Medically Significant Fungi

Laboratory Diagnosis of Fungi

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Disclaimer

- This presentation was meant to provide students with both didactic and laboratory skills as they apply to clinical mycology. It is meant for educational purposes only and does not represent Cleveland Clinic views or practices.
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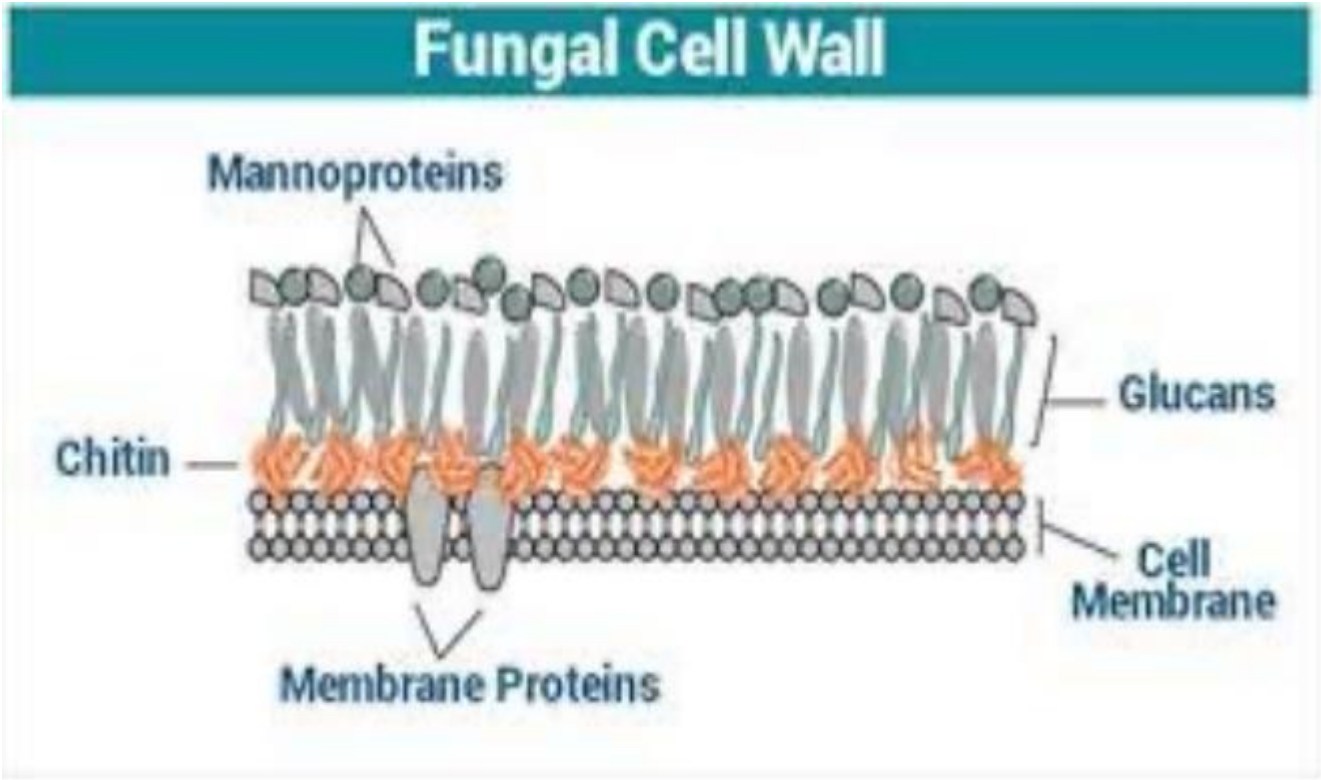
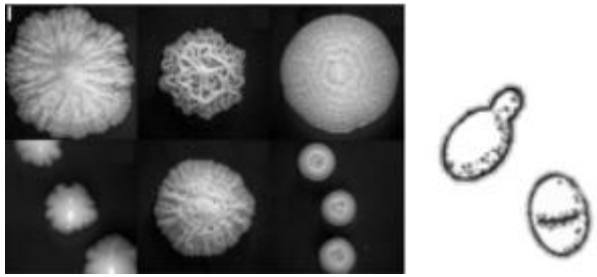
- Most of the information was adopted from the Textbook of Diagnostic Microbiology by Mahon & Lehman (see citation) but condensed for bite sized learning.

Mycology: The Study of Fungi

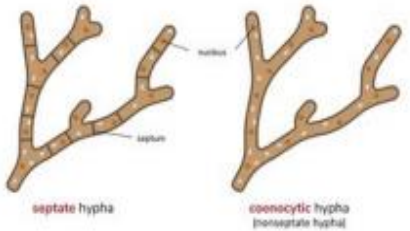
- Eukaryotic
- Lack chlorophyll
- Cell walls contain chitin
- Obligate aerobes

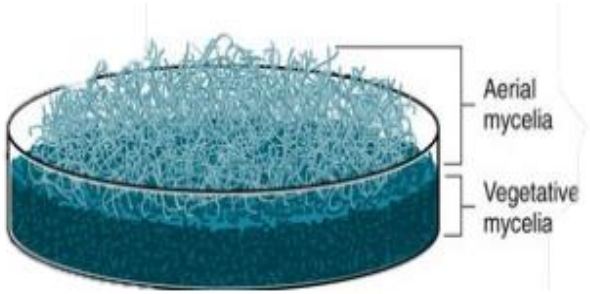


Mycology: General Characteristics



Yeast
- Unicellular
- Bacterial-like colonies
- Reproduce by budding to form blastoconidia or fission





Molds
- Multicellular
- Fuzzy or woolly
- Hyphae
  - Vegetative
  - Aerial
    - Hyaline/Moniliaceous vs. Phaeoid/Dematiaceous
    - Septate vs. Sparsely Septate/Pauciseptate/Mucoraceous

Definitions
- Dimorphism: thermally dimorphic fungi are yeasts at 37℃ and mold at 30℃
- Polymorphism: one organism but yeast forms and mold forms in the same culture

Yeast or Mold?


Yeast or Mold?


Yeast or Mold?

Yeast or Mold?

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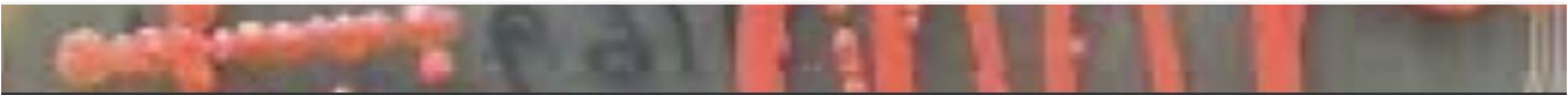
Yeast or Mold?

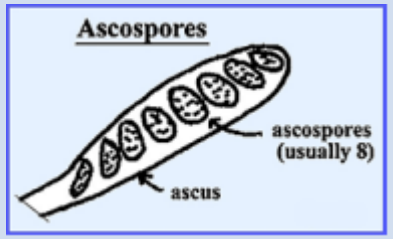
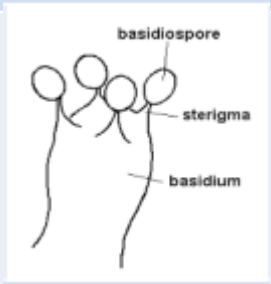
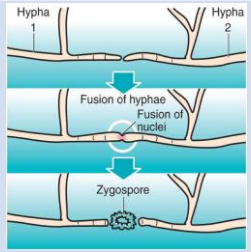



Yeast or Mold?
Gram stain

Yeast or Mold?

Gram stain

Mycology: Taxonomy

Phylum	Sexual Reproduction	Asexual Reproduction
Ascomycota		Conidia
Basidomycota		Conidia
Mucoromycota		Sporangiospores
Deuteromycota	N/A	Conidia

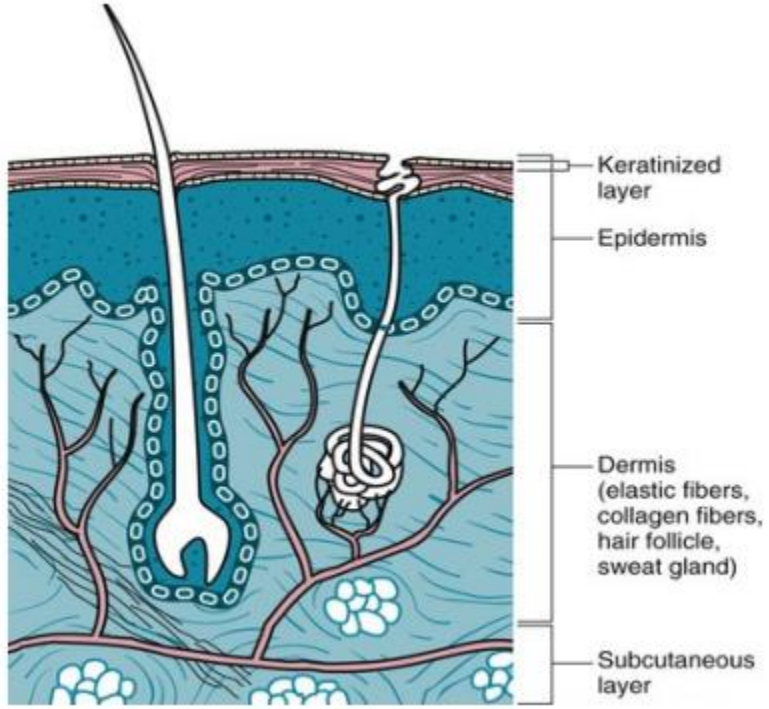
Rarely do we see sexual forms (**teleomorph**) in the laboratory.

Most of our identification is made from the asexual form (**anamorph**).

Some teleomorphs can have more than one anamorph. In this situation, the anamorphs are called **synanamorphs**.

Mycology: Mycoses

Mycosis	Tissue involved
Superficial	Outer “dead” layers of skin and hair
Cutaneous	Keratinized portions of hair, skin, and nails
Subcutaneous	Muscle, bone, and connective tissue
Systemic	Any tissue, especially of pulmonary, lymphatic, and circulatory systems
Opportunistic	Any organ or tissue



Laboratory Diagnosis of Fungi: Safety

- Airborne conidia pose a hazard
- Class 2 biological safety cabinet should be used to reduce exposure of personnel to fungal elements
  - processing of specimen
  - reading cultures growing mold
- All media must be parafilmed

Laboratory Diagnosis of Fungi: Specimen Collection, Handling, Transport

- Almost any tissue or body fluid can be submitted for fungal culture
- Most common specimens
  - Blood, bone marrow, CSF
  - Respiratory secretions
  - Hair, skin, nails
- Transported and processed as soon as possible (<2hrs)
  - Pathogenic fungi grow slowly
  - Could be overgrown by contaminating bacteria

Laboratory Diagnosis of Fungi: Specimen Collection, Handling, Transport

Specimen	Collection/inoculation
Hair	Used sterile forceps to remove infected hair and place directly onto a sterile petri dish.
Skin	Clean skin with 70% isopropyl alcohol, scrape skin from the outer edge of lesion, inoculate onto agar.
Nails	Clean nails with 70% isopropyl alcohol before clipping or scarping. Sterile scissors use to cut nails into small strips and then are embedded into the agar.
Blood and bone marrow	The isolator tube is used to lysis WBCs and RBCs and concentrated by centrifugation. The sediment is inoculated onto media. Automated blood culture systems are also designed for the recovery of fungi.
Cerebrospinal fluid	Concentrate by centrifugation.
Abscess fluid, wound exudates, and tissue	Abscess fluid and wound exudates can be plated directly onto media. Tissue should be gently minced before inoculation.
Respiratory specimens	First morning sputum. All specimens should be collected in a sterile container.
Urine	First morning voided urine concentrated by centrifugation. Sediment is inoculated onto fungal media.

Grinding tissue could kill filamentous fungi and prevent their recovery in culture.

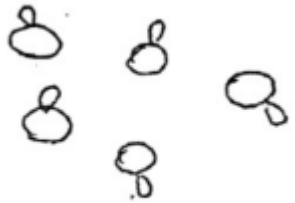
Laboratory Diagnosis of Fungi: Direct Microscopic examination of Specimens

- 10% KOH & heat
  - Breaks down keratin and skin layers revealing fungi present in specimen
  - Specimen + KOH applied to microscope slide with coverslip
  - Heated on slide warmer
  - Read using 200x and 400x on a light microscope
- Calcofluor white & heat
  - Binds to chitin
  - Fluoresce apple green or blue-white
  - Specimen + Calcofluor white combined on microscope slide with coverslip
  - Heated on slide warmer
  - Read using 200x and 400x on a fluorescent microscope

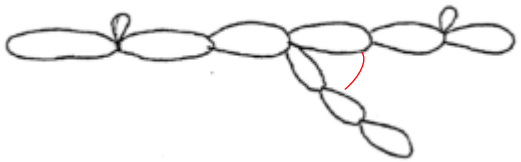
Laboratory Diagnosis of Fungi: Direct Microscopic examination of Specimens

- Structures you can see on KOH or Calcofluor white
  - Yeast
    - Budding yeast (blastoconida) [A]
    - Pseudohyphae [B]
  - Mold
    - Septate hyphae [C]
    - Mucoraceous hyphae (pauciseptate) [D]
- Final smear report = quantitation + structure

[B]



[A]



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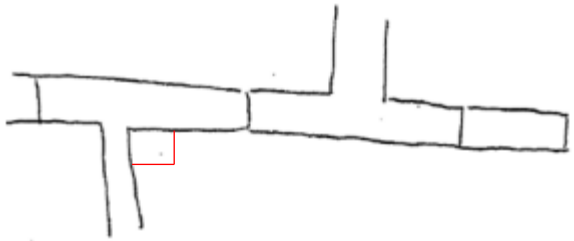
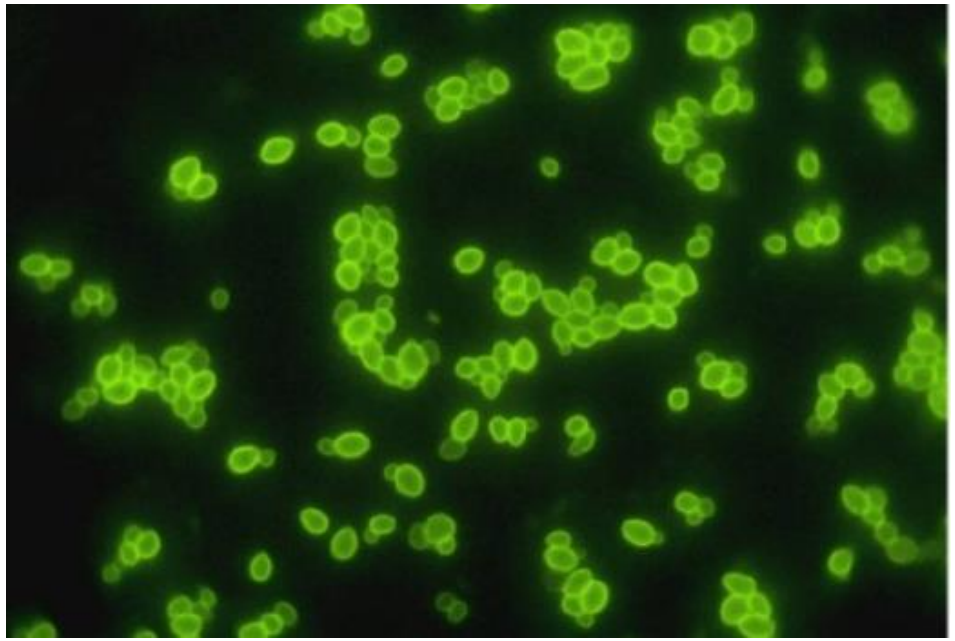


[C]

[D]

Laboratory Diagnosis of Fungi:
Direct Microscopic examination of Specimens

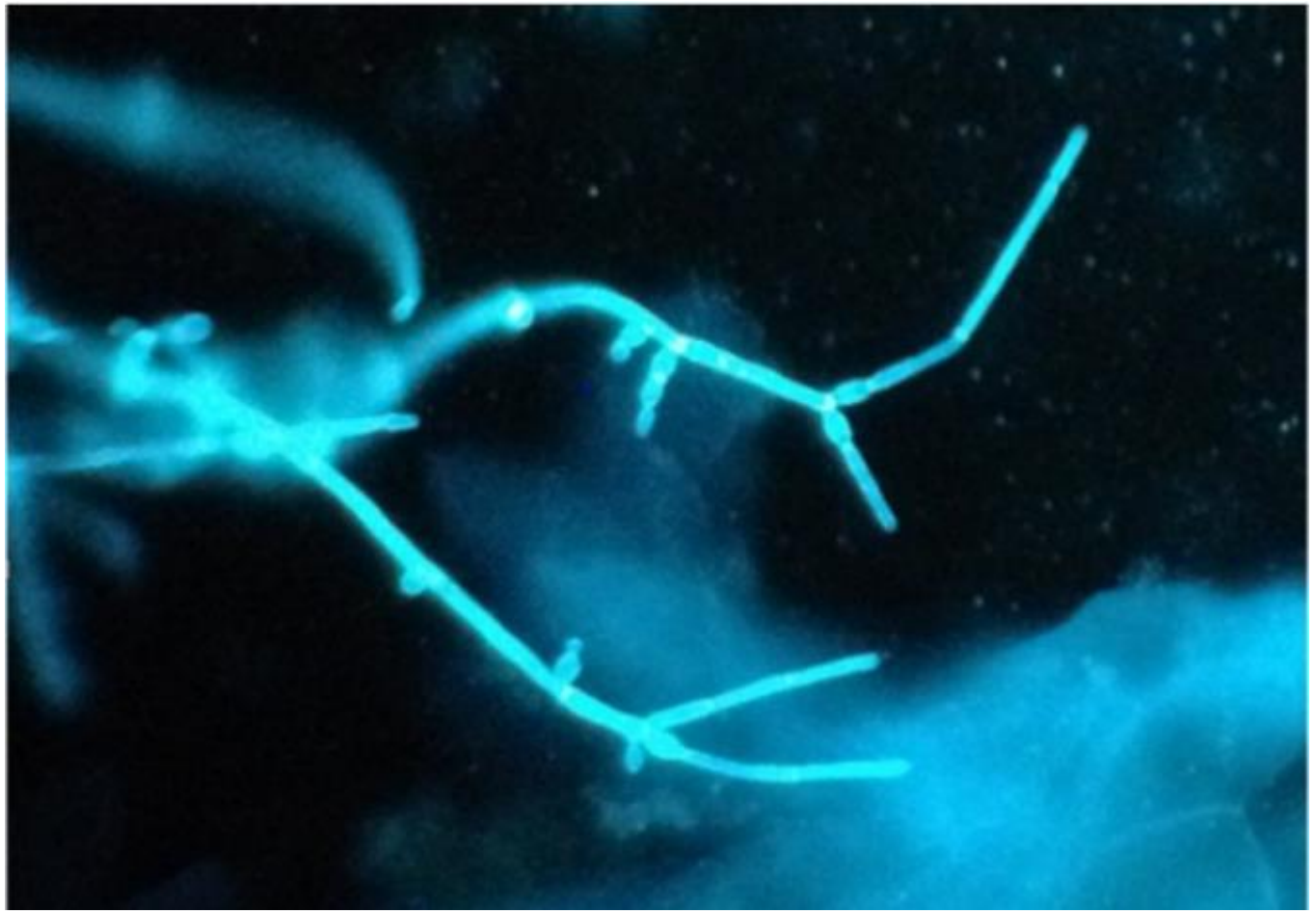
KOH



Calcofluor white

Highlight the structure(s)

- Budding yeast (blastoconida)
- Pseudohyphae
- Septate hyphae
- Mucoraceous hyphae (pauciseptate)



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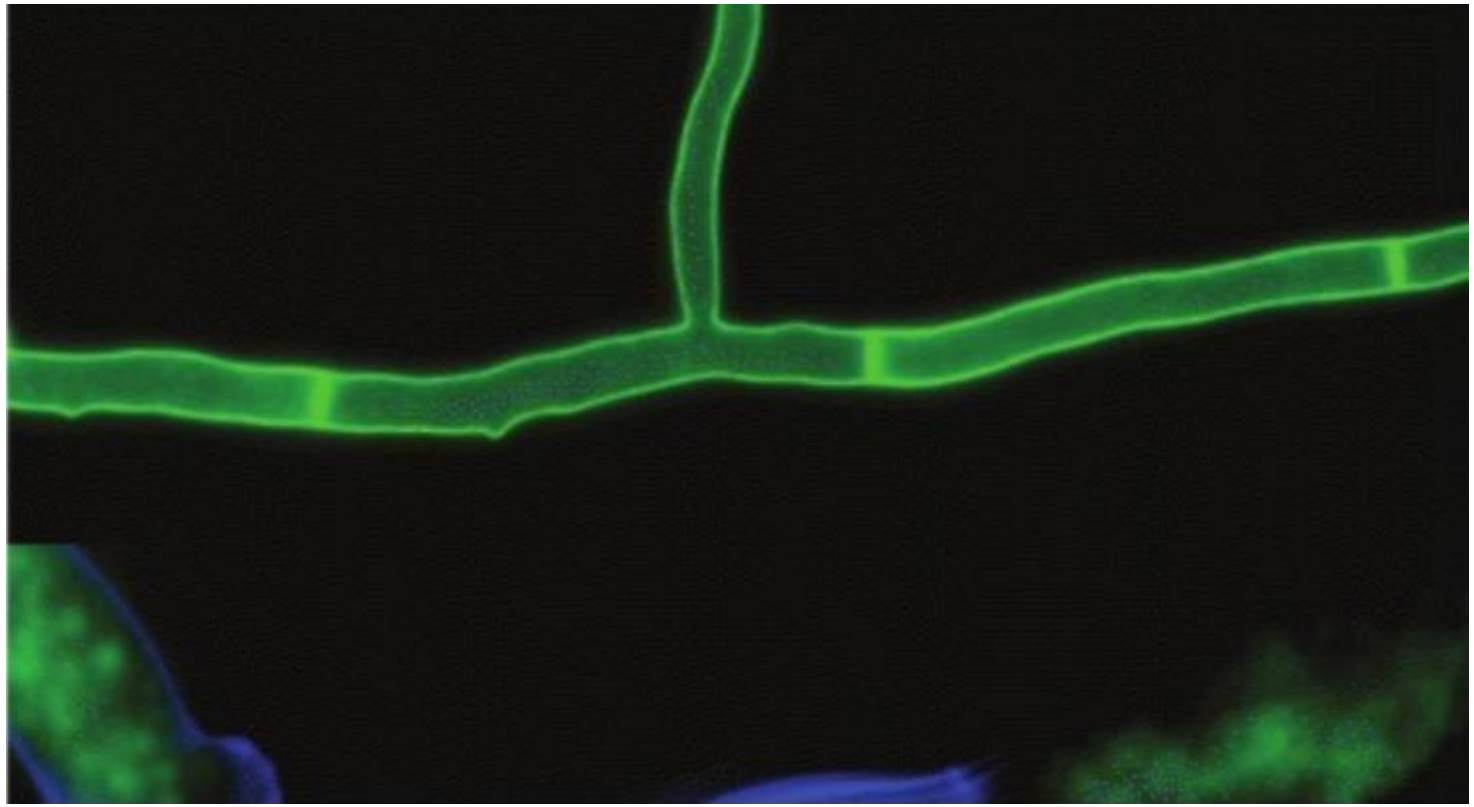

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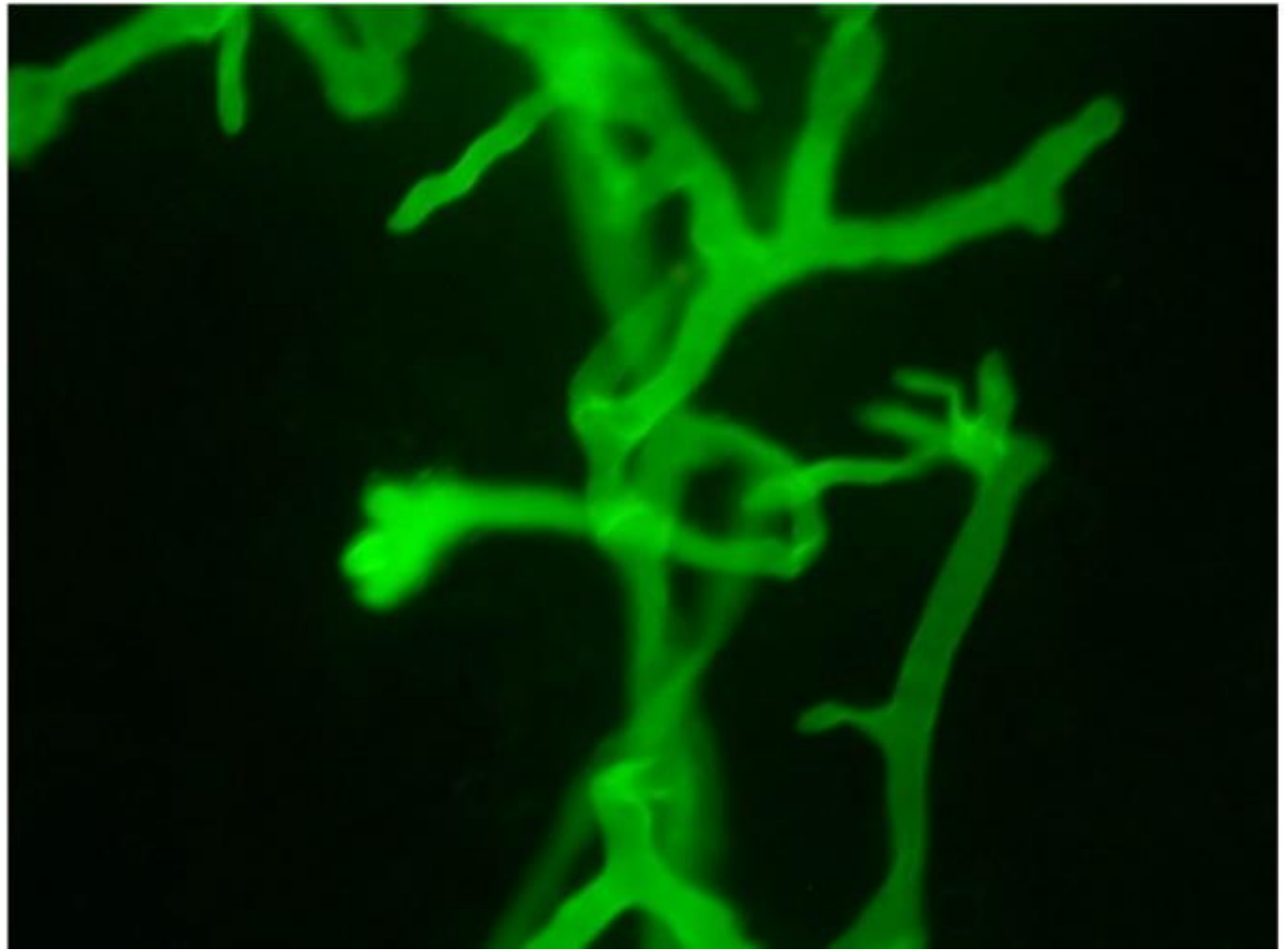

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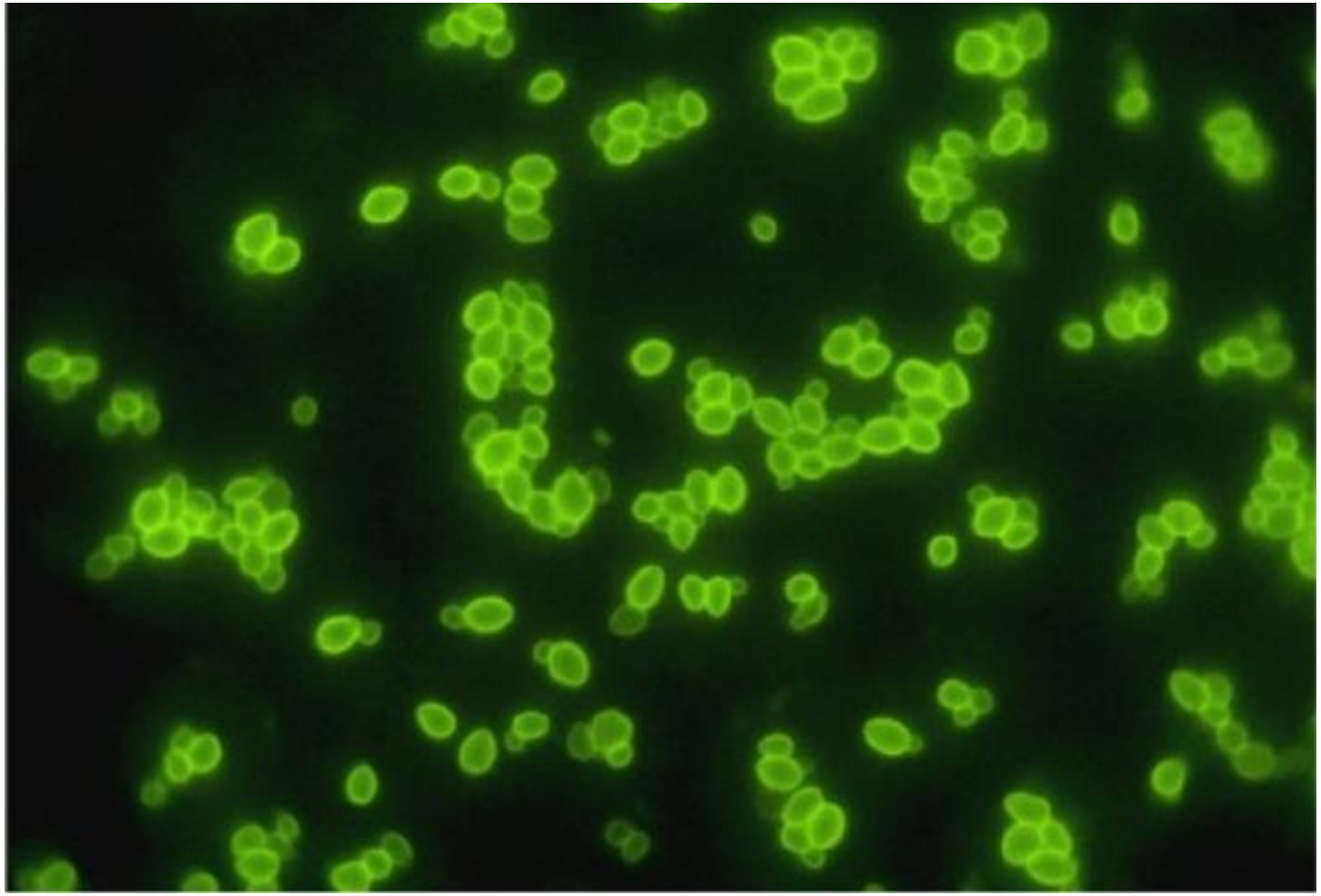
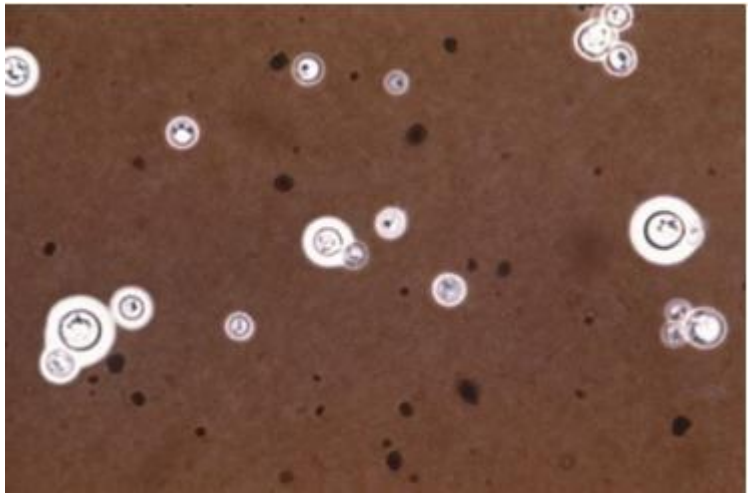


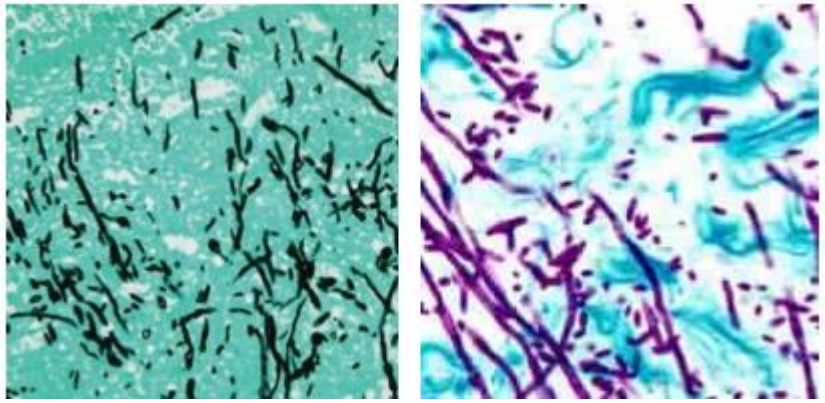
Calcofluor white

Laboratory Diagnosis of Fungi:
Direct Microscopic examination of Specimens



India ink

Capsulated yeast



Gomori methenamine siliver (GMS)

Periodic acid-Schiff (PAS)

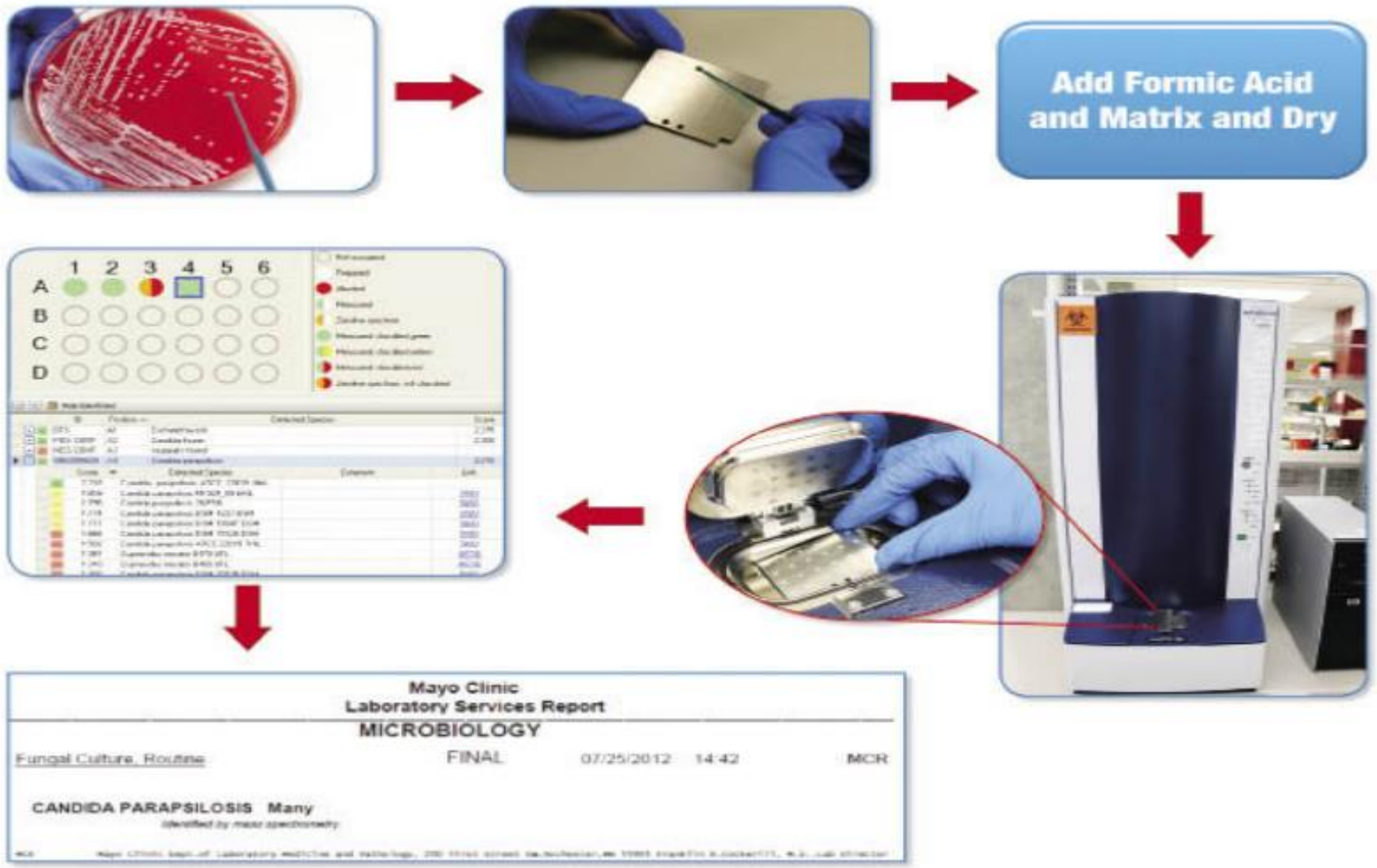
Fungi in tissue (histology stains)

Laboratory Diagnosis of Fungi: Primary Plating Media

- Nonselective
  - Sabouraud dextrose agar (SDA) or Potato dextrose agar (PDA)
- Selective
  - Sabouraud dextrose agar or Potato dextrose agar with chloramphenicol
  - Mycosel agar (mycobiotic) with cycloheximide & chloramphenicol
- 30℃ O_2 for 4 weeks
  - Examined MWF ≦ 7days old
  - Examined Tues. > 7days

PDA

Laboratory Diagnosis of Fungi: Fungal Identification (Yeast)



Laboratory Diagnosis of Fungi: Fungal Identification (Yeast)

Procedure 8

Germ Tube Production for Yeast Identification

Purpose

To differentiate among the pathogenic yeast based on the ability to produce a germ tube

Principle

When grown in serum or plasma at 35° C, some yeasts have the ability to form hyphae. This is an important characteristic in the identification of *Candida albicans*.

Specimen

Isolated cultures of test yeasts

Medium

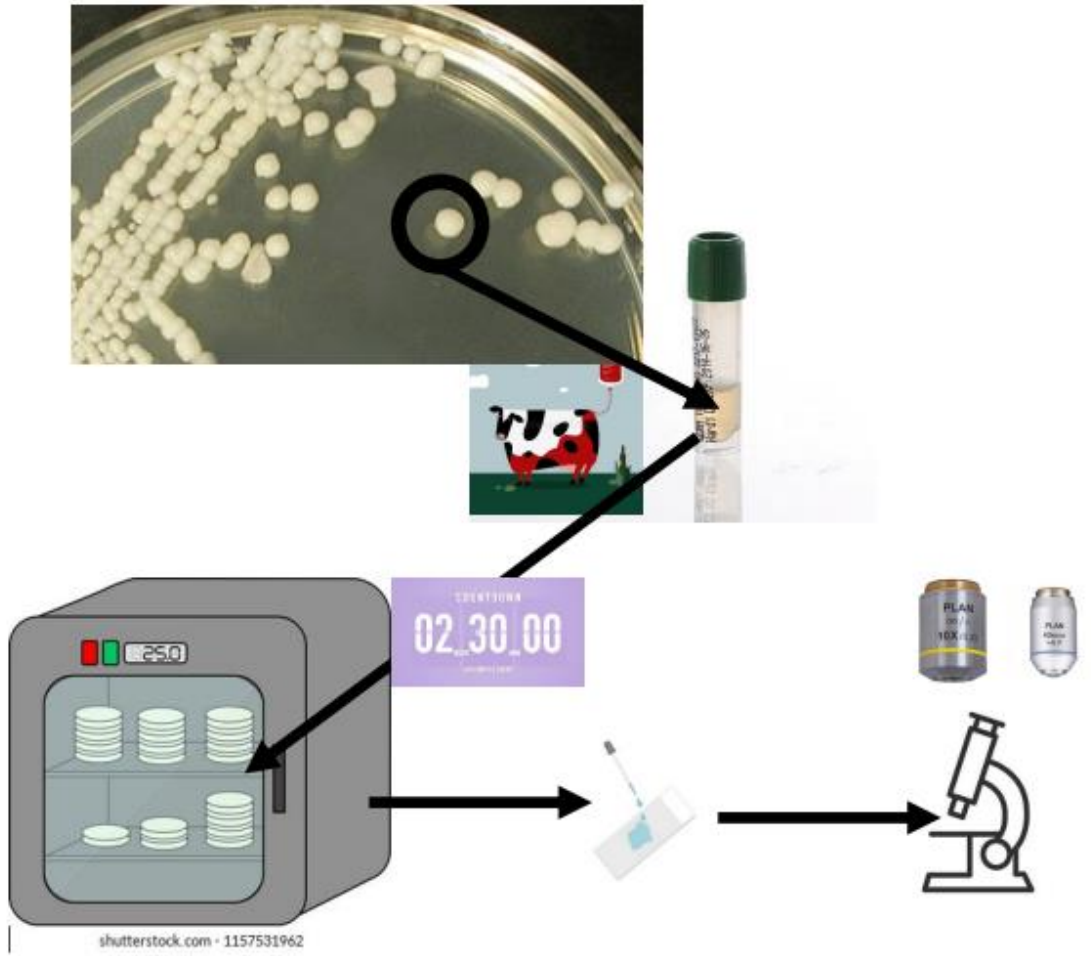
Rabbit plasma or serum or fecal calf serum

Procedure

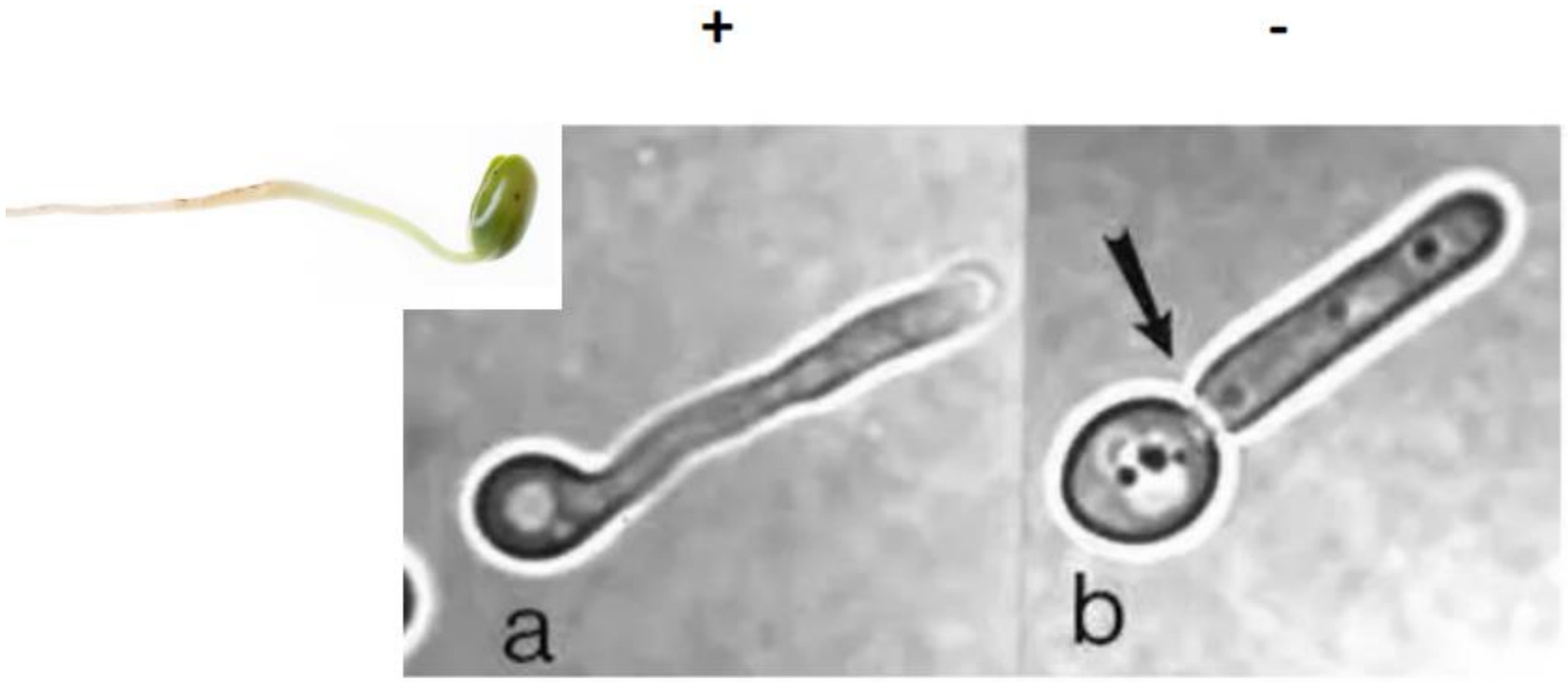
1. Make a light suspension by adding one yeast colony to 0.5 mL of sterile serum. Germ Tube Solution (Remel, Lenexa, KS), composed of fetal bovine serum and trypticase soy broth, may be used as an alternative. This alternative substrate eliminates the risk of human immunodeficiency virus and hepatitis viruses that can be present in human serum.
2. Incubate the suspension at 35° C for 2.5 to 3 hours.
3. Place one drop of the suspension on a microscope glass slide and add a coverslip.
4. Observe microscopically for the presence of germ tubes.

Controls

A known germ tube positive isolate of *C. albicans* can serve as the positive control; *Cryptococcus* spp. can be used as a negative control.



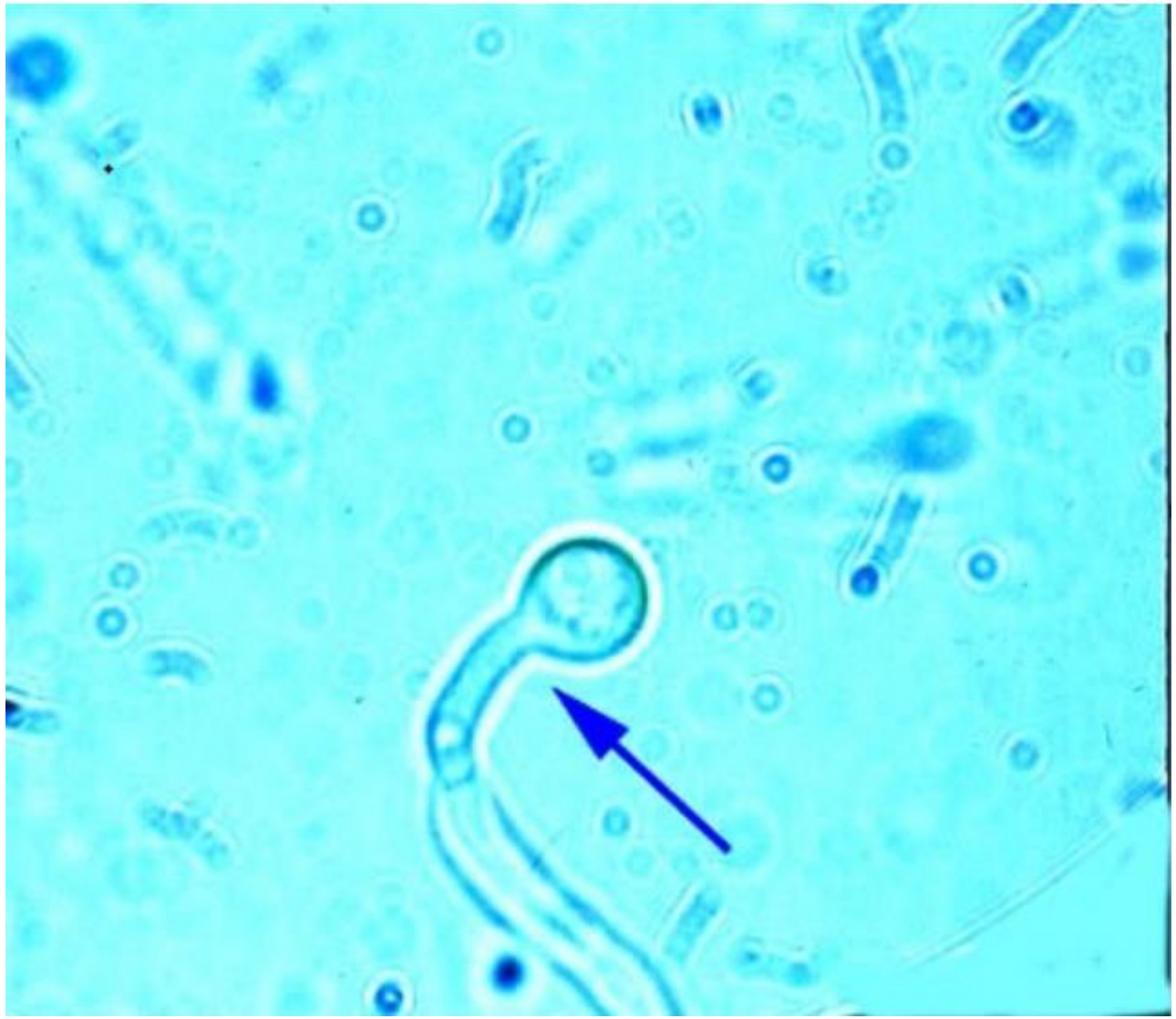
Laboratory Diagnosis of Fungi: Fungal Identification (Yeast)



a. Germ tube formation in *Candida albicans*. b. *Candida tropicalis* blastoconidial germination with constriction

Germ tube
or
pseudogerm
tube?

Germ tube
or
pseudogerm
tube?



Germ tube
or
pseudogerm
tube?

Laboratory Diagnosis of Fungi: Fungal Identification (Yeast)

Urease Test

Procedure

i. Rehydrate urea broth vial with 3 ml sterile saline or sterile deionized water.
    1. The color of the rehydrated media should be amber. If it is pink, do not use.
    2. Once reconstituted, rehydrated broth can be stored at 2-8°C and must be used on the day it is prepared.
ii. Number each isolate for test (1, 2, 3, etc.). For each patient or control to be tested, pipet 200µL of urea broth into a well on the microtiter plate.
iii. Using a sterile wooden applicator stick, heavily inoculate the urea broth with test isolate. Add the controls to the end of the run.
iv. Seal wells with clear scotch tape.
v. Incubate aerobically (O_2 incubator) at 35-37°C for 2-4 hours.
vi. Observe any color change from amber to pink/red.

-

+

Laboratory Diagnosis of Fungi: Fungal Identification (Yeast)

Procedure 7

Cornmeal Agar for Yeast Identification

Purpose

To aid in the identification of pathogenic yeasts by microscopic morphology

Principle

Isolated yeasts are inoculated onto the surface of an agar plate. The inoculum is covered with a coverslip and incubated for 3 days. The yeasts on the plate are examined microscopically. Microscopic morphology is an important characteristic in the identification of yeasts.

Specimen

Isolated cultures of test yeasts

Medium

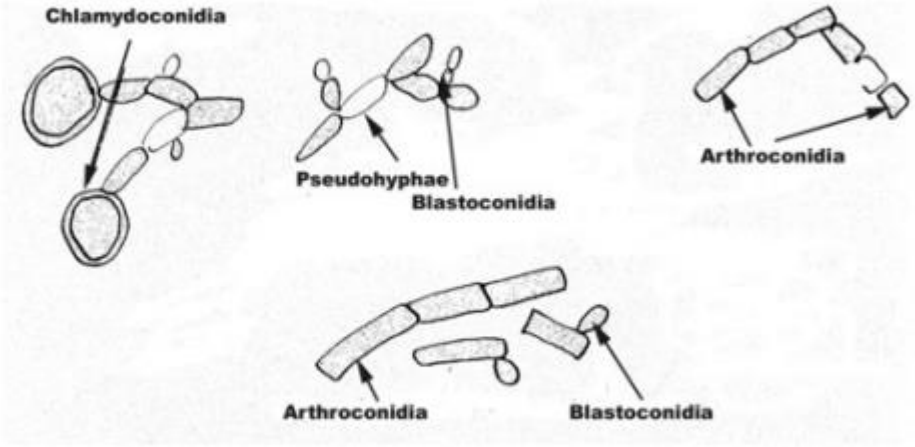
Cornmeal-Tween 80 plate; one third or one fourth of a plate can be used for each yeast.

Procedure

1. Pick up a small amount of a yeast colony with an inoculating loop.
2. Make one streak of the yeast in the center of the agar surface. Do not cut the agar.
3. Make three or four streaks across the original streak to dilute the inoculum, being careful not to cut into the agar.
4. Cover the inoculum with a sterile coverslip and incubate at room temperature in the dark for 3 days.
5. Remove the lid from the Petri dish and examine the yeast with the low-power (×10) and high-power (×40) objective lenses for the presence of hyphae, pseudohyphae, arthroconidia, chlamydoconidia, and blastoconidia.

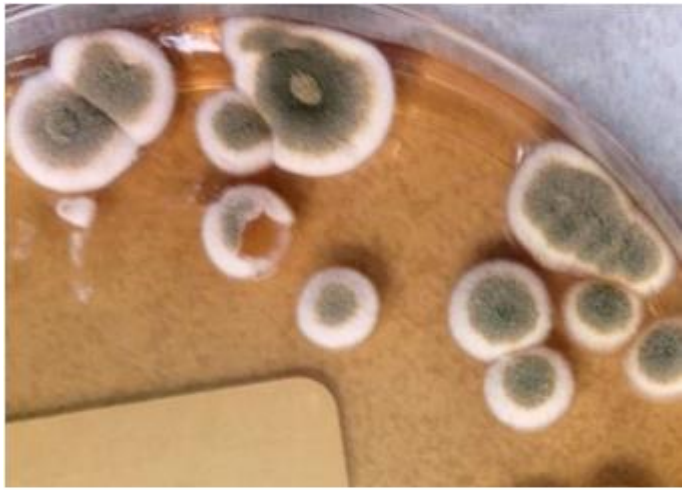
Controls

Candida albicans is used to demonstrate hyphae and chlamydospores.
Cryptococcus spp. are used to demonstrate blastoconidia.



Laboratory Diagnosis of Fungi: Fungal Identification (Mold)

- Macroscopic examination cultures
  - Growth rate
  - Forward color: color of aerial mycelium [A]
  - Reverse color: color(**dark**= dark brown/black or **light**=any color but brown/black) of vegetative mycelium [B]



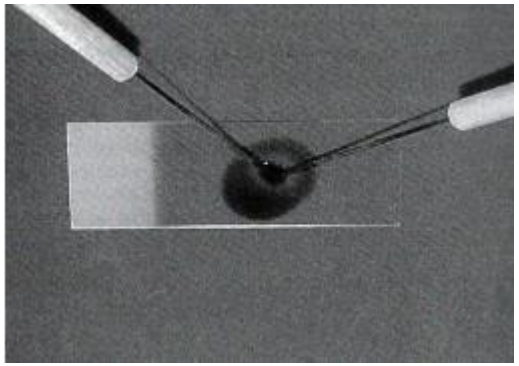
[A]

[B]

Laboratory Diagnosis of Fungi: Fungal Identification (Mold)

- Microscopic examination w/ lactophenol cotton blue
  - Aerial hyphae is obtained by either…
    - Cellophane tape mount [A]
    - Tease mount [B]
    - Slide culture [C]
  - Aerial hyphae is microscopically described as..
    1. Hyaline vs. Phaeoid
    2. Septate vs. Sparsely Septate
    3. Reproductive structures

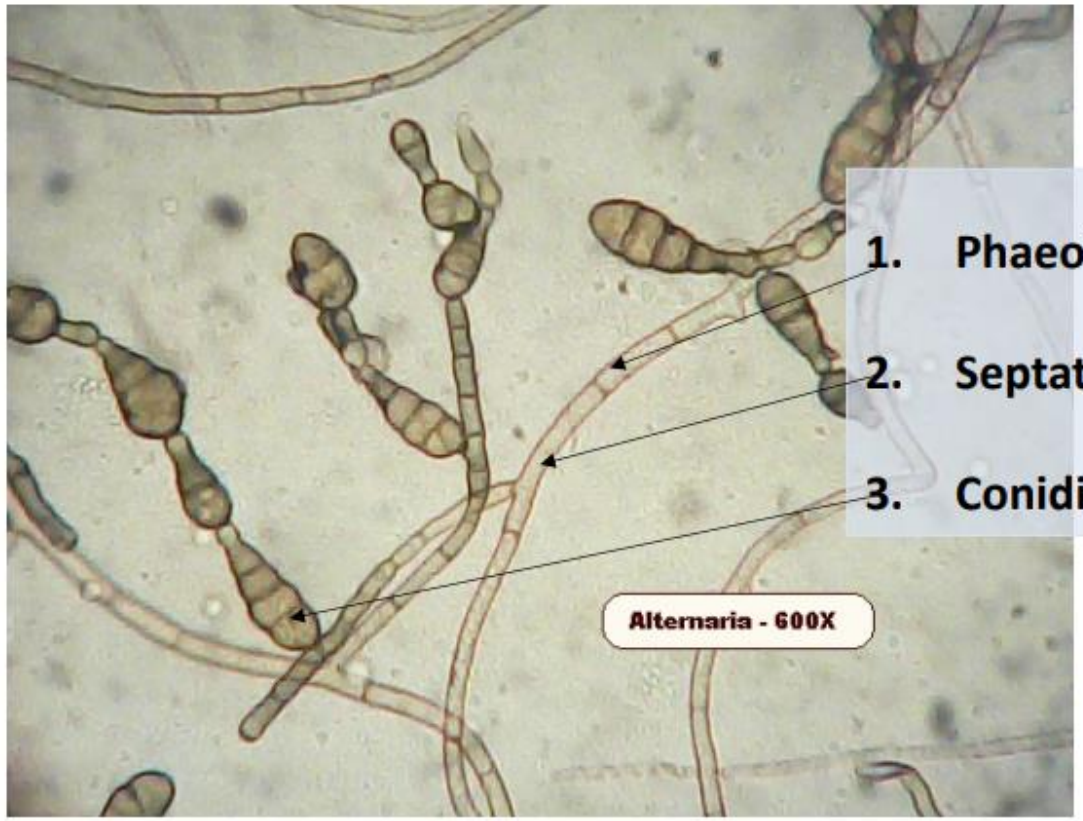
[A]



[B]

[C]

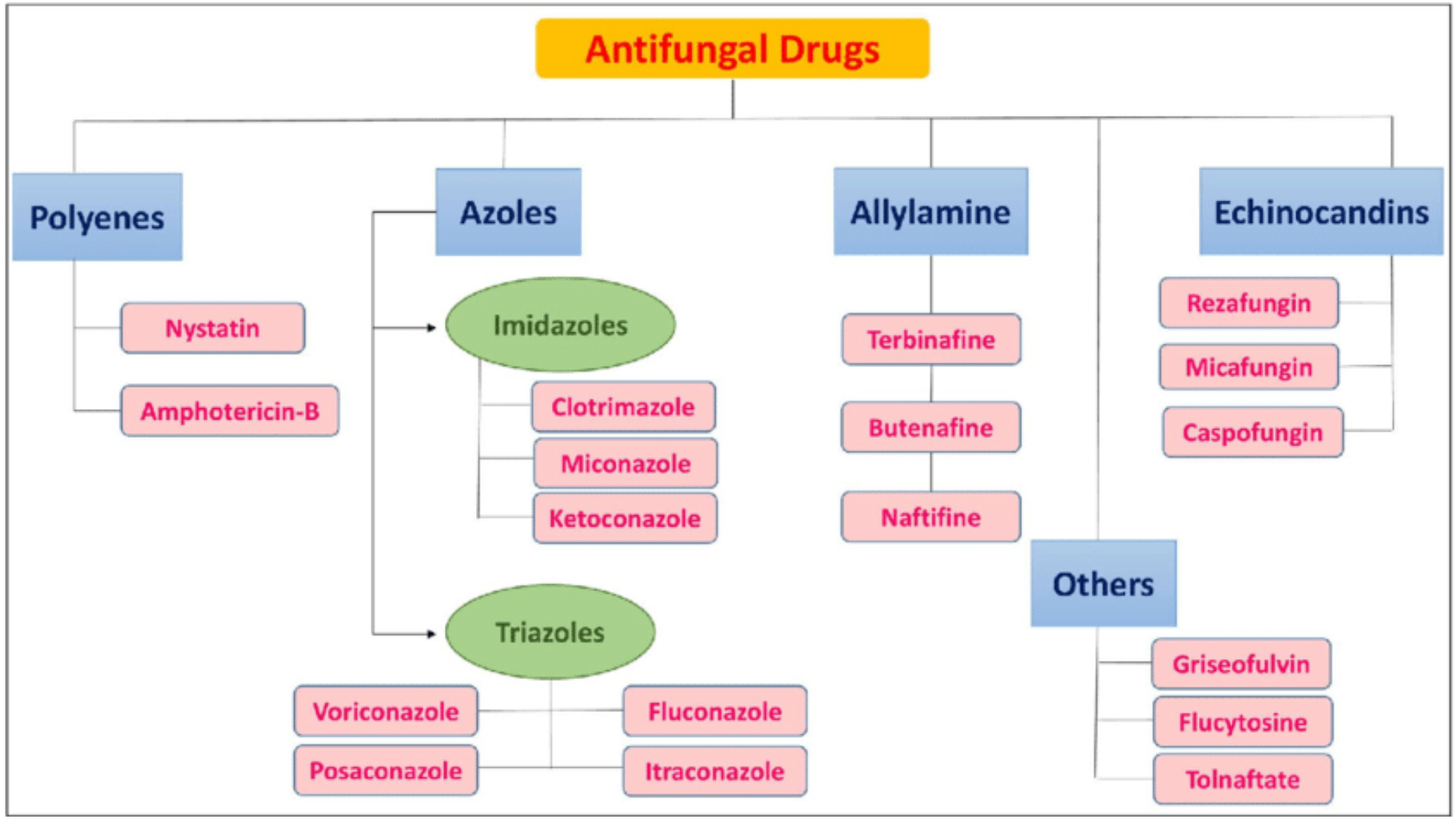
Laboratory Diagnosis of Fungi: Fungal Identification (Mold)



Lactophenol cotton blue

Cells that produce conidia are called conidiogenous cells. The conidiogenous cell can be a conidiophore. At other times, the conidophore only supports the conidiogenous cells. Other conidiogenous cells include phialides (vaselike) and annellides (ringed).

Laboratory Diagnosis of Fungi: Antifungals



Citations

- Mahon, C. R., & Lehman, D. C. (2023). *Textbook of Diagnostic Microbiology* (7th ed., pp. 639-707). Elsevier.