Advanced Medical Mycology

# Laboratory diagnosis of fungal infections

MSc level

Prof. Dr. Inaam Alrubayae

❖ **Specimen:**

- ✓ ***According to the site of infection.***
- ✓ ***For example, skin scales, nails, hair clippings for dermatophyte examination.***

❖ **Microscopic examination of these specimens using KOH 10%:**

- ✓ ***KOH dissolves keratin but does not affect fungi. Branching hyphae are detected among epithelial cells.***
- ✓ ***Fungal stains such as lactophenol cotton blue could be used.***

# ❖Culture:

## Medium

- Sabouraud' dextrose agar.
- 4% glucose, 1% peptone, 2% agar and PH 5.5.
- Chloramphenicol + Cycloheximide

## Technique

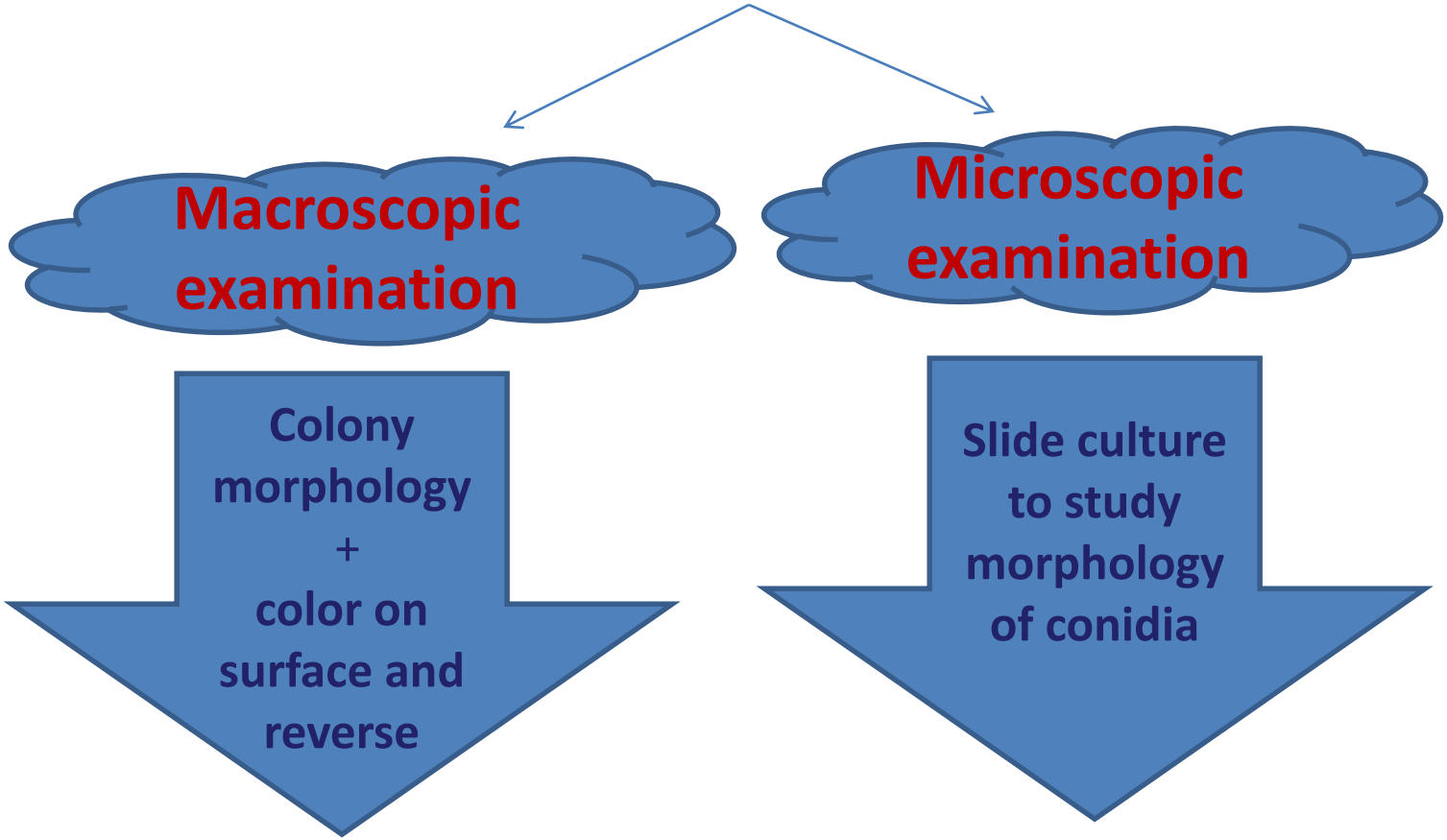
- Two cultures are incubated separately at:
- One at the room temperature (25 degree).
- The other at body temperature (37 degree).

## Duration

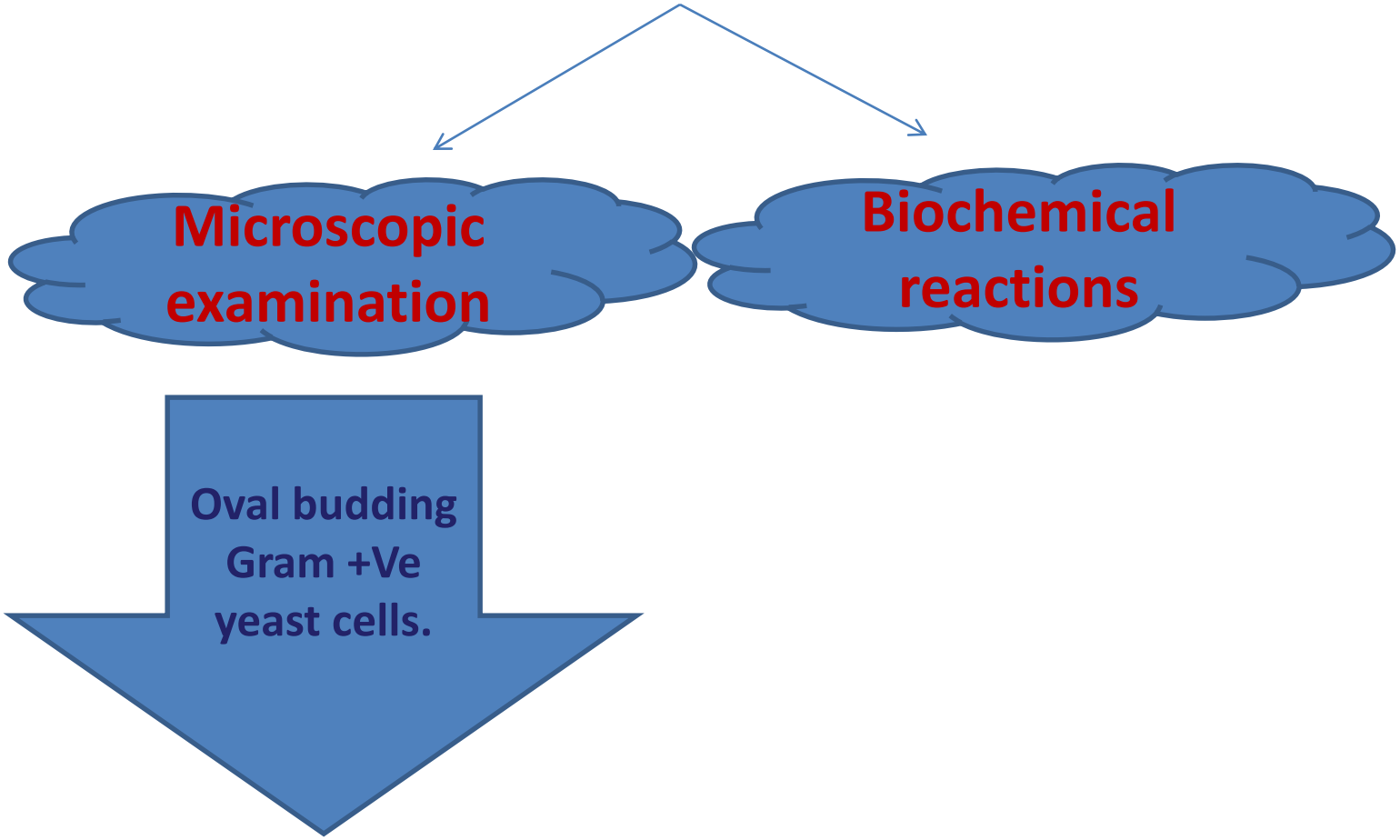
- Most yeasts grow at 37 degree.
- Molds grow at room temperature for up to 4 weeks.

❖ **Identification of the isolated fungi on culture is done by:**

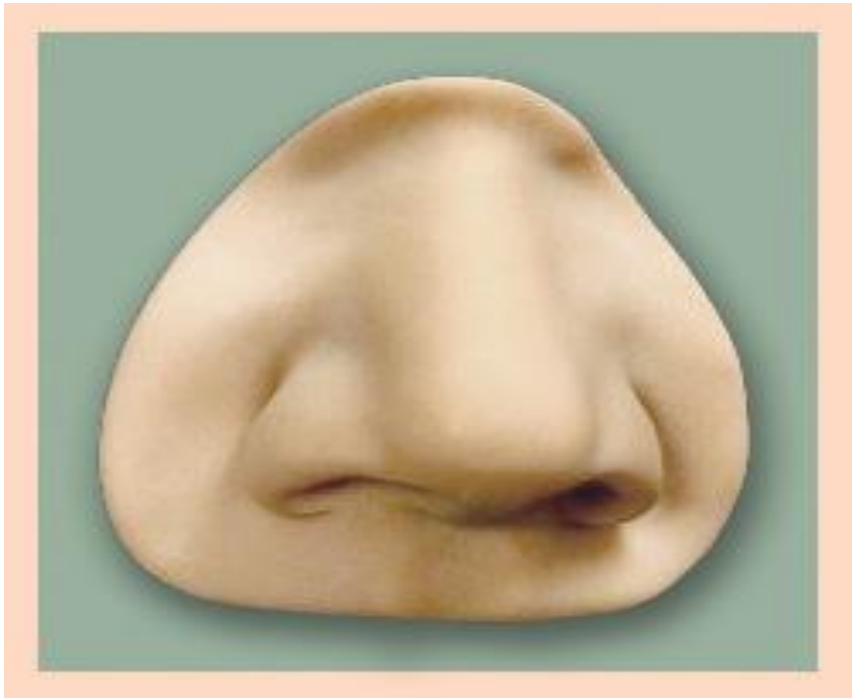
**For molds:** ***identification is done by :*** ✓

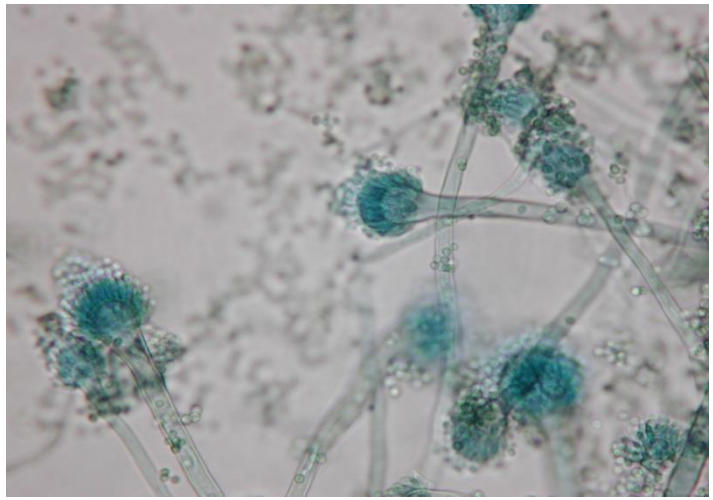


**For yeasts:** ***identification is done by :✓***



# Fungi are in the air



# Types of specimens

- *Skin ,hair ,& nails
- *Sputum , exudates, urine ,blood, C.S.F ,tissue biopsies.

# Common fungal primary recovery culture media

- **SDA (Sabouraud dextrose agar )**
- **BHA (Brain-heart infusion agar)**
- **PDA (Potato Dextrose agar)**
- These media used for primary recovery of saprophytic & dimorphic fungi.

**Dermatophyte test medium**
***Mycosel**
These media used for identification of dermatophytes
.

# Differential media

**Corn meal agar** with Tween 80 &Trypan blue for identification of *Candida albicans*

*__**Potato dextrose agar**__ for demonstration of pigment produced by *Trychophyton rubrum*

*__**Niger seed agar**__ for identification of *Cryptococcous neoformans.*

*__**Urease medium**__ for detection of *Cryptococcus* spp, and *Trichosporon* spp.

## Direct examination

## Potassium hydroxide (10 % to 20 %)

The most rapid method for direct examination of infected material . Gently heating the slide will increase the rate of clearing & the fungus should be more readily observed.

# Cultivation of fungi

*__Sabouraud glucose agar__ with antibiotics (chloramphenicol) and cycloheximide will support most of the pathogenic fungi.

*Place a small amount of hyphae or spores or both on the center of the agar medium in a petri dish using a stiff 22 –gauge nichrome wire in an inoculating needle holder

# Temperature and incubation period

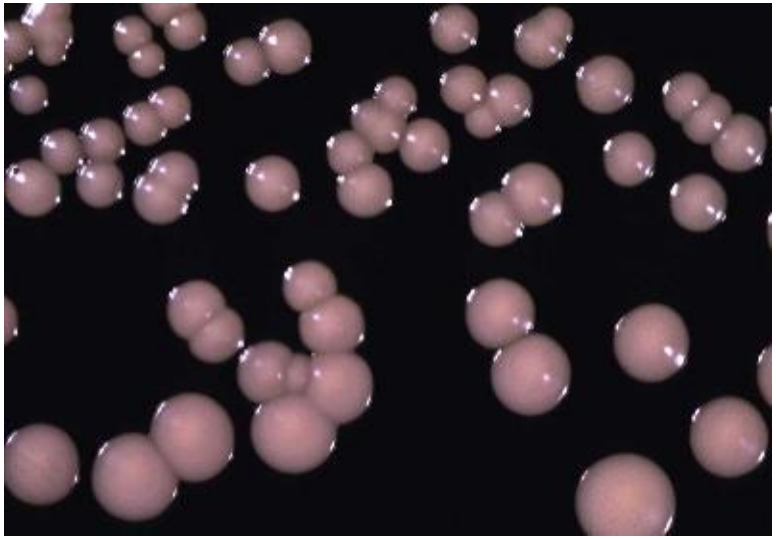
- **Temperature requirement**
  - Majority of fungi – 37°C
  - Superficial mycosis – 30°C
  - Dimorphic fungi – 25°C & 37°C

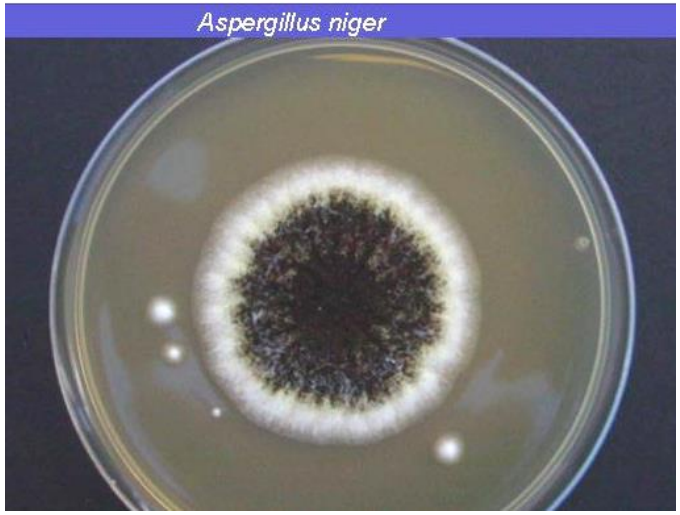
- **Incubation time**
  - At least 4 weeks
  - Usually positive cultures are obtained in 7-10 days
  - Candida & Aspergillus - 24 to 72 hrs

Observe the development of the colony over a period of several weeks , noting rate of growth , texture , pigmentation on the surface & reverse side , as well as folds or ridges on the surface.

# Identification of fungal cultures

- Colony morphology – colour, texture, pigment production





# Specimens should be cultured on agar slants:

- Safe
- Require less space
- More resistant to drying during prolonged incubation
- Blood cultures should be inoculated in to biphasic blood culture bottles

# Direct mounts

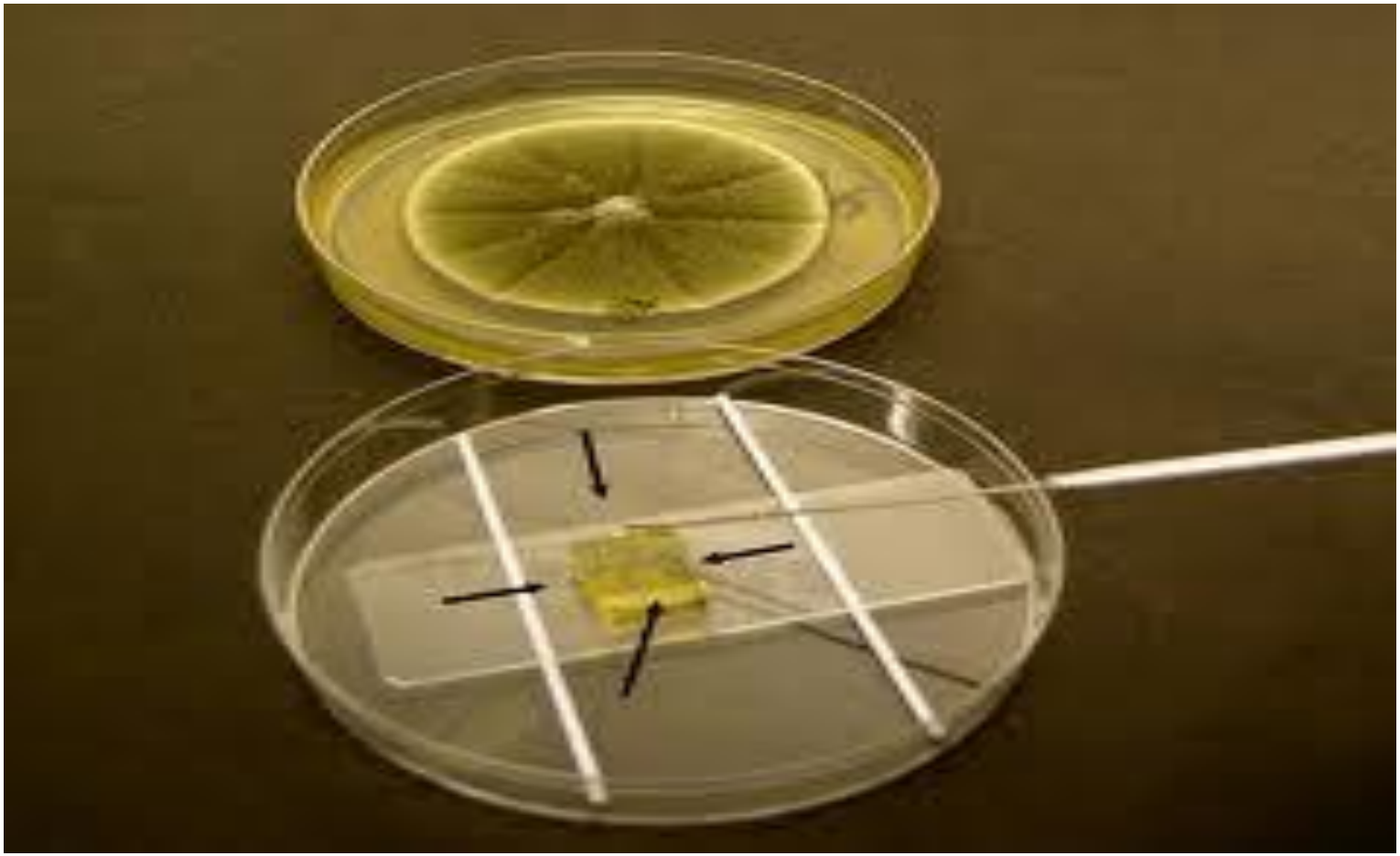
- *Using sterile technique ,remove a small portion of the colony from near the center.
- *Place material in a drop of **lactophenol** with or without cotton blue mounting medium .
- The cotton blue dye is desirable for light-colored or colorless fungi.

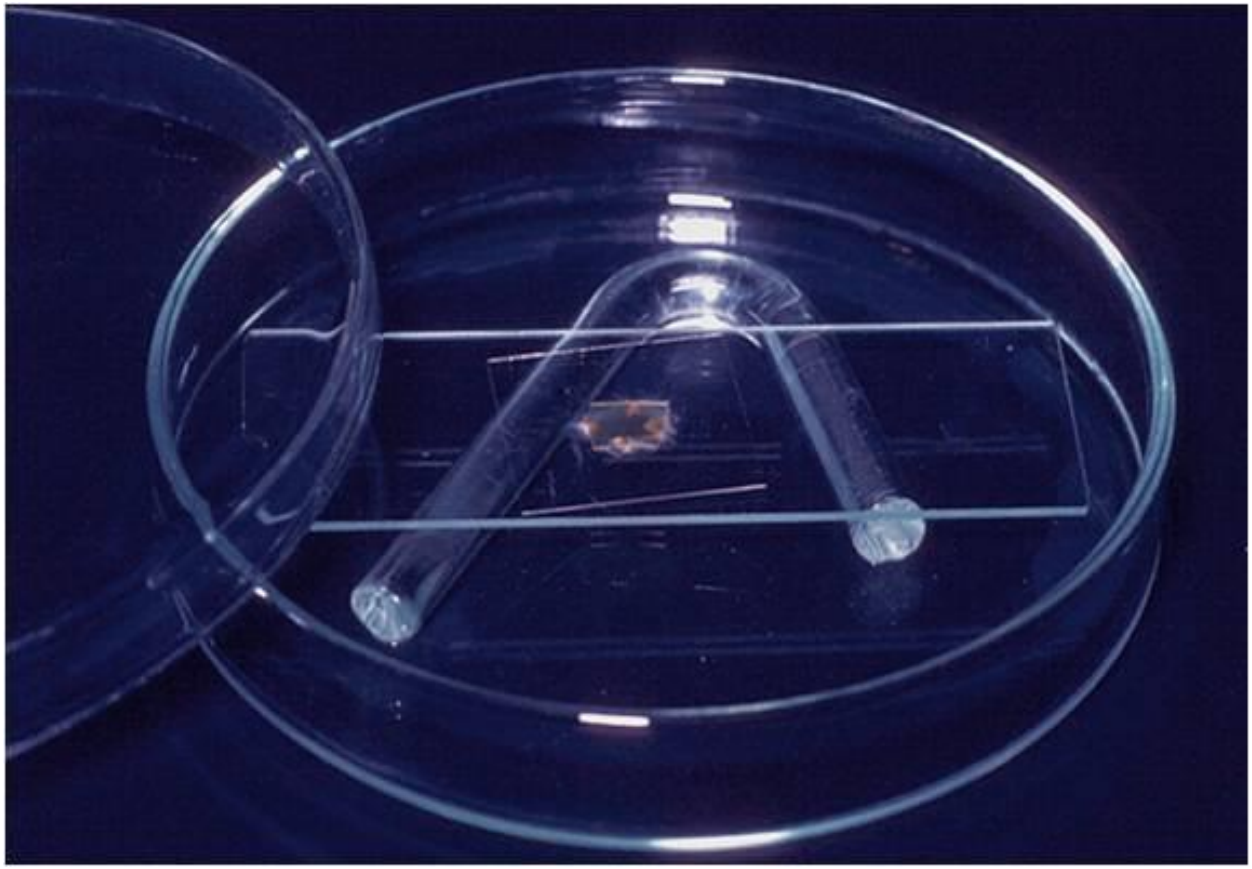
- If a material is dense ,tease a part with two sterile needles & add a cover slip.
- *Examine under low magnification of the microscope , then higher magnification for smaller organisms.

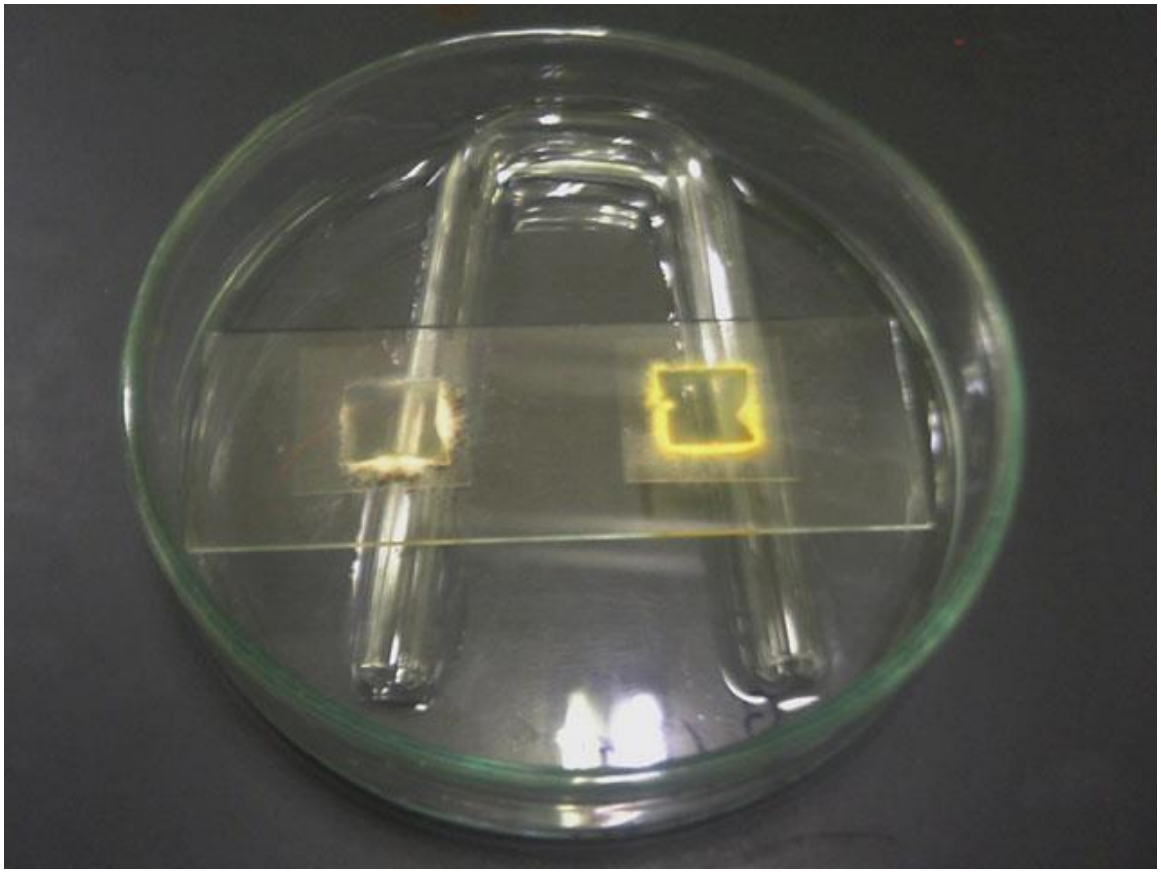
# Slide culture

- **In** order to avoid disturbing the arrangement of the fungus structures ,slide culture techniques can be utilized& permanently stained mounts made from the slide and cover glass

# Slide culture technique







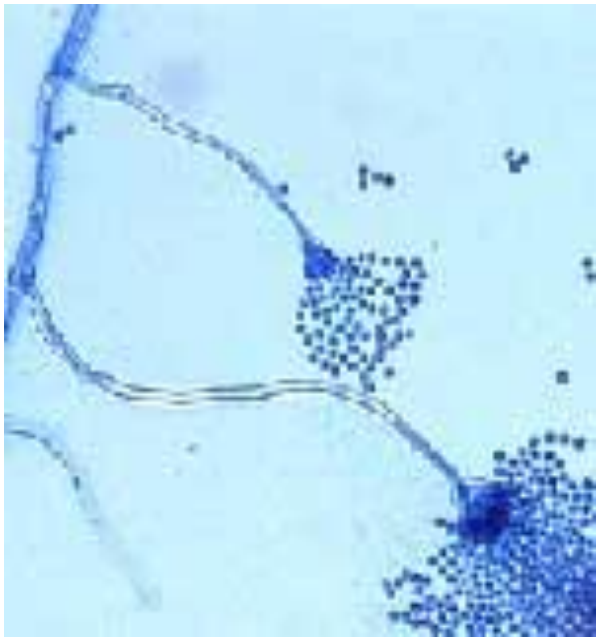
# Mycology

**Saprophytes:**

**Hyaline Members -**

***Aspergillus spp*.**

- **Growth rate varies, colors vary, surface velvety to cottony.**
- **Mycelium - septate and hyaline with unbranched conidiophores**
- ***A. fumigatus* is considered a potential pathogen, especially if from a pulmonary source.**

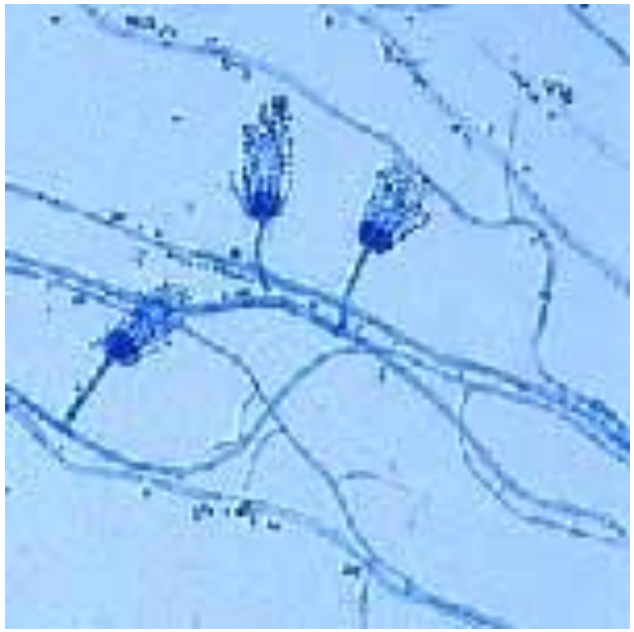


# *Penicillium* spp.

**Saprophytes:**

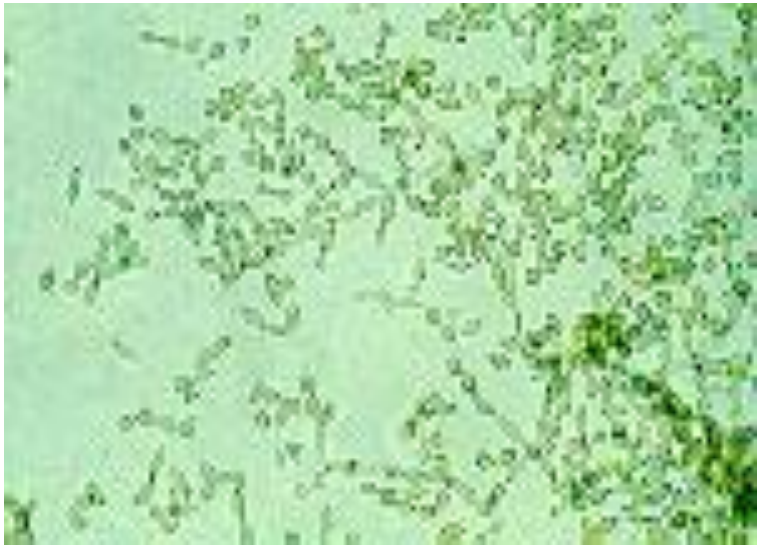
**Hyaline Members -**

- **Commonly rapid growing; white to bluish-green.**
- **Conidiophores characteristically form a brush-shaped structure.**
- **Sterigmata are flask shaped.**



# *Cladosporium* spp.

- **Saprophytes:**
- **Dematiaceous Members -**
- **Rapid growth. Green colonies, reverse is black.**
- **Septate, dematiaceous mycelium.**
- **Conidia are borne in chains.**



# Zygomycetes

**Saprophytes:**

**Aseptate Members -**

- **All are susceptible to cycloheximide.**
- **Rapid growers.**
- **Some have root-like structures termed rhizoids.**
- **Spore bearing structures are called sporangiophores.**

# *Rhizopus* spp.

- **Saprophytes:**
- **Aseptate Members -**
- **Rhizoids are present.**
- **Sporangiophores**

# Description of Yeasts

- Unicellular, reproduce by budding
- Bud= Blastoconidia [Konis(G)= dust]
- Pseudohyphae Vs True hyphae
- A few produce ascospore [Ascus(G)= bag]
- Chlamydospore [Chlamyds(G)= mantle]

# Description of Yeasts

- Most common fungi isolated
- Ubiquitous in environment
- Normal inhabitants of body
- Clinical Significance
  - Repeated recovery
  - Direct microscopic demonstration in the infected tissue
- Opportunistic pathogens
- Candidiasis is the most common fungal infection

# Description of Yeasts

- Colonies
  - Smooth, glabrous [Glab (L)= Bald]
  - Moist or dry
  - White or creamy
- Microscopic morphology
  - Cornmeal-Tween 80 agar by using Dalmau method

# Identification of Yeasts

- Depends primarily on the body site
  - Patient population
  - Cost issues

- An optimum scenario
  - Lower respiratory tract > *C. neoformans*
  - Throat culture > *C. albicans*
  - Genital culture > *C. albicans, C. glabrata*
  - Blood and other body site > Complete identification

- If complete identification is not done hold the isolate for a week

# Identification of Yeasts

- If colony is mucoid (*C. neoformans*)
  - Wet preperation
  - India Ink preperation
  - Urease slant

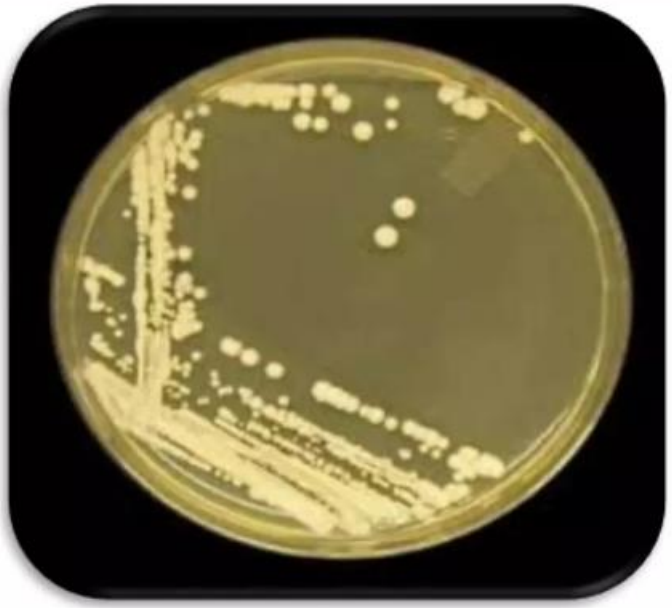
  - Cornmeal-Tween 80 or other (Glucose free)
  - Caffeic acid disk test
  - Confirm the first isolate with Commercial Yeast ID kits

# Identification of Yeasts

- If colony is non mucoid
  - Germ tube test
  - +ve Germ tube test
    - *C. albicans*
    - *C. dublinensis*
  - -ve Germ tube test
    - Small relatively slow growing colonies, small oval cells on wet preperation
      - Perform RAT for *C. glabrata*
  - If the isolate is not C. albican or C. glabrata
    - API 20C Aux
    - ID 32 C
    - Rapid yeast plus
    - Yeast identification panel

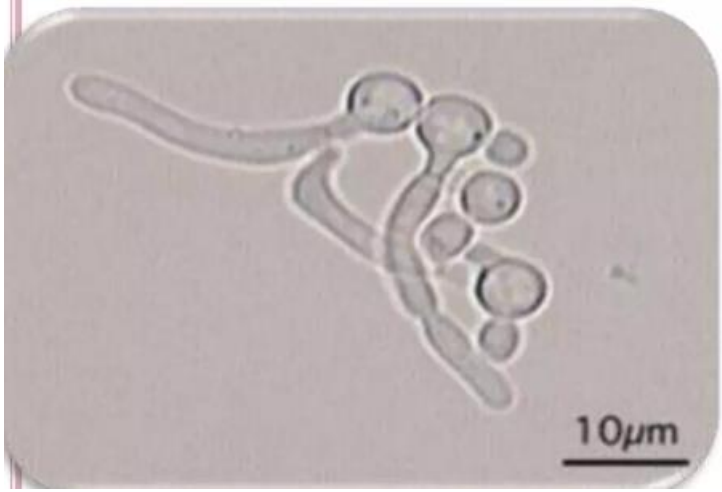
# *Candida albicans*

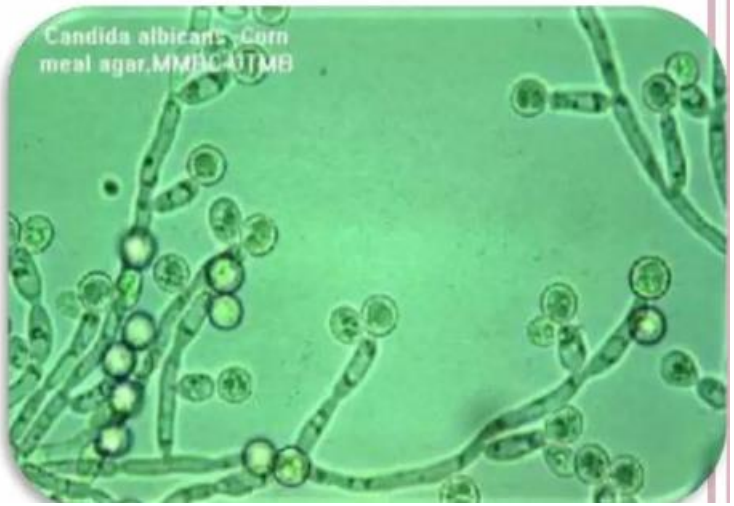
- Growth
  - 3 days
- Colonies
  - White or creamy
  - Pasty
  - Feet at the border



# *Candida albicans*

- Microscopy
  - Routine primary media
    - Round – oval yeast cells
  - Cornmeal – Tween 80 agar (25 $^{0}$ C)
    - Pseudo hyphae + some hyphae
    - Clusters of blastoconidia at septa
    - Single terminal Chlamydospores (inhibited at 37 $^{0}$C)
    - Germ tube test positive



# *C. dublinensis*

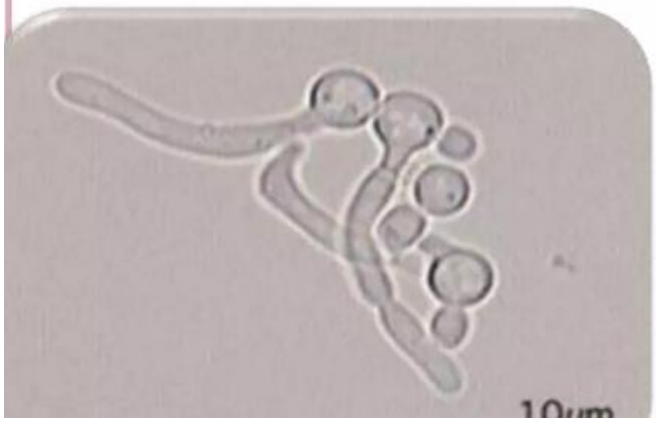
- Found throughout the world
- Recurrent erythematous oral candidiasis in HIV patients
- Dissseminated disease in non HIV immunocompromised patients
- Resistant to Fluconazole

# *C. dublinensis*

- Growth
  - 3 days
- Colonies
  - White or creamy
  - Pasty
  - Feet at the border

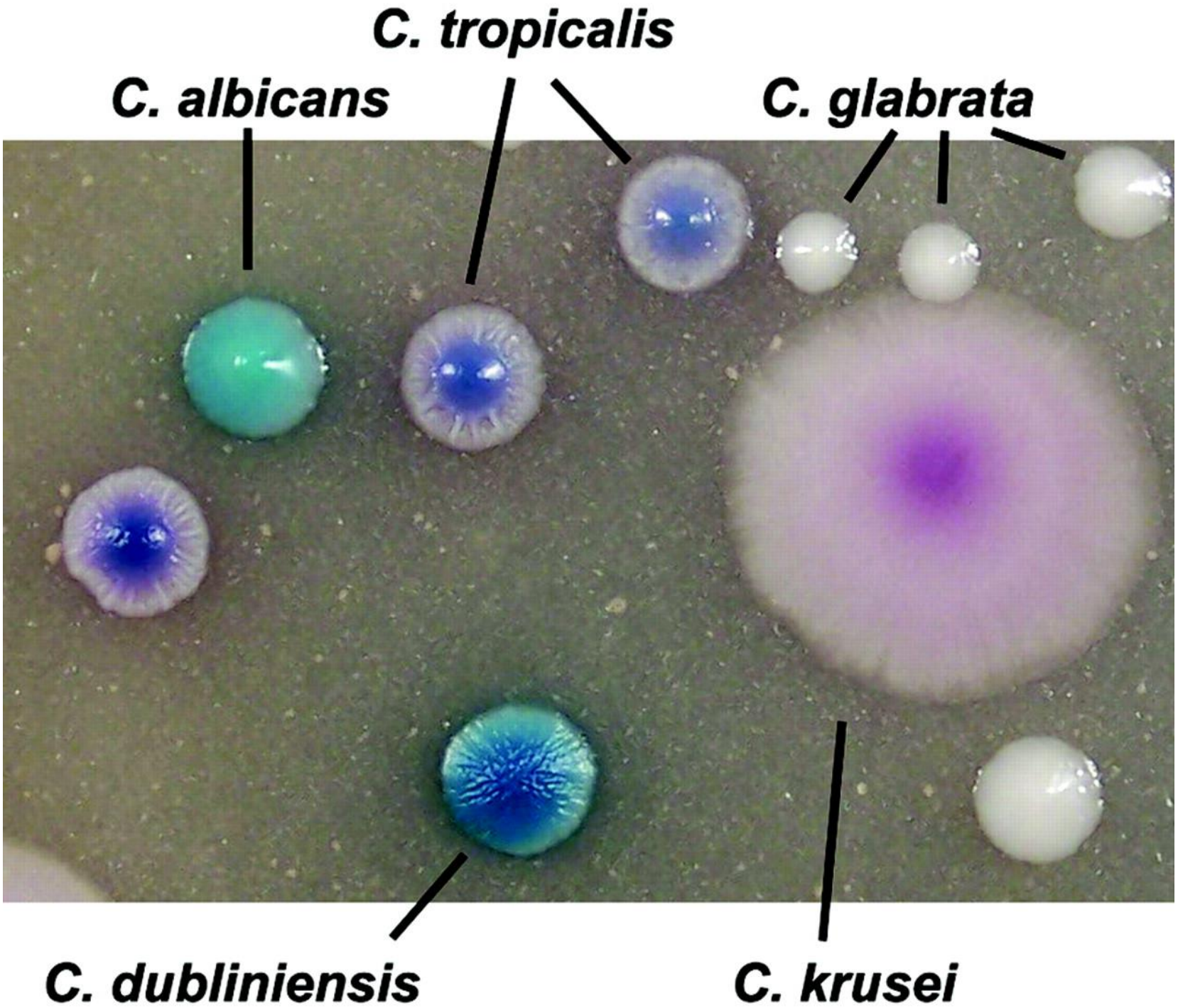
# *C. dublinensis*

- Microscopy
  - Routine primary media
    - Round – oval yeast cells
  - Cornmeal – Tween 80 agar (25 $^{0}$ C)
    - Pseudo hyphae + some hyphae
    - Clusters of blastoconidia at septa
    - Terminal  Chlamydospores in pairs or clusters(inhibited at 37 $^{0}$C)
    - Germ tube test positive




# *C. albicans* Vs *C. dublinensis*

| | Colonies on CHROMagar at 37 $^0$C | Growth at 42-45 $^0$C at 48 h | Chlamyd-ospores | Colonies on Staib agar | XYL | TRE |
|---|---|---|---|---|---|---|
| *C. albicans* | Light green or light bluish green | + | Usually single | Smooth and shiny | + | + |
| *C. dublinensis* | Usually dark green (distinct at 72 h) | _ or poor | Usually in pairs or small clusters | Rough with hyphal finge | _ | _ |


C. tropicalis
C. albicans
C. glabrata
C. dubliniensis
C. krusei


GOOD LUCK