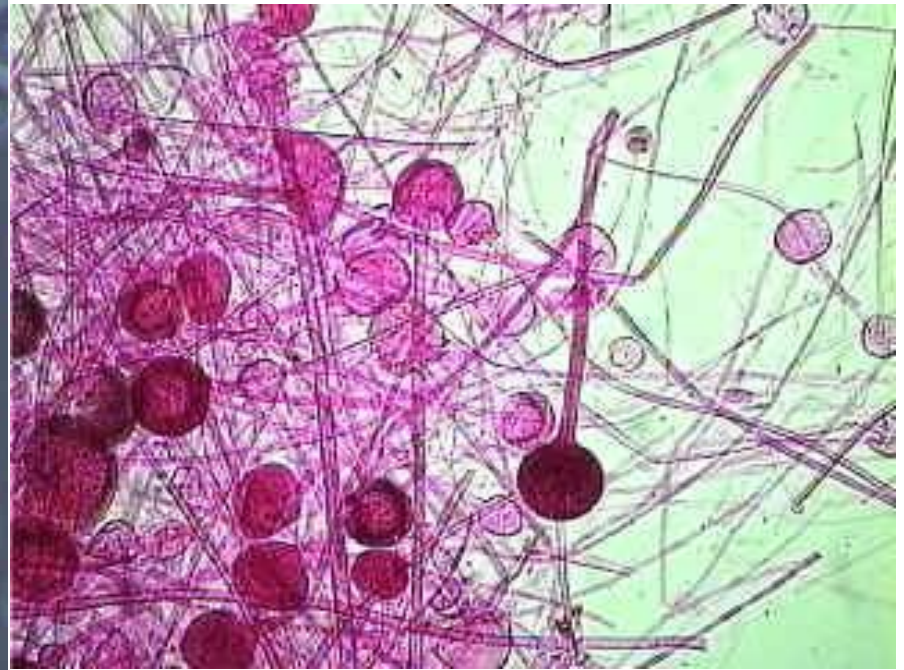
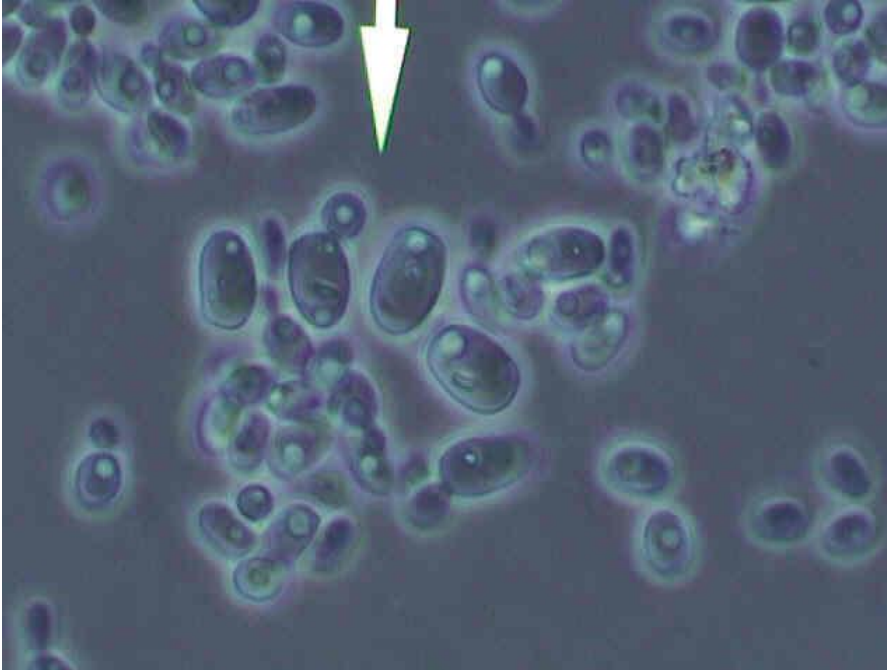
<http://www.guident.net>

Structure of Fungi

Fungi can be

Unicellular = Yeasts

Multicellular = Molds



Mycosis: diseases caused by Fungi

Examples of Superficial Mycosis include:

- Yeast infections caused by *Candida* species
- Athlete's foot (*Dermatophytes*)
- Jock itch
- Scalp or hair infection (*Piedra*)
- Finger or toenail infection
- Ringworm

Examples of Systemic Mycosis include:

- *Aspergillosis*
- *Blastomycosis*
- *Coccidioidomycosis*
- *Cryptococcosis*
- *Histoplasmosis*

Laboratory Tests for Fungal Infection

Fungal tests are used to help:

- Detect and diagnose a fungal infection.
- Guide treatment.
- Monitor the effectiveness of treatment.

Superficial Mycosis

For many superficial skin and yeast infections, a **clinical examination** of the affected person and **microscopic examination** of the sample may be sufficient to determine that a fungal infection is present. The specific organism is **not always identified**. The doctor has several topical and oral antifungal treatment options and bases the choice(s) on practice guidelines and experience.

Tests for Superficial Mycosis

| TEST NAME | SAMPLE TYPE(S) | DESCRIPTION | USES | TIME FOR RESULTS |
|---|---|---|--|------------------|
| KOH prep (potassium hydroxide solution) | Skin scrapings, hair or nail clippings, tissue, vaginal swab, body fluids, sputum | The sample is placed on a slide and the chemical solution dissolves non-fungal elements; reveals yeast cells and fungal hyphae (branching filaments) on a microscope slide; examined by a doctor or trained laboratorian. | Primary screening tool; detects fungi but does not tell what specific fungus is present. | Rapid |
| Calcofluor white stain | Skin scrapings, hair or nail clippings, vaginal swab, body fluids, sputum | Stain binds to fungal elements in a sample and fluoresces under ultraviolet light; allows visualization on microscope slide; more sensitive means of visualizing fungi. | Detects fungi but does not tell what specific fungus is present. | Rapid |
| Fungal culture | Skin, nail, hair, body fluids, tissue, vaginal swab, sputum, blood | A sample is placed on or into nutrient media and incubated to grow any fungi present in sample. | Primary tool to diagnose a fungal infection; grows fungi for identification tests and subsequent susceptibility testing. | Weeks |

Systemic Mycosis

For persistent, deeper, or systemic infections, several tests may be used for diagnosis and monitoring:

- 1. Fungal cultures** are used to identify the specific fungi present. Many fungi are slow-growing, so it may take weeks for results. Susceptibility testing performed on fungi isolated from a culture is used to determine which antifungal is best to use for treatment.

Cont. Systemic Mycosis

2. Tests for fungal antigens and antibodies

- Used to determine if an individual has, or recently had, a specific fungal infection.
- They are more rapid than fungal cultures but only test for a specific fungal species, so the doctor has to know what fungal organism to test for.
- Many people have fungal antibodies from a prior exposure to the organism so that a single antibody test may not confirm the presence of a current infection (acute and convalescent samples).

3. **Molecular tests** may be performed to identify fungi grown in culture and sometimes may be used to detect a specific fungus directly in the sample collected.

Tests for Systemic Mycosis

| TEST NAME | SAMPLE TYPE(S) | DESCRIPTION | USES | TIME FOR RESULTS |
|-------------------------------------|---|---|--|---|
| Susceptibility testing | Sample of fungus isolated in culture | Follow-up to fungal culture; when a pathogenic fungus has been identified, susceptibility testing is sometimes ordered to determine the most effective antifungal agent(s) to use. | Guide treatment | Days to weeks after culture |
| Antigen testing | Blood, urine, CSF , body fluids | Detects proteins associated with a specific fungus. This type of test is available for a variety of fungi. | Diagnose infection by specific fungus | Day(s); rapid tests are available for some fungi (e.g., <i>Cryptococcus</i> , <i>Histoplasma</i> species) |
| Antibody testing | Blood, CSF, body fluids | Detects immune response to a specific fungus; may be ordered on a single sample or on acute and convalescent samples collected 2 to 3 weeks apart. | Diagnose current or recent infection by specific fungus; monitor treatment | Day(s) or weeks |
| Molecular tests for DNA, RNA | Sample of fungus isolated in culture, blood, CSF, body fluids | Detects genetic material of a specific fungus. | Detects some fungi; not yet widely available, some in research settings only | Days to weeks |

Specimens for Superficial Mycosis

- Scrapings of scale, best taken from the leading edge of the rash after the skin has been cleaned with alcohol.
 - Skin stripped off with adhesive tape, which is then stuck on a glass slide.
 - Hair which has been pulled out from the roots.
 - Brushings from an area of scaly scalp.
 - Nail clippings, or skin scraped from under a nail.
 - Skin biopsy.
 - Moist swab from a mucosal surface (inside the mouth or vagina) in a special transport medium.
 - A swab should be taken from pustules in case of secondary bacterial infection.
- They are transported in a sterile container or a black paper envelope.

Specimens for Systemic Mycosis

- Respiratory secretions.
- CSF.
- Blood.
- Urine.
- Pus.
- Ocular specimen.
- Tissue.
- Bone marrow.

➤ They are transported in a sterile container.

I. Direct Microscopy

The specimen is examined by microscopy by one or more of these methods:

- Potassium hydroxide (KOH) preparation with or without parker super chrome blue-black ink usually for hard specimens e.g. hair, skin,...
- Unstained wet-mount.
- Stained dried smear: India ink, Calcoflour white, and Silver stains.
- Histopathology of biopsy with special stains, e.g., periodic acid-Schiff (PAS) and Geimsa.

Yeast Microscopy

- ✓ Fungal elements are sometimes difficult to find, especially if the tissue is very inflamed, so a negative result does not rule out fungal infection.

A yeast infection can be identified by the presence of:

- Yeast cells, which may be dividing by budding .
- Pseudohyphae (branched filaments similar to those of a dermatophyte) forming a pseudomycelium.

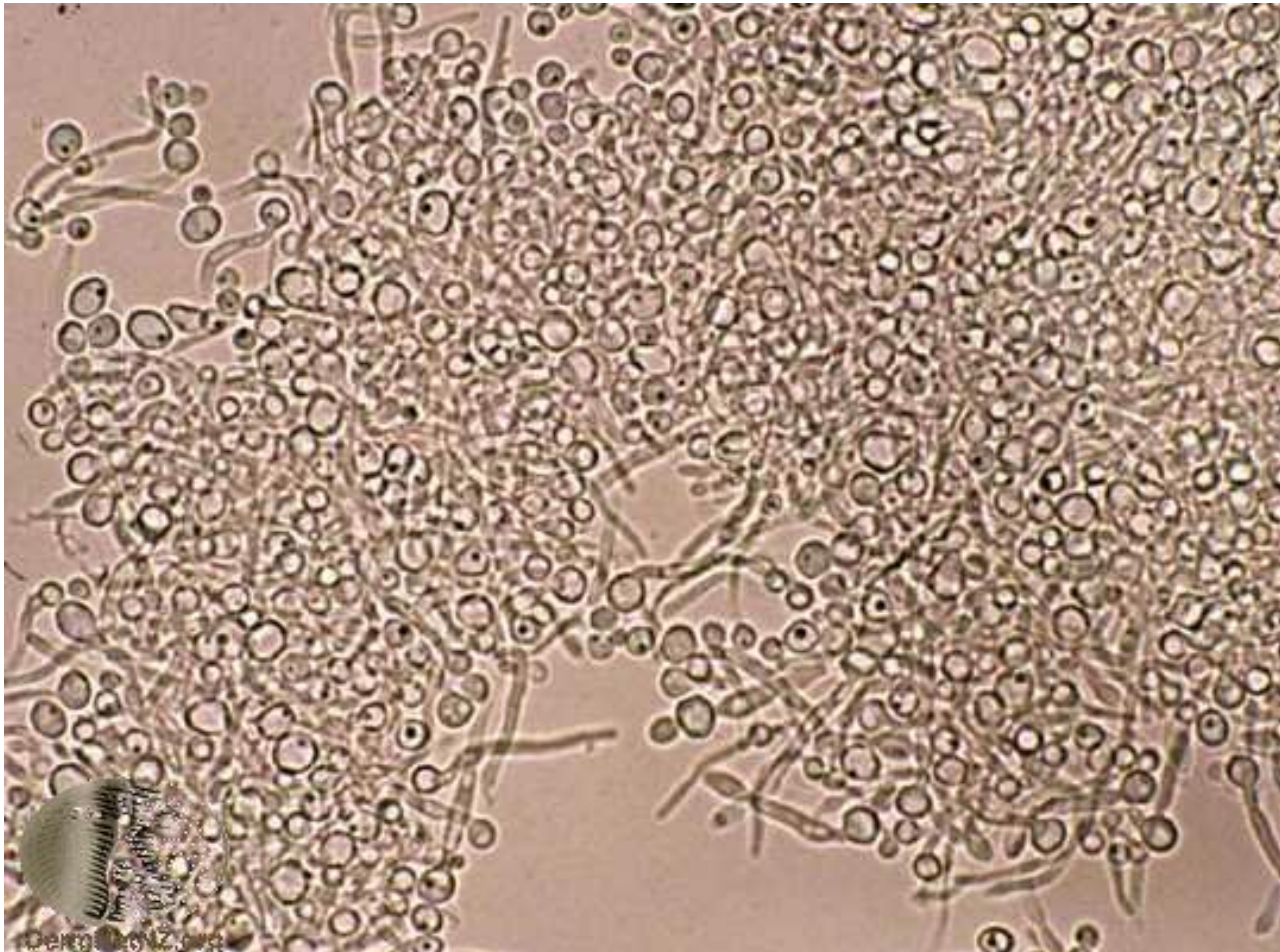
Dermatophyte Microscopy

Microscopy can identify a dermatophyte by the presence of:

- Fungal hyphae (branched filaments) making up a mycelium.
- Arthrospores (broken-off spores).
- Arthroconidia (specialised external spores).
- Spores inside a hair (endothrix) or outside a hair (ectothrix).

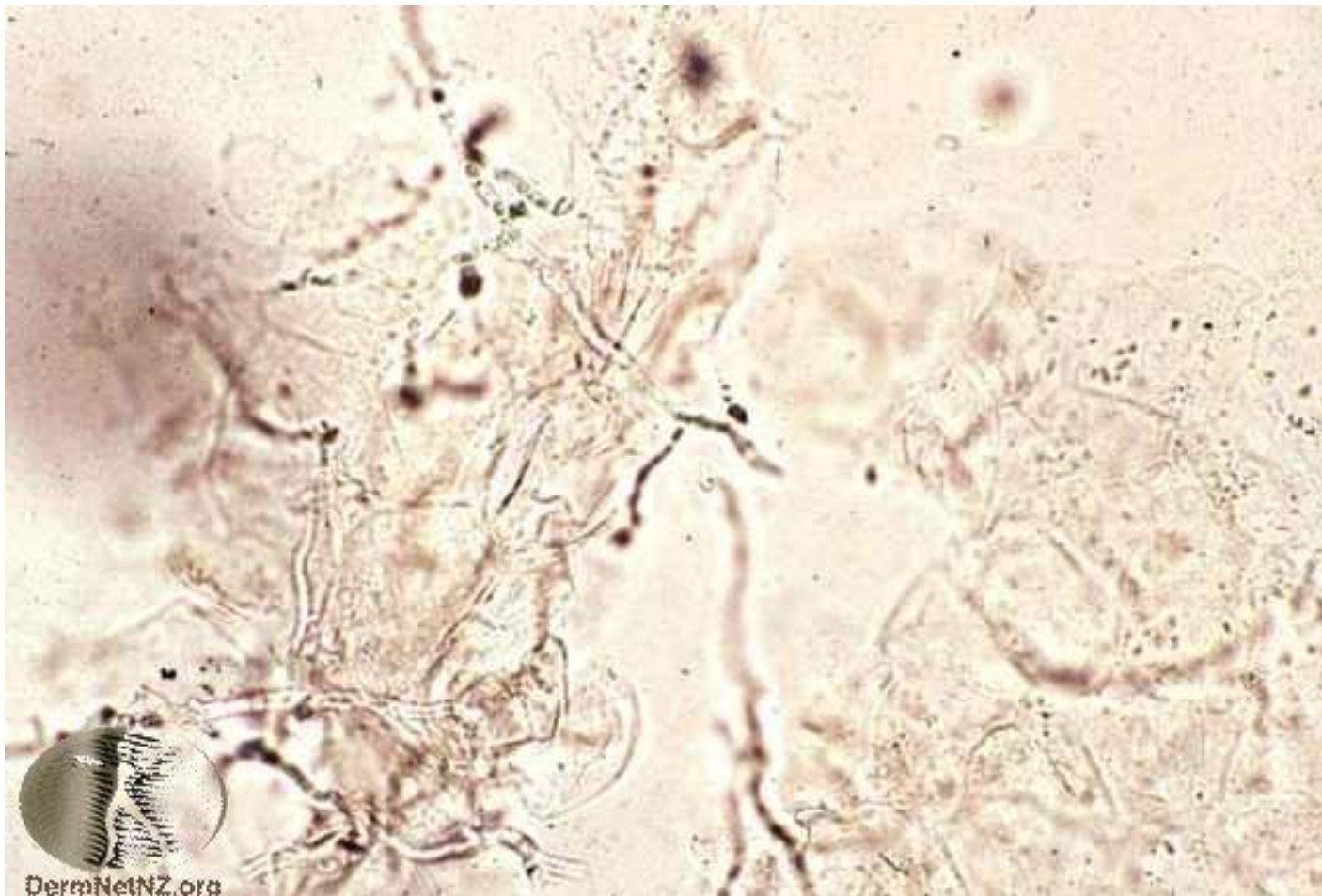
KOH Preparation

Candida showing pseudohyphae



KOH Preparation

Microsporum canis showing hyphae

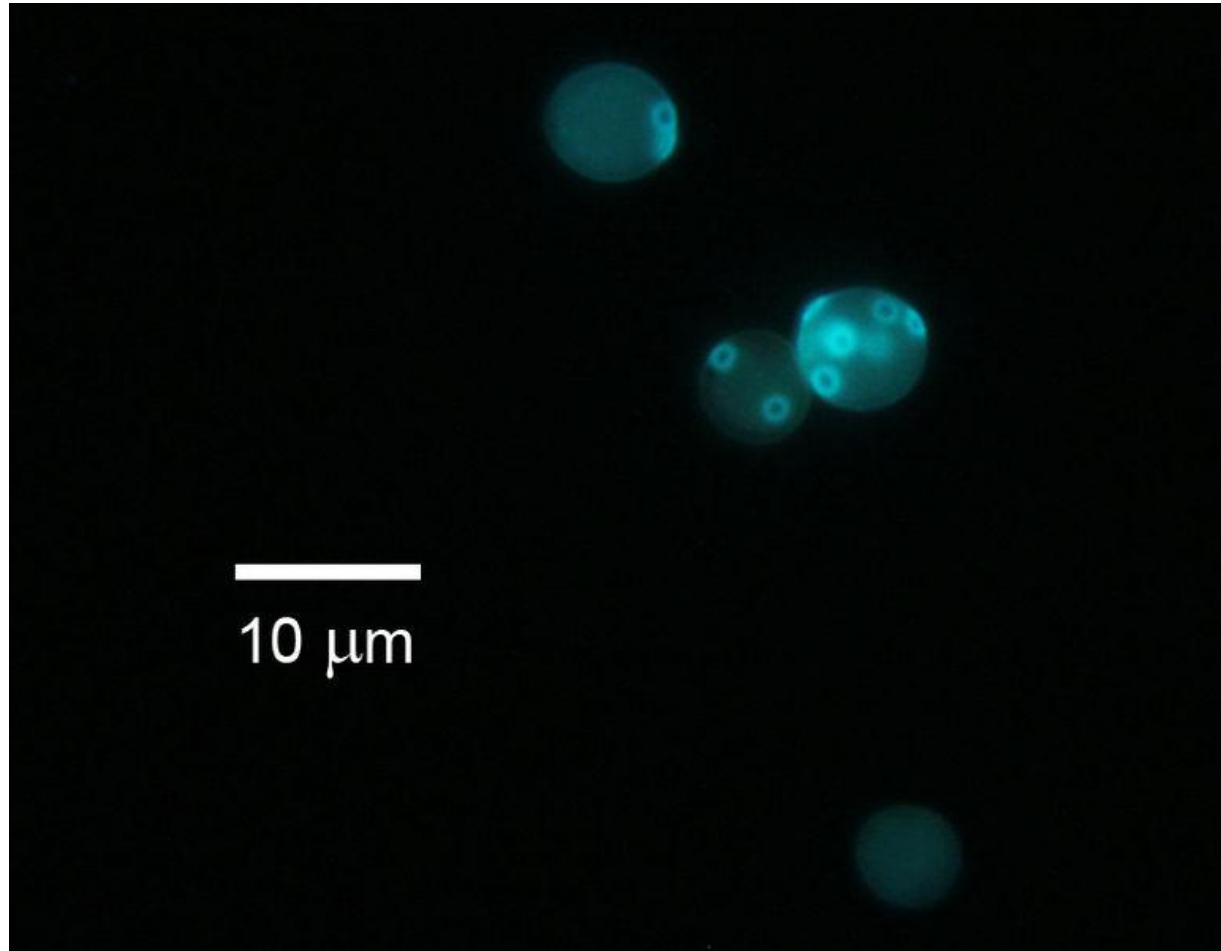


Preparation with Calcoflour White and KOH

- The dye binds to β 1-3, β 1-4 polysaccharides in fungi then fluoresces under Fluorescence microscope.
- Yeast cells, pseudohyphae and hyphae display a chalk-white or brilliant apple-green fluorescence against background material.
- **Disadvantages:**
 1. Need for a Fluorescence microscope.
 2. Inability of the dye to detect the endospores.
 3. Difficulty in interpreting vaginal secretions.

Calciflour Stain

showing yeast

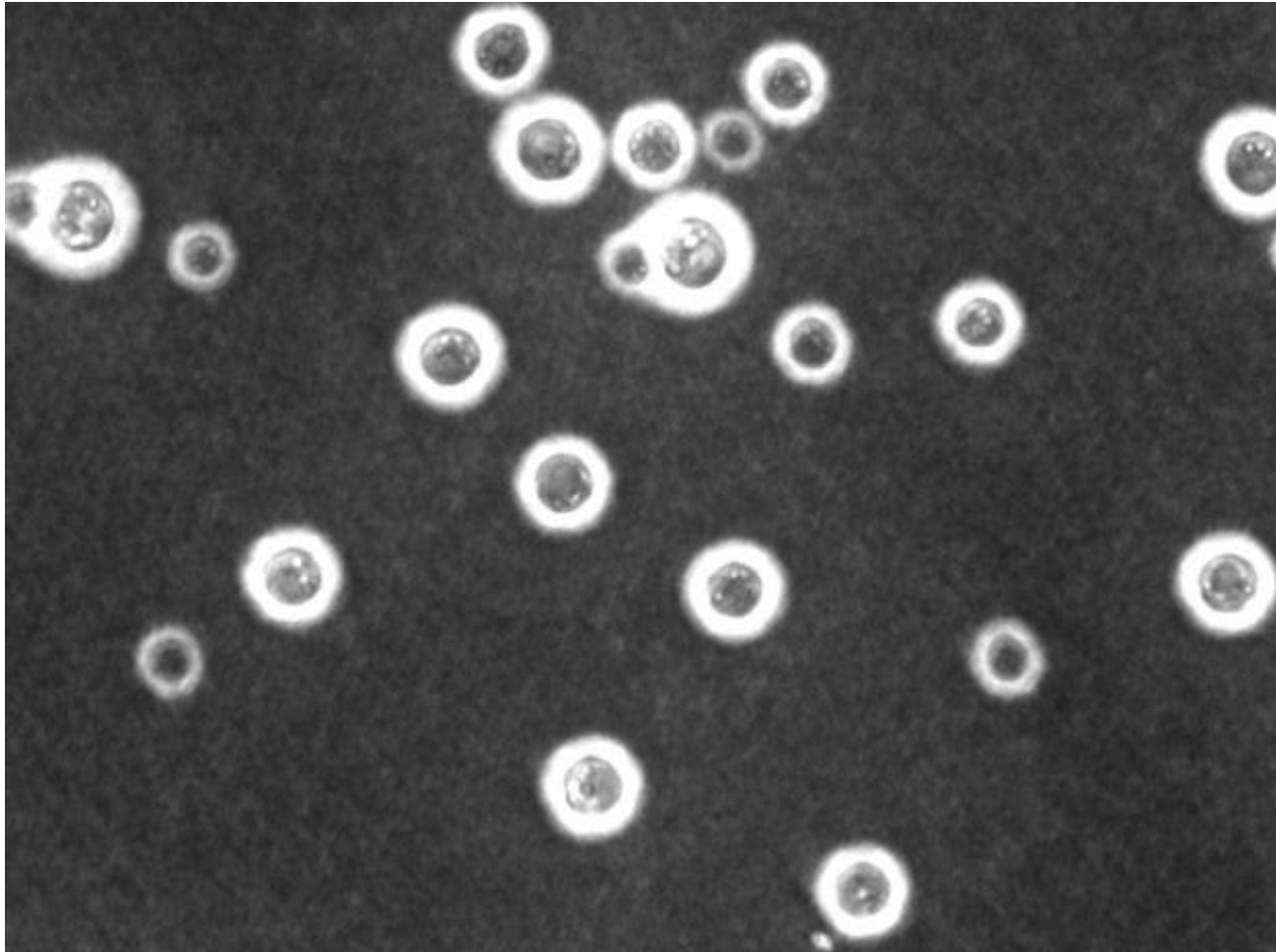


Preparation with India Ink

- India ink is useful for indicating the presence or absence of fungal capsules.
- The technique is particularly helpful for detecting *Cryptococcus neoformans* in CSF.
- Because India ink serves as a negative stain, the encapsulated yeast cells can readily be detected against the dark back ground.
- Because tests for antigen also have false-positive results, the tests can be used to complement one another.

India Ink Preparation

C. neoformans showing the capsule

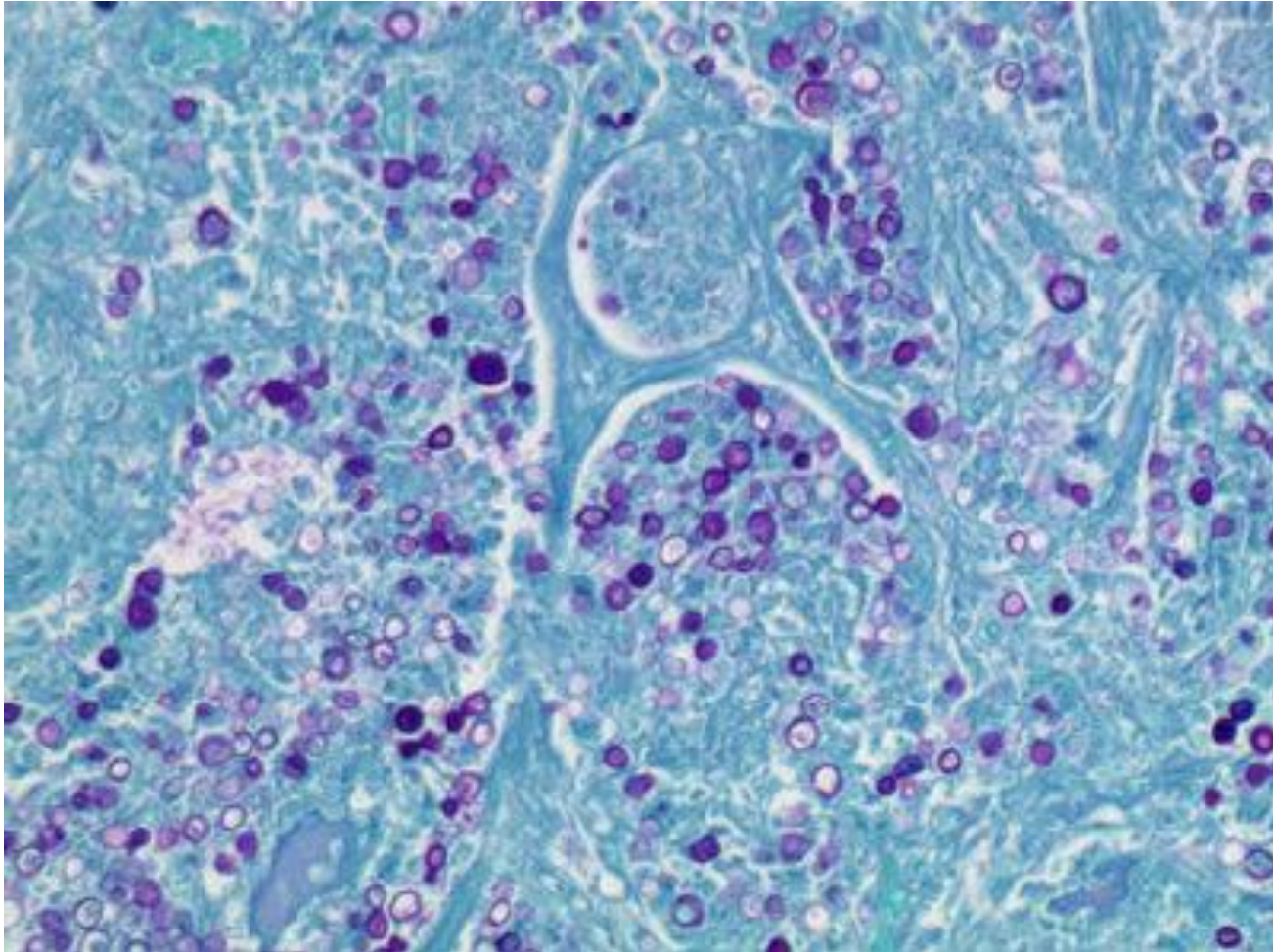


Preparation with Periodic Acid-Schiff (PAS) Stain

- In a direct examination of clinical specimen, PAS stain is sometimes used when a KOH preparation do not reveal fungi that are suspected to be present.
- Light green is preferred as a counter stain because the fungus appears deep purplish red against the contrasting background color.
- The PAS reaction stains certain polysaccharides found in the fungal cell wall.
- For laboratories with a fluorescent microscope, calcoflour white is preferred over PAS.

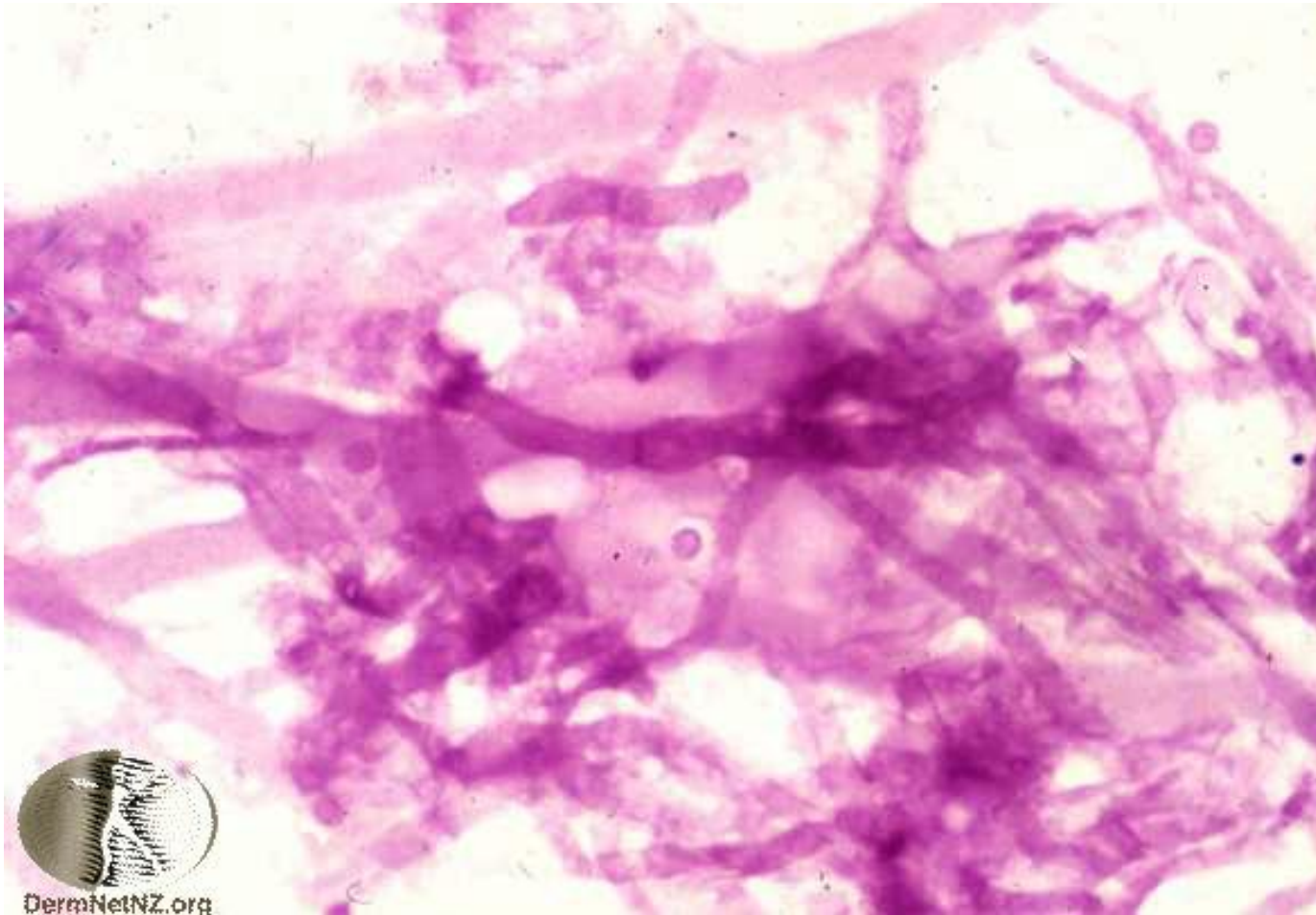
PAS

showing purple fungi



PAS

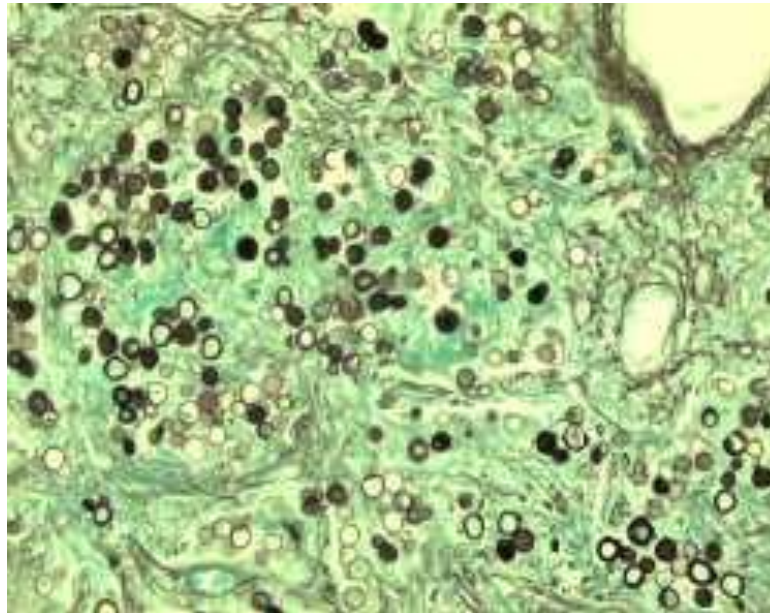
***Aspergillus* seen in a skin biopsy**



Silver Stain

Gomori's Methenamine

- The best staining but its not used routinely because it takes about 2 hours. Used to detect *Pneumocystic carinii* (PCP)
- GMS stain is used for fungi and bacteria. The fungi and bacteria are turned black, while everything else is stained green with Light green SF solution.



II. CULTURE

Culture identifies which organism is responsible for the infection:

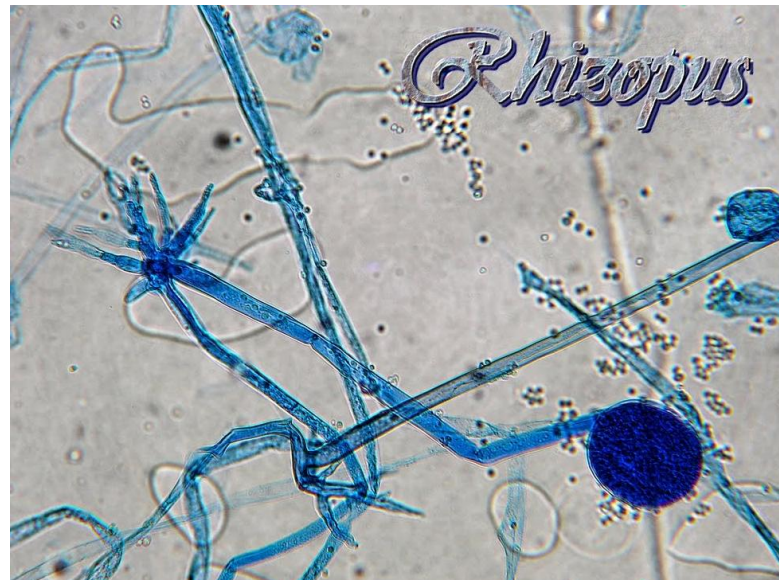
- To find out the source of infection.
- To select the most suitable treatment.
- Growing the fungus in culture may take several weeks, incubated at 25-30°C.
- The specimen is inoculated into a medium such as **Sabouraud's dextrose agar** containing cycloheximide and chloramphenicol to suppress bacteria.
- The cycloheximide is left out if a mould requires identification.

Cont. Culture

- Use brain-heart infusion to prevent dimorphic fungi “incubate at 37°C”.
- Look for growth every day during the 1st week, once or twice/week during 2nd week, and once/week during 3rd & 4th weeks.
- Keep the culture up to 6 weeks.
- **If +ve for mould** = Stain with Lactophenol.
- **If +ve for yeast** = culture on Corn-Meal Agar + do Germ Tube test.

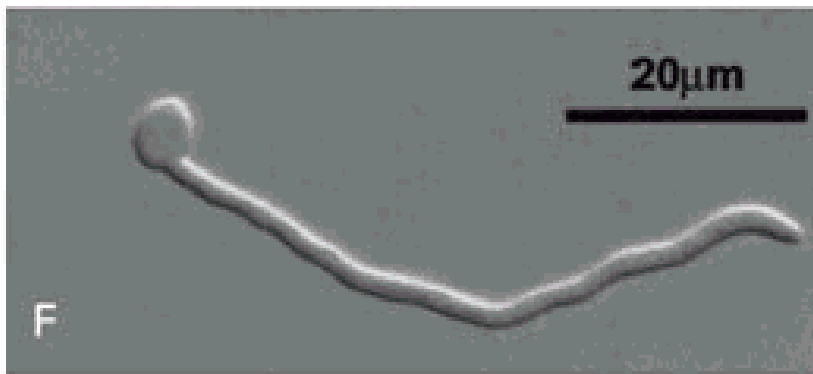
Lactophenol Cotton-Blue Stain (LPCB)

- The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures, and cotton blue which stains the chitin in the fungal cell walls.



Germ Tube Test

- The Germ Tube Test is a screening procedure used to differentiate *Candida albicans* from other yeast.
- Approximately 95 - 97% of *Candida albicans* isolated develop germ tubes when incubated in a proteinaceous media.



Germ Tube (no constriction)



Pseudohyphae (constriction)

A negative culture may arise because:

- The condition is not due to fungal infection.
- The specimen was not collected properly.
- Antifungal treatment had been used prior to collection of the specimen.
- There was a delay before the specimen reached the laboratory.
- The laboratory procedures were incorrect.
- The organism grows very slowly.

Culture of yeasts and moulds may be due to harmless colonisation rather than infection. The infection may be secondary to an underlying skin disease.

Blood Tests

- Blood tests are not useful for the diagnosis of superficial fungal infections. But in subcutaneous and systemic infection, several tests may be useful.
- Culture
- Antibodies (histoplasmosis, coccidioidomycosis)
- Antigen (cryptococcosis, aspergillus, candidosis, histoplasmosis)