

Processing of specimens

Processing of clinical specimens

- 1. Macroscopic examination**
- 2. Microscopic examination**
- 3. Culture**
- 4. Biochemical reactions**
- 5. Serology**

Types of Specimens in Mycology lab

- Skin, nail, hair.
- Blood
- CSF
- Biopsy tissue
- Sputum
- Urine
- Body fluids
- Bone marrow
- Bronchial brush
- Abscesses

Important Notes

- Body fluid specimen (urine, CSF, etc...) must be centrifuge first.
- For mucoid specimens (like sputum) add N-Acetyl L- Cysteine (NALC).
- Tissue specimen must be grind with sterile saline.

Direct Mount Stain

- 10% KOH
- Giemsa stain
- H&E stain
- Gram stain
- Silver stain(GMSS)
- Peroidic Acid Shiff
(PAS)
- ZN stain
- Negative stain

Yeast Identification

The Medically Important Yeasts

- *Candida sp.* >> Candidiasis
- *C. albicans* >> Candidiasis
- *Trichosporon beigelii* >> Trichosporonosis, Candidiasis
- *Geotrichum condidium* >> Geotrichosis
- *Saccharomyces servacies* >> Candidiasis
- *Rhodotorula sp.* >> Candidiasis
- *Cryptococcus neoformance* >> Cryptococcosis

Candida albicans

- Specimen>> depend on site of infection (swabs, urine, blood, CSF, respiratory specimen).
- DM with 10%KOH>> budding yeast cells & pseudohyphae.
- Culture on SDA, CMA>> rapid growth, creamy moist colonies.

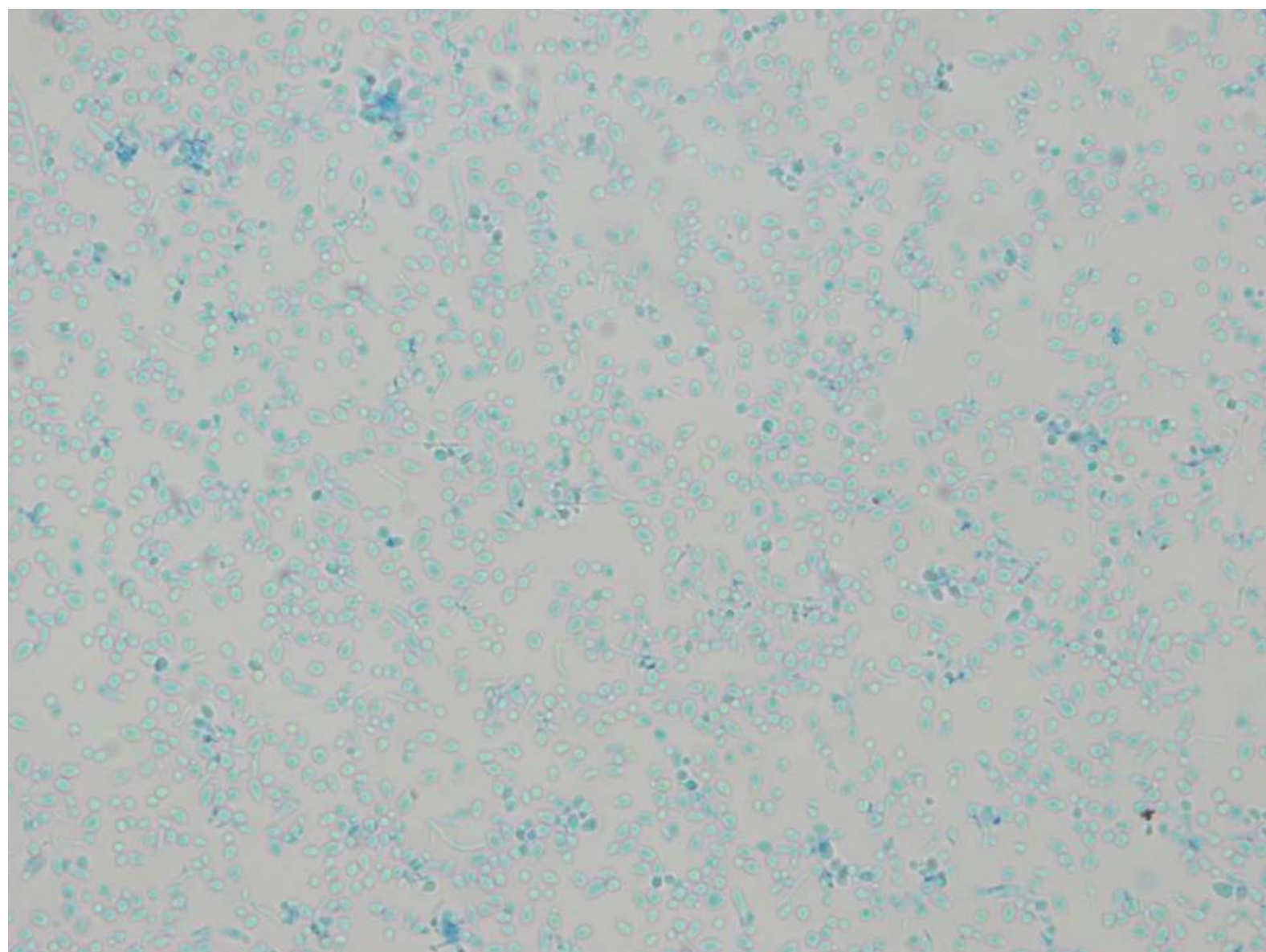
Candida albicans

- Microscope ex. With LPCB:

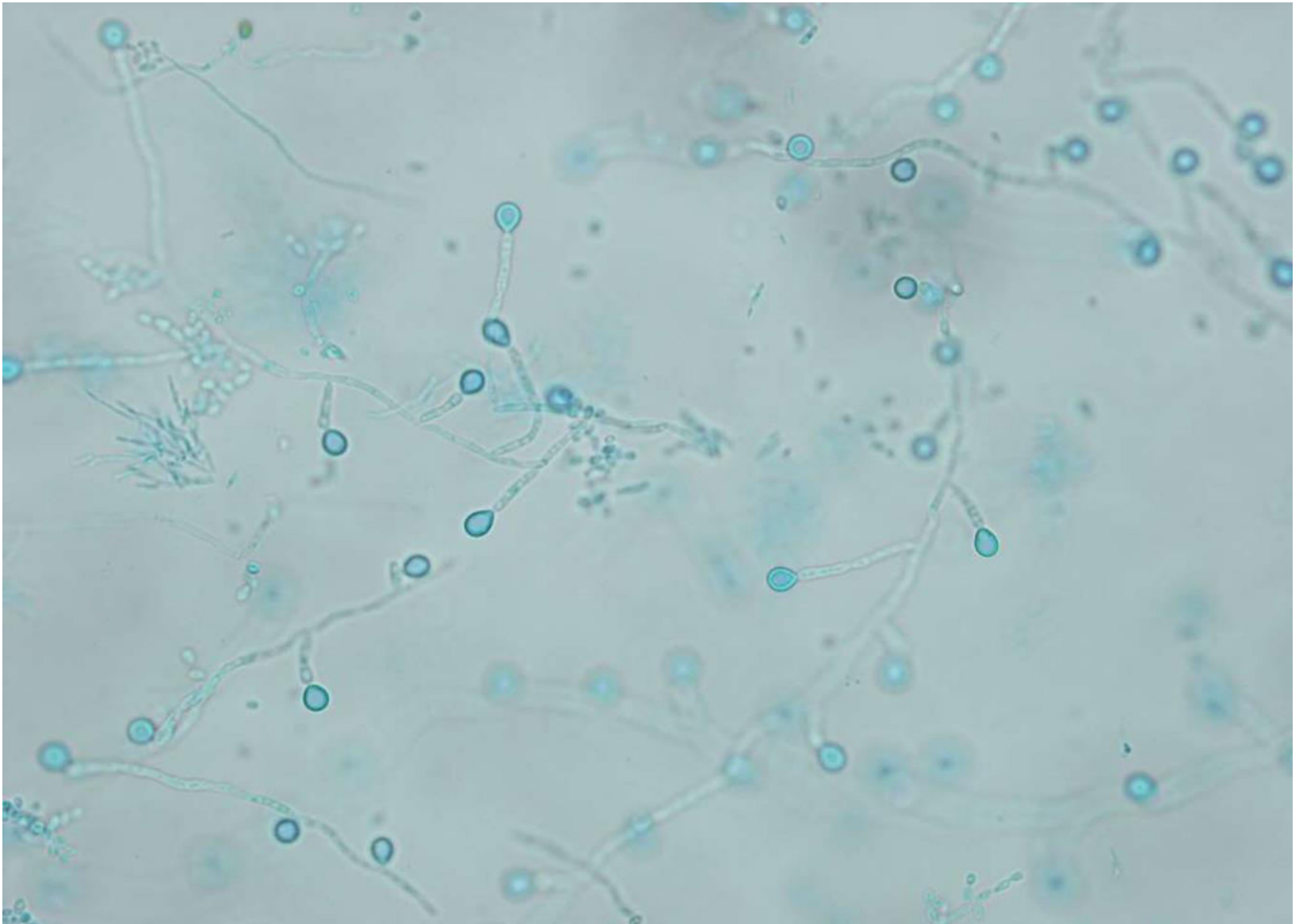
LPCB from SDA>> yeast cells

LPCB from CMA>> yeast cells, **chlamydospore**

NOTE: On CMA, all yeast will give pseudohyphae and yeast cells, but *C.albicans* is the only yeast that give Chlamydospore



Chlamydospore of *C. albicans*



Candida albicans

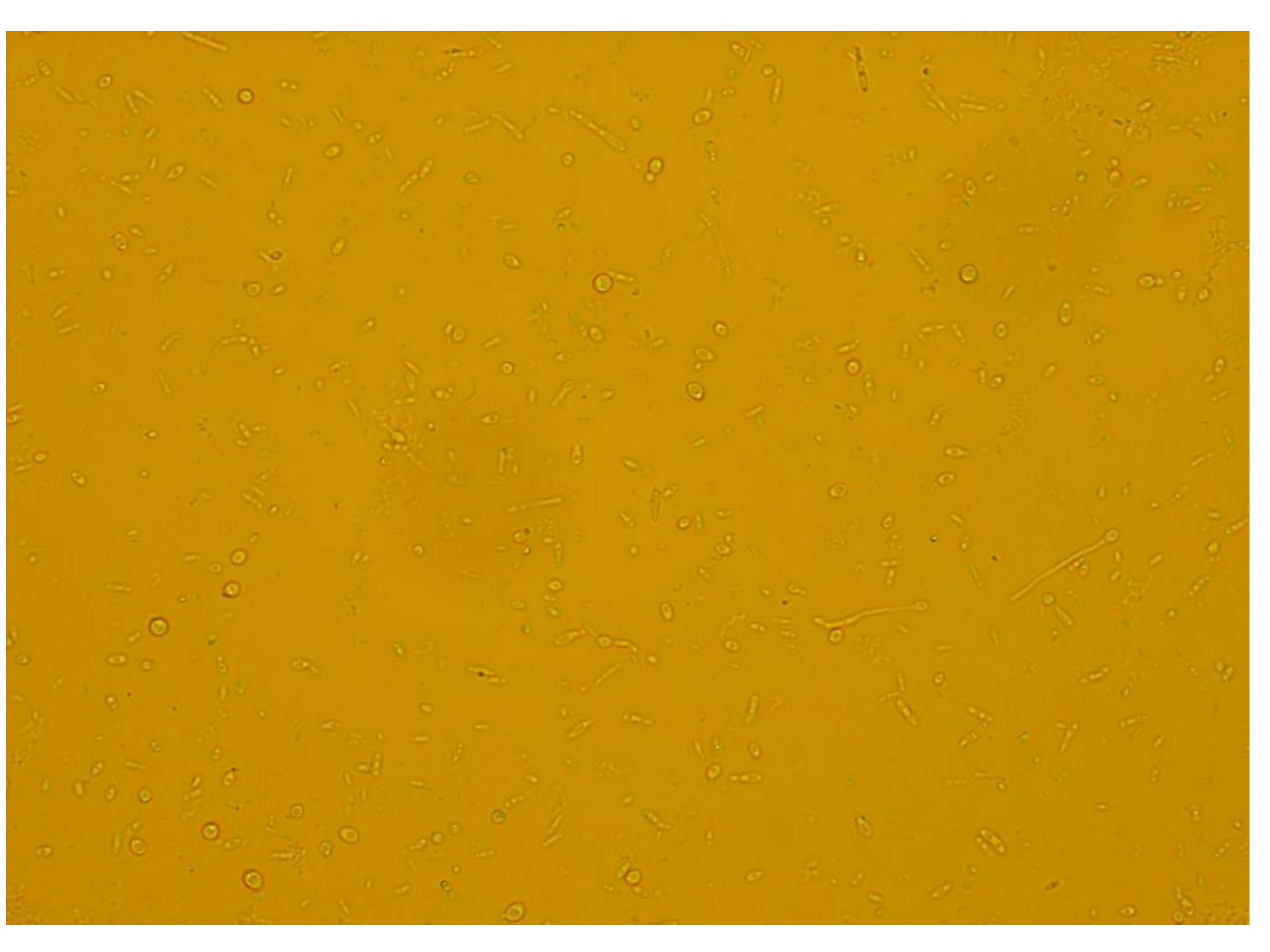
- **Germ Tube Test (GTT):**

Procedure>> add yeast colony to 0.5 or 1 ml serum and incubate it at 37°C for 1:30 - 3 hrs.

Result>>

If there is germination >> GTT positive

If there is no germination>> GTT negative

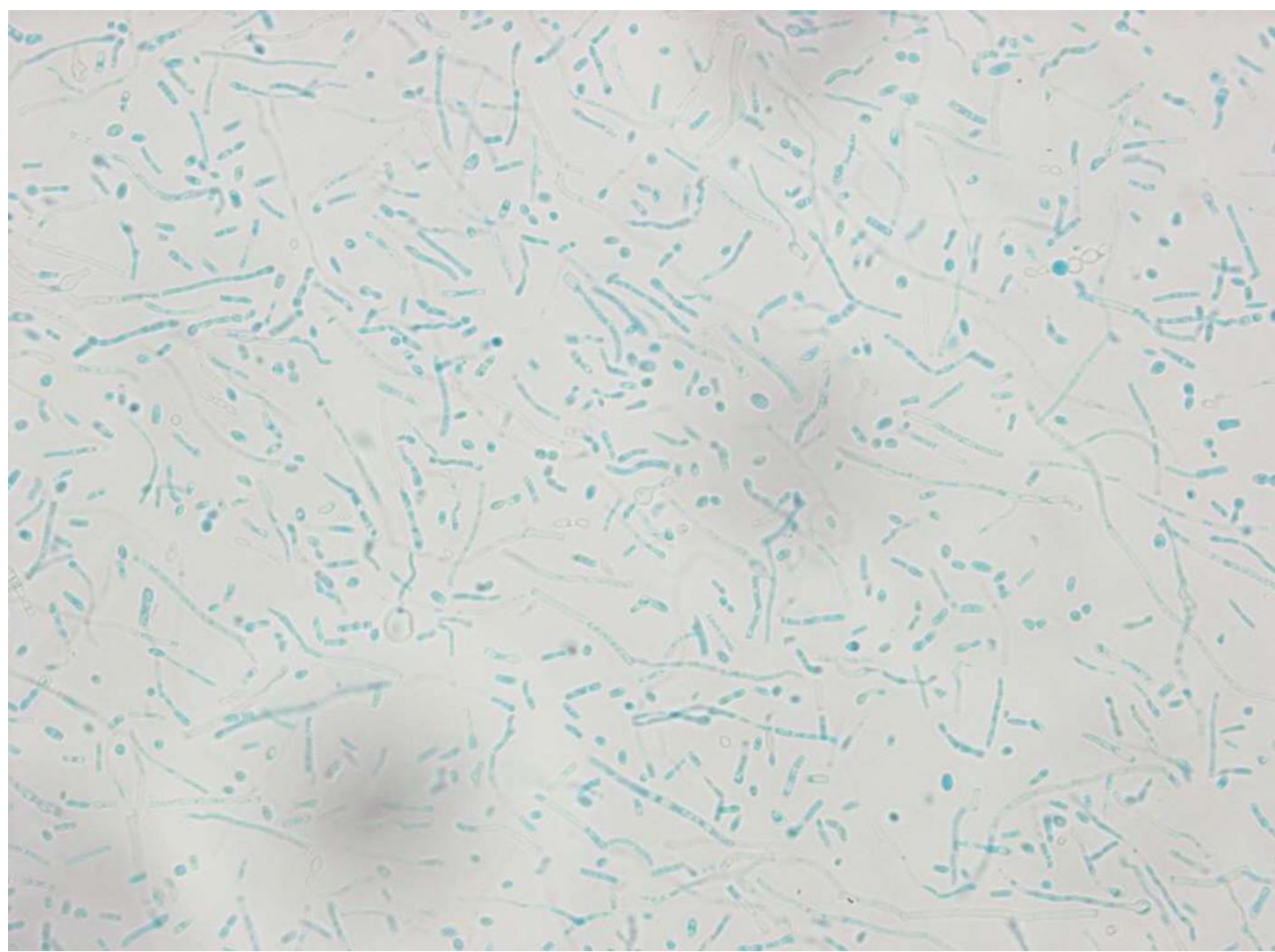


Candida albicans

- If:
 - 1- GTT is positive, and
 - 2- Chlamydospore production is positive>> The yeast is *C. albicans*
- If not>> other yeasts
- To identify any yeast in the clinical lab use **API 20C test**

Trichosporon beigeli & *Geotricum candidum*

- DM with 10%KOH>> budding yeast cells & pseudohyphae.
- Culture on SDA, CMA>> rapid growth, creamy moist colonies.
- Microscope ex. With LPCB:
LPCB from SDA>> yeast cells, **arthrospores** and true hyphae
LPCB from CMA>> no chlamydospore
- Urease positive
- GTT negative
- Make API 20C test



API 20c

- API 20c >> Analytical profile index 20 different carbohydrate.
- Use for yeast identification.
- Principle>> Carbohydrate assimilation.
- Result:
 - hazy or turbid tube>>> there is growth (+)
 - Clear tube >> no growth (-)

API 20c

Procedure:

- Make suspension.
- By using sterile pipette place (inoculate) 2-3 drops in the API 20c tubules.
- Place the strip in an incubation tray contain water to provide humid atmosphere and the lid are placed.
- Incubate for 24-48 hrs

Urease Test

- This test is used to detect the enzyme urease, which breaks down urea into ammonia
- Urea $\xrightarrow{\text{urease}}$ $\text{NH}_3 + \text{CO}_2$
- if the yeasts grow in the media have urease enzyme, they will destroy the urea in the media, so the media color will change from acidic (yellow) to alkaline (pink).
- Indicator: phenol red

Urease Test



VITEK System

- Fully automated system dedicated to the identification and susceptibility testing of microorganisms. e,g: identify yeast in 15 h due to a sensitive fluorescence-based technology.
- The ID-YST card consists of 47 biochemical reactions.
- VITEK system is a rapid and accurate method for the identification of medically important yeasts

Cryptococcus neoformance

- Specimen>> CSF, body fluid and tissue
- DM with India ink>> encapsulated budding yeast cells
- Culture on SDA, BHI agar>> grow fast 1-2 days
- Serology lab

