



2. LF UK

Diagnostics in Medical Mycology

2LF UK

Practicals 2024

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Diagnostics of mycoses

Complex assessment of

- Patient's risk factors
- Clinical findings
- Laboratory findings
- Imaging
- Histopathology

How does microbiology contribute?

Depends on clinical material available

Microscopy (direct, clinical specimen)

- Rapid, urgent processing
- Primarily sterile specimen – proof of infection

Culture

Identification of the fungus, susceptibility testing – supportive in treatment choice

Biomarkers – antigen, DNA

- Patients with defined risk factors
- Detection may precede clinical signs – preemptive treatment

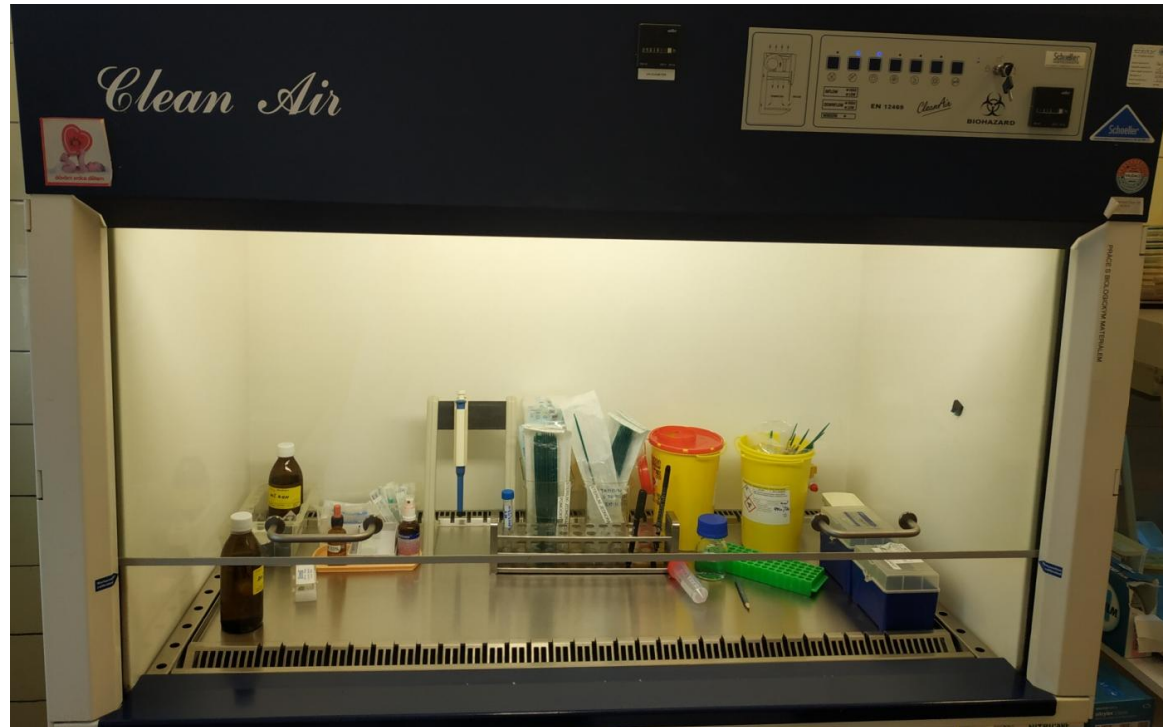
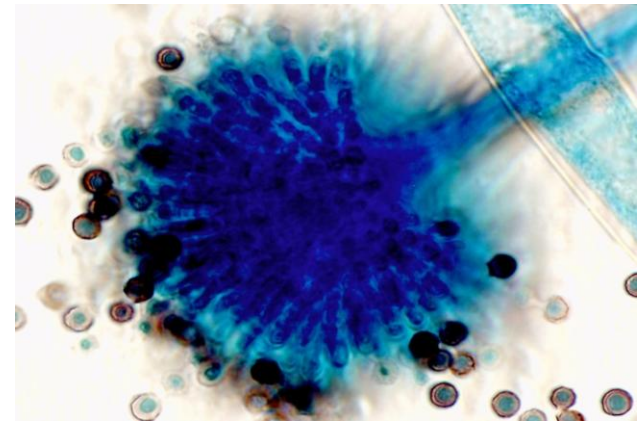
Safety measures – molds

BSL2 (endemic mycoses BSL3), safety box needed

- To protect lab personnel
- To avoid laboratory contamination

Do not open Petri dishes with molds unless in safety box

Never sniff mold cultures



Clinical specimens for medical microbiology examination

Specimen from the site of infection is optimal

Dermatophytes – superficial infection, material is always available

Decontaminate the site with 70% ethanol before specimen collection

Collect the specimen from the site of transition between healthy and affected tissue.

Transport on a sterile slide and in a sterile container or inoculated on the solid medium.

- Nails – scrape the nail and the material from underneath the nail with a sterile scalpel.
- Hair - pluck hair to collect hair roots.
- Skin lesions – scrape skin scales with a scalpel.

Superficial (skin, mucosal) candidiasis – swab from the site of infection

Invasive mycoses – liquid or solid material required

- **Biopsy from the site of infection is optimal** – collection not always possible
- Yeasts – blood culture, liquids, pus, urine...
- Molds – respiratory tract often affected - sputum, BALF, paranasal cavity liquid/wash
- *Pneumocystis jirovecii* – optimal BALF, 4th portion

Diagnostic methods in mycology

Microscopy

- Gram stain
- Wet mount/KOH
- Fluorescence – optical brightener, immunofluorescence
- Giemsa stain
- India ink - capsule

Culture

Sabouraud agar, Sabouraud broth, normal atmosphere, different temperatures and time

Biomarker detection

- Antigen – ELISA, Lateral flow assay
- DNA (PCR)

Candidiasis – diagnostic methods

Microscopy - Gram stain, wet mount

Culture

- **Sabouraud agar**, (Sabouraud broth, chocolate agar – selected specimens) ambient atmosphere, 5 days
- Special blood culture vials for blood culture, prolonged incubation

Identification

- **MALDI-TOF**
- Chromogenic agar
- Sugar fermentation/assimilation
- Micromorphology – rice agar
- **Hyphae growth in *Candida albicans*** – incubation in horse serum 2 – 4 hours, 37 °C

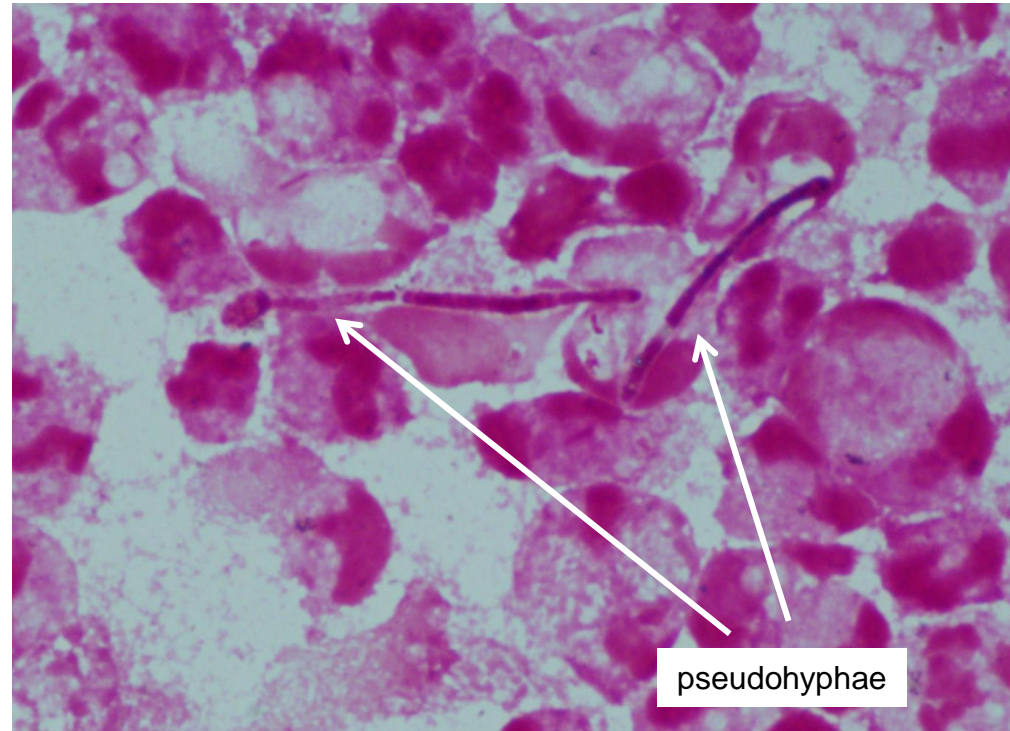
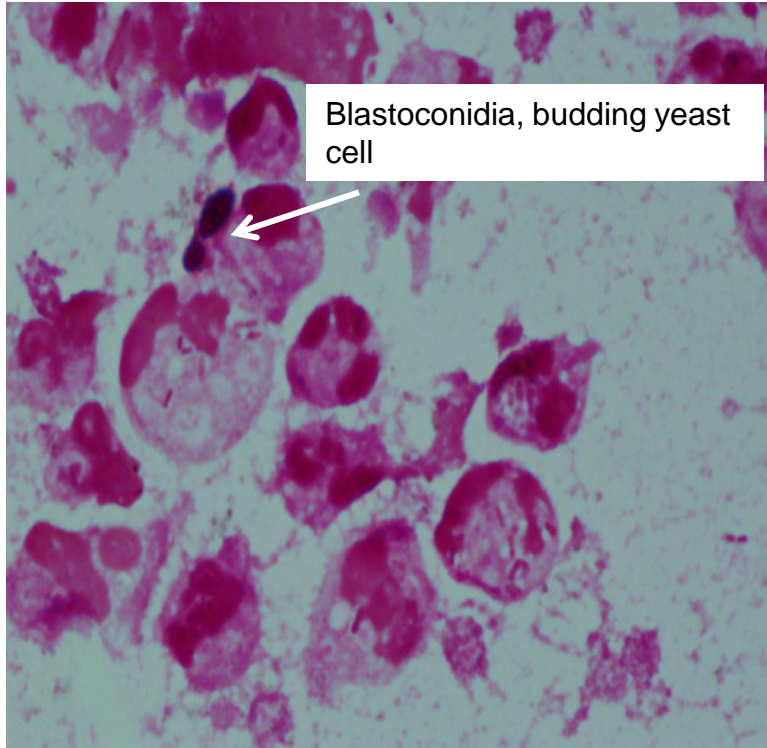
Antifungal susceptibility testing

- Similar as in bacteria, **special culture media**
- Clinical breakpoints only available in most common species

Biomarkers

- Antigen: beta-D-glucan in serum, mannan/antimannan in serum (disseminated candidiasis)

Yeasts, Gram stain



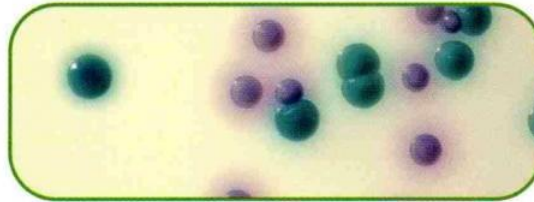
Yeast (*Candida* sp.) – culture, Sabouraud agar, 37 °C, 48 h



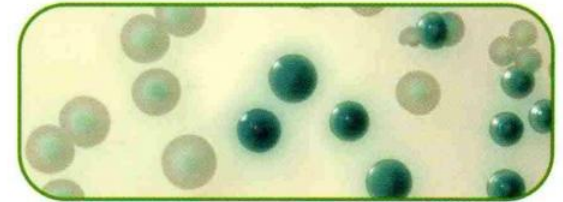
Chromogenic agars

Analysis of mixed cultures – mucosal lesions, monitoring

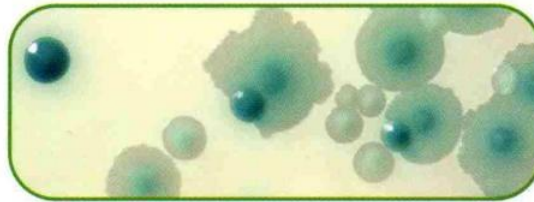
Bonne visualisation des cultures mixtes



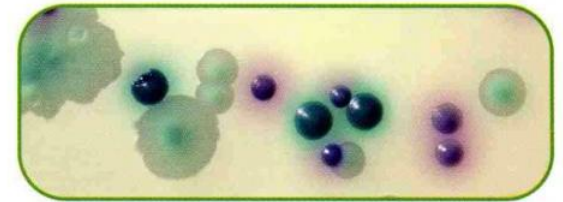
C. albicans + C. tropicalis



C. tropicalis + C. glabrata



C. tropicalis + C. glabrata + C. krusei



C. albicans + C. tropicalis + C. glabrata + C. krusei

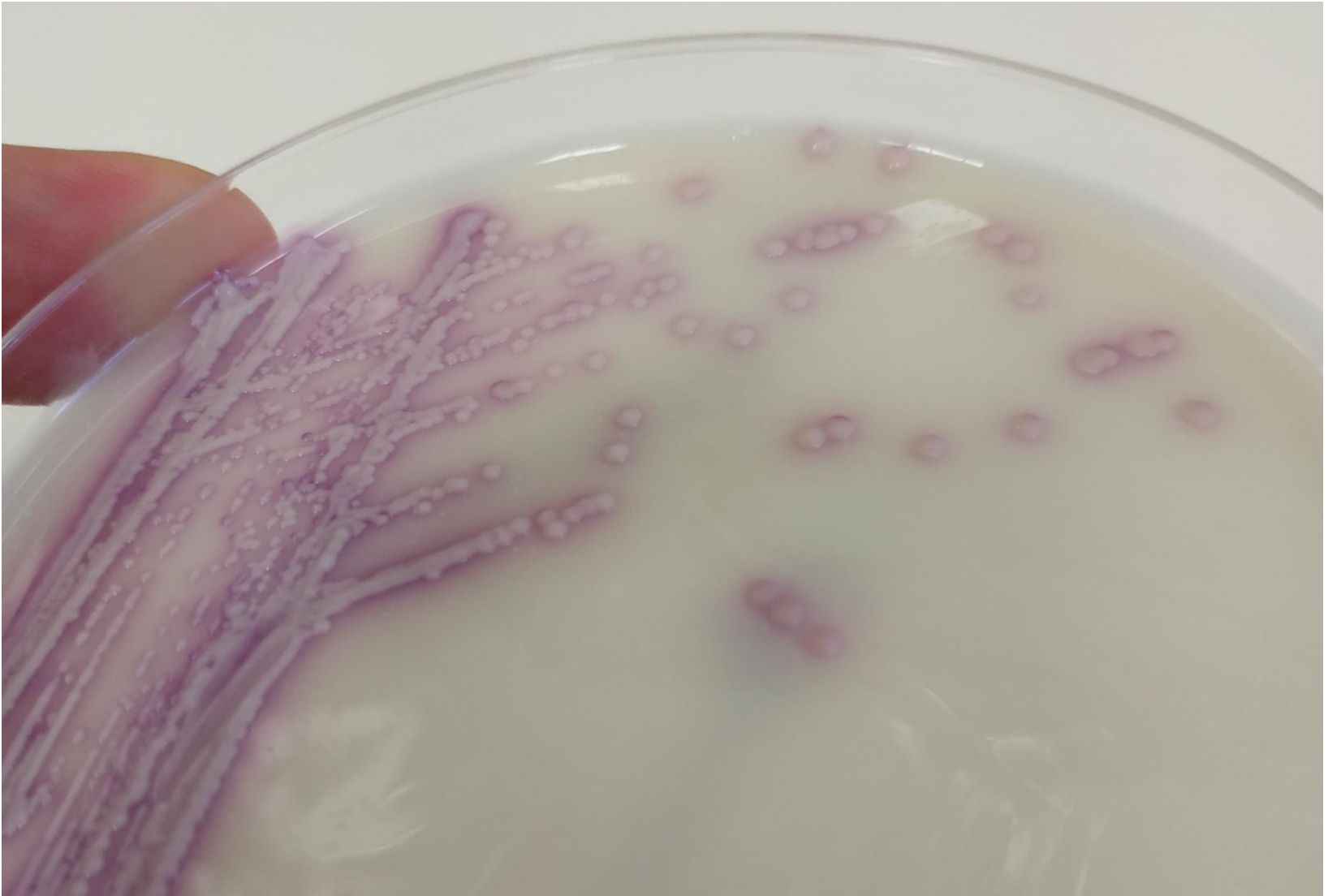
CandiSelect™ 4	20 boîtes Ø 90 mm	code 63746
Auxacolor™ 2	20 galeries	code 56513
Fungitest™	10 galeries	code 60780
Témoin d'opacité (Auxacolor™ 2, Fungitest™)	2 flacons	code 56499
Sabouraud + Cmp + Genta	20 boîtes Ø 90 mm	code 63774

BIO-RAD

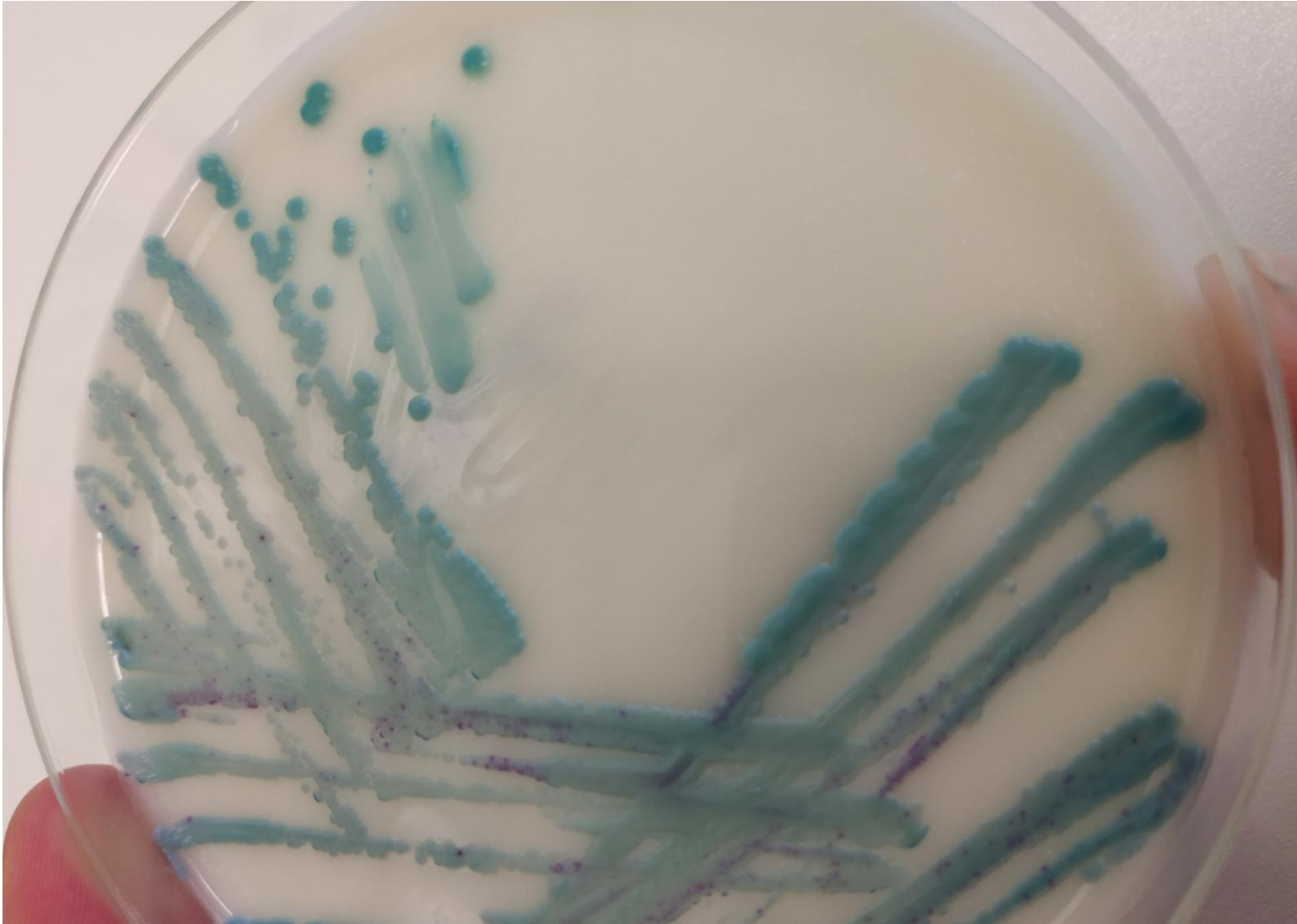
**Bio-Rad
Laboratories**



Candida albicans, Chromogenic selective-diagnostic agar, 37 °C, 48 h

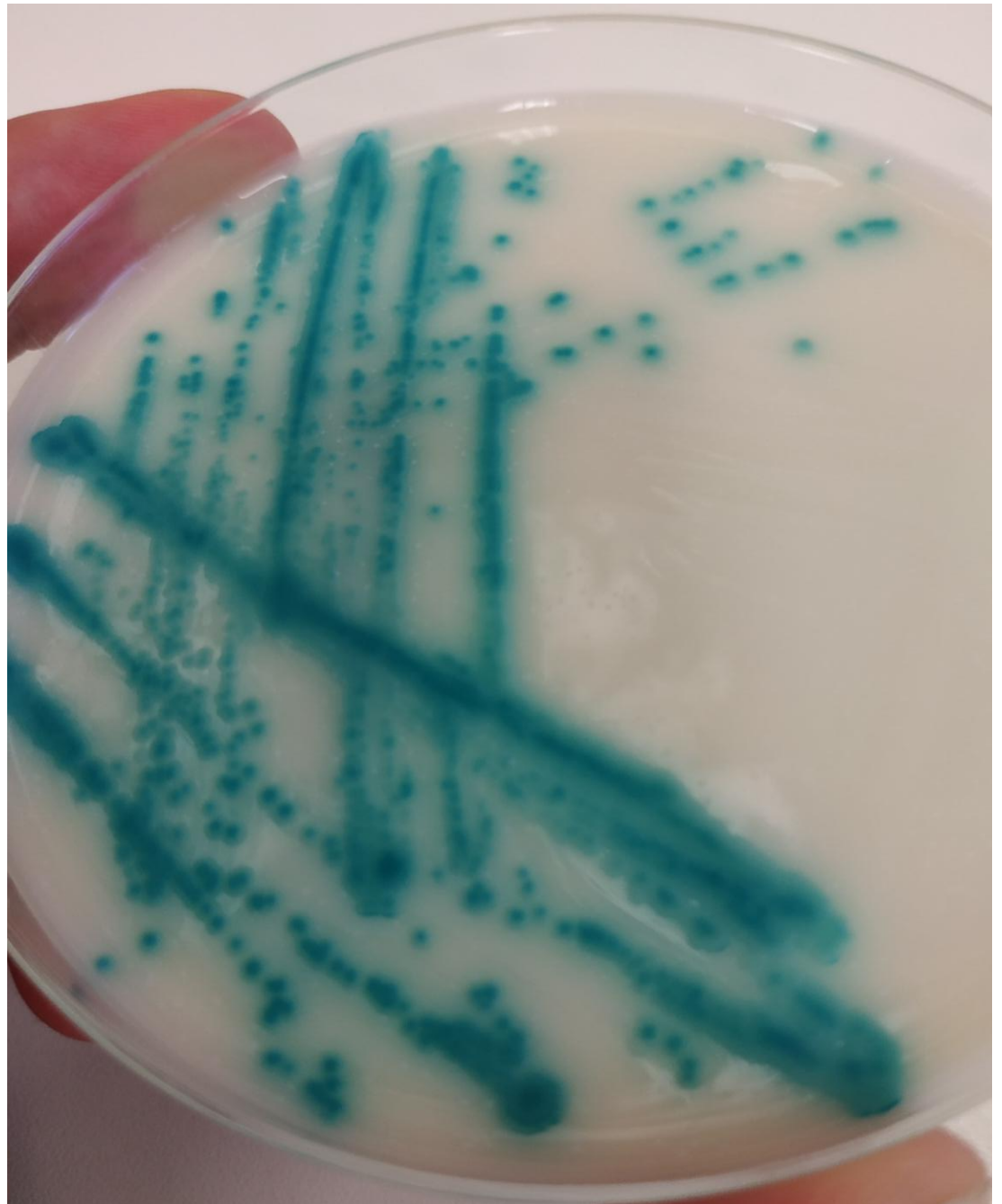


Candida glabrata Chromogenic selective-diagnostic agar, 37 °C, 48 h

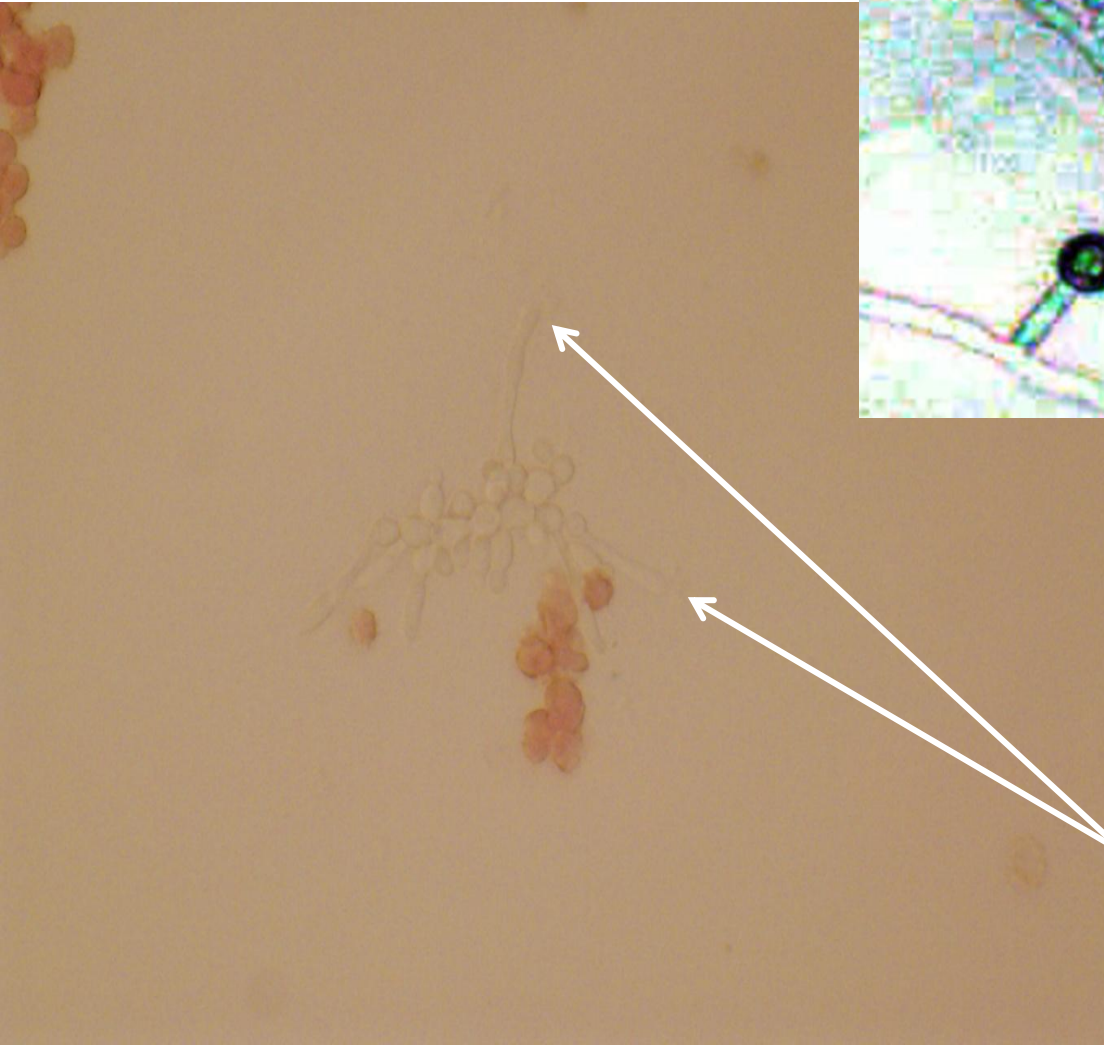
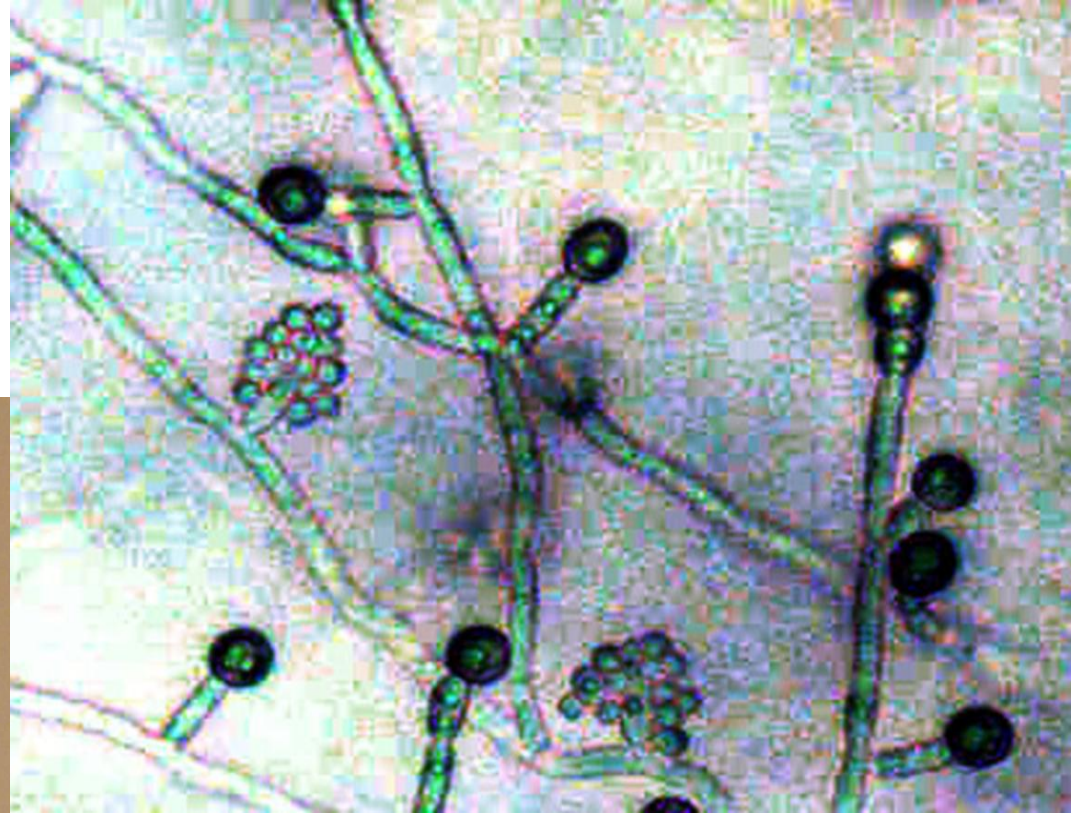


Candida krusei

Chromogenic
selective-diagnostic
agar, 37 °C, 48 h

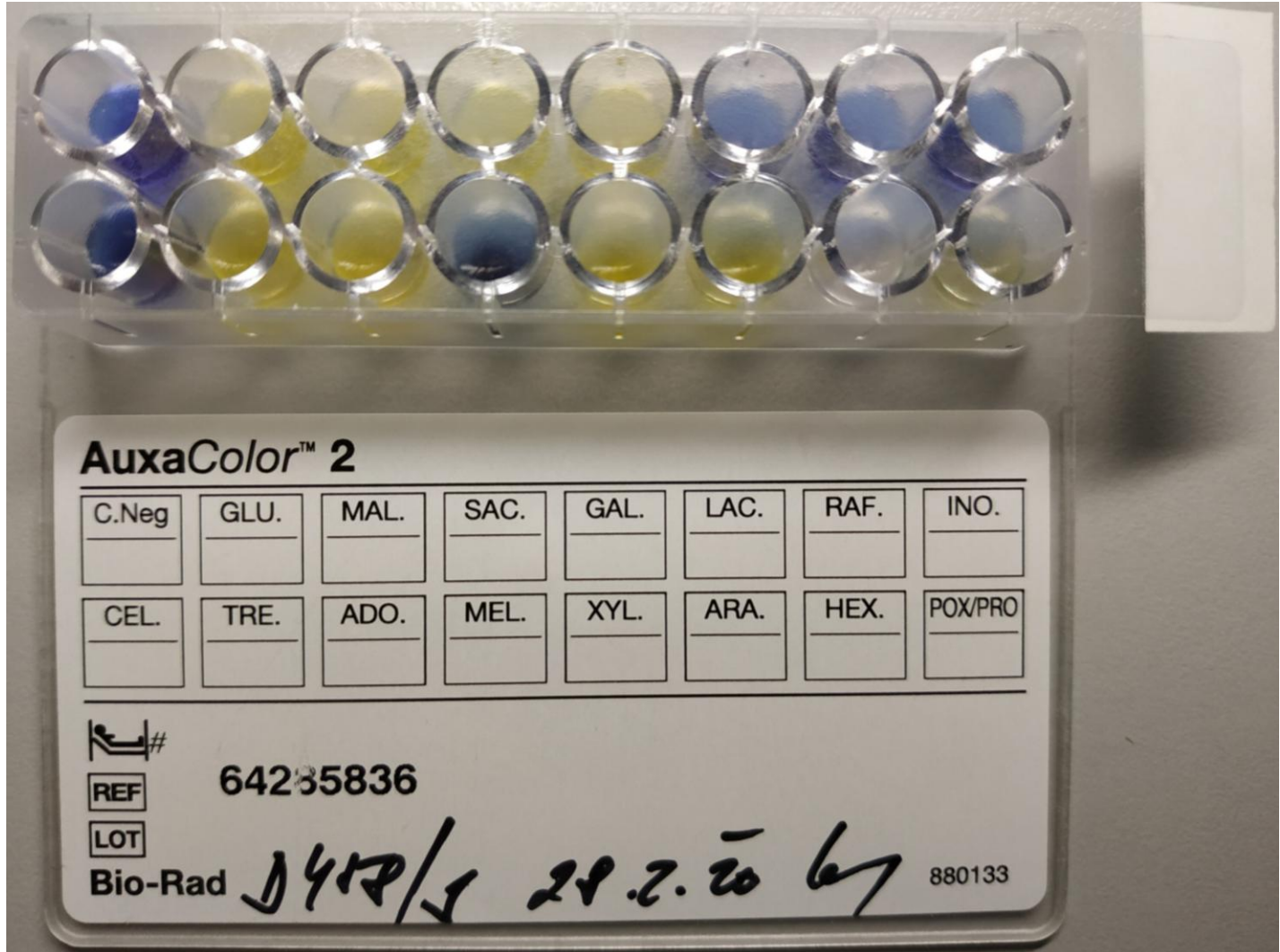


Micromorphology, rice agar, *C. albicans*



Hyphae growth, horse serum,
wet mount
C. albicans

Yeast culture – biochemical identification (sugar assimilation test)



Candida – antifungal susceptibility testing

Clinically significant isolates and monitoring in high-risk patients

Disc diffusion method

Mueller-Hinton agar supplemented with glucose and methylene blue

Procedure must be followed precisely

Incubation 24 h, 35 °C

Clinical breakpoints available for most frequent species only.

Interpretation – candidiasis

Oral and vaginal mucosa – yeasts belong to microflora, treatment indicated in patients with typical clinical signs (mucositis, thrush, vaginitis)

Lower respiratory tract – Colonization or contamination from oral mucosa in most cases, most cases of pneumonia are haematogenous

Blood culture – always significant, test for endocarditis and endophthalmitis and control blood sample collection indicated in every case of candidemia

Negative blood culture does not exclude the diagnosis of disseminated candidiasis

Primarily sterile body liquids (CSF, pleural, pericardial, peritoneal or intraarticular liquid) **or tissue specimens** – always significant

Urine – difficult to distinguish contamination or catheter colonization from significant candiduria, clinical and biochemical signs must be evaluated, quantity is less relevant in compare with bacteriuria

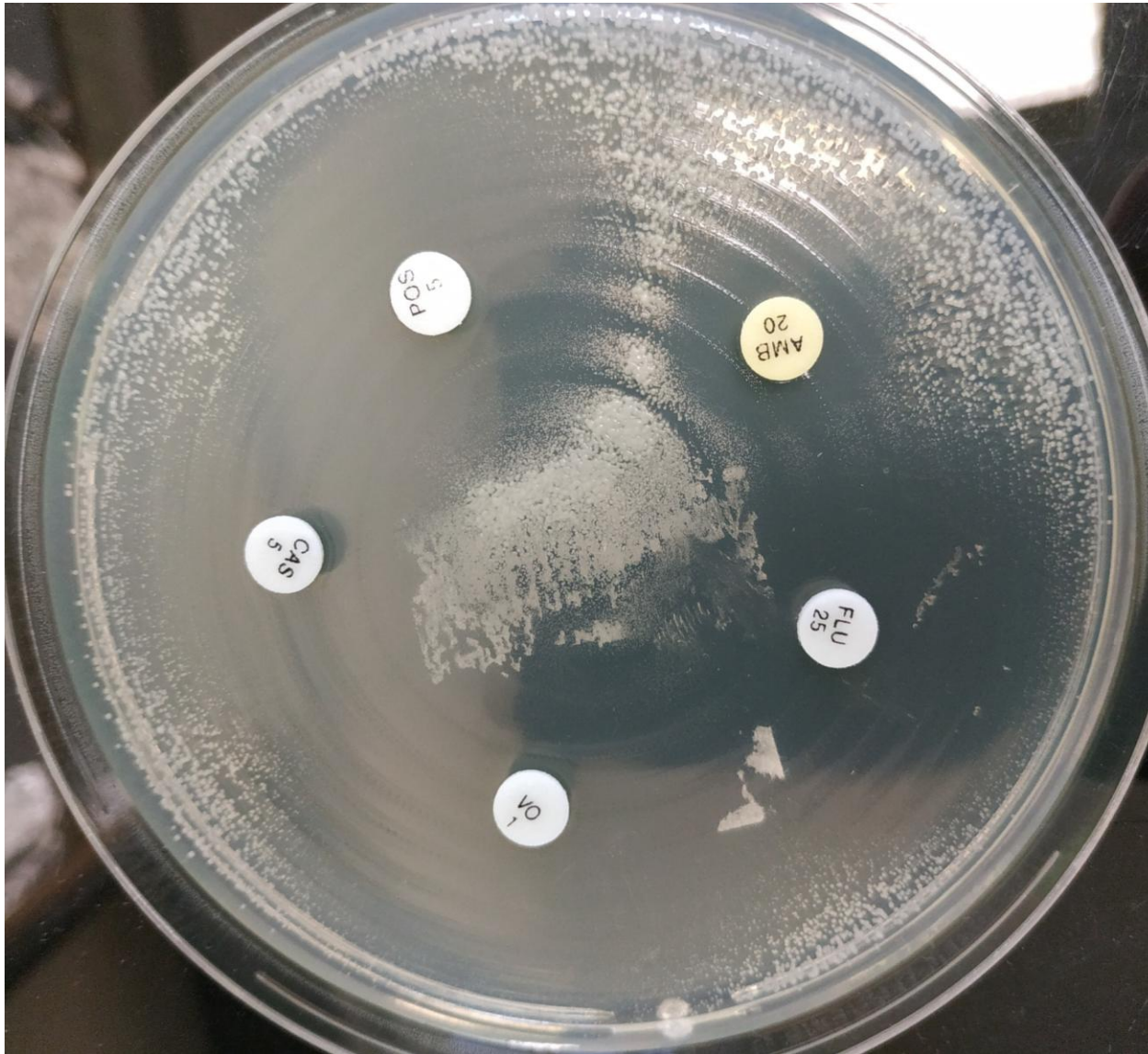
***Candida krusei*, disc diffusion method**

- Mueller-Hinton agar supplemented with glucose and methylene blue
- Incubation 24 h, 35 °C



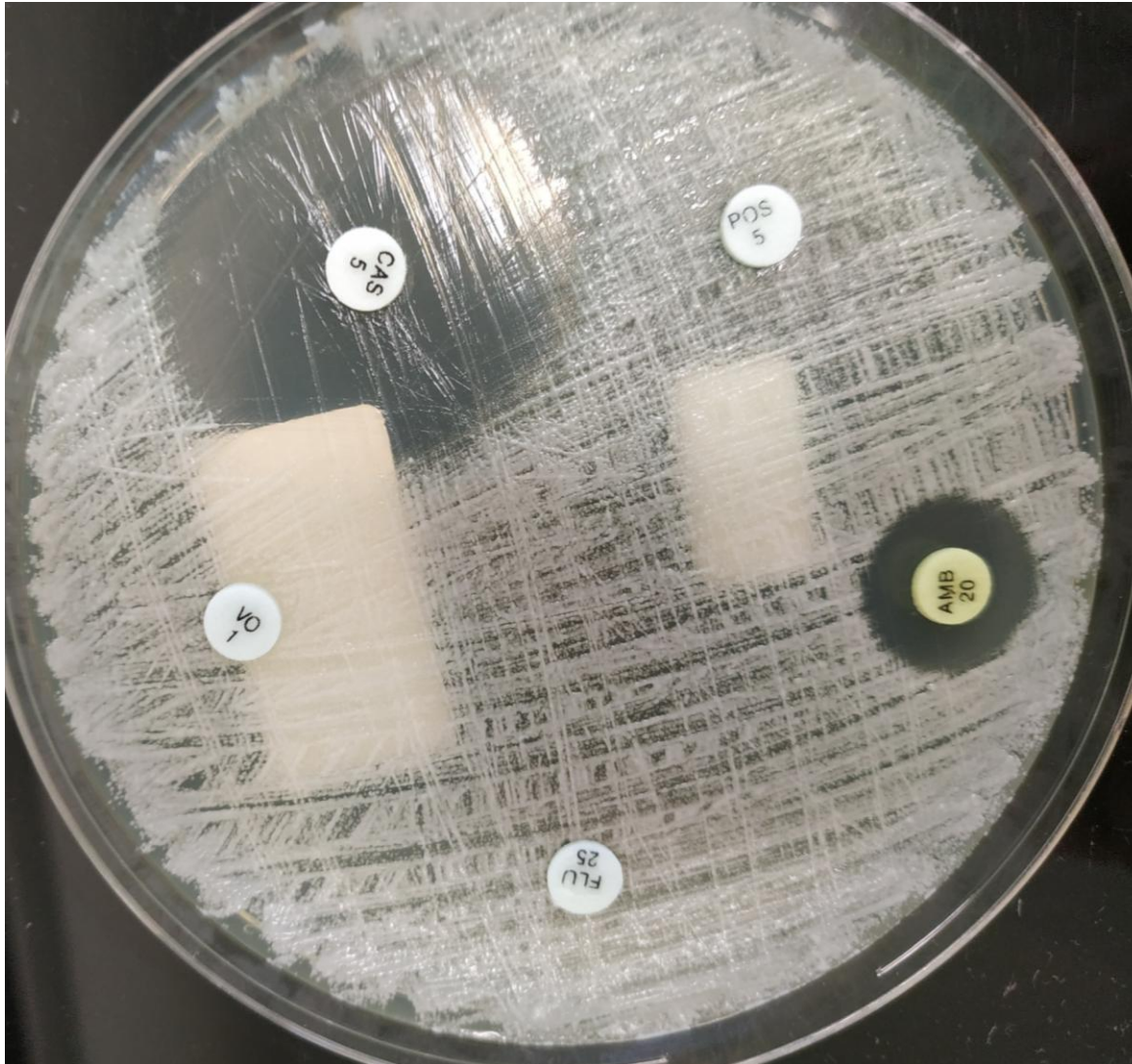
***Candida albicans*, disc diffusion method**

- Mueller-Hinton agar supplemented with glucose and methylene blue
- Incubation 24 h, 35 °C



***Candida glabrata*, disc diffusion method**

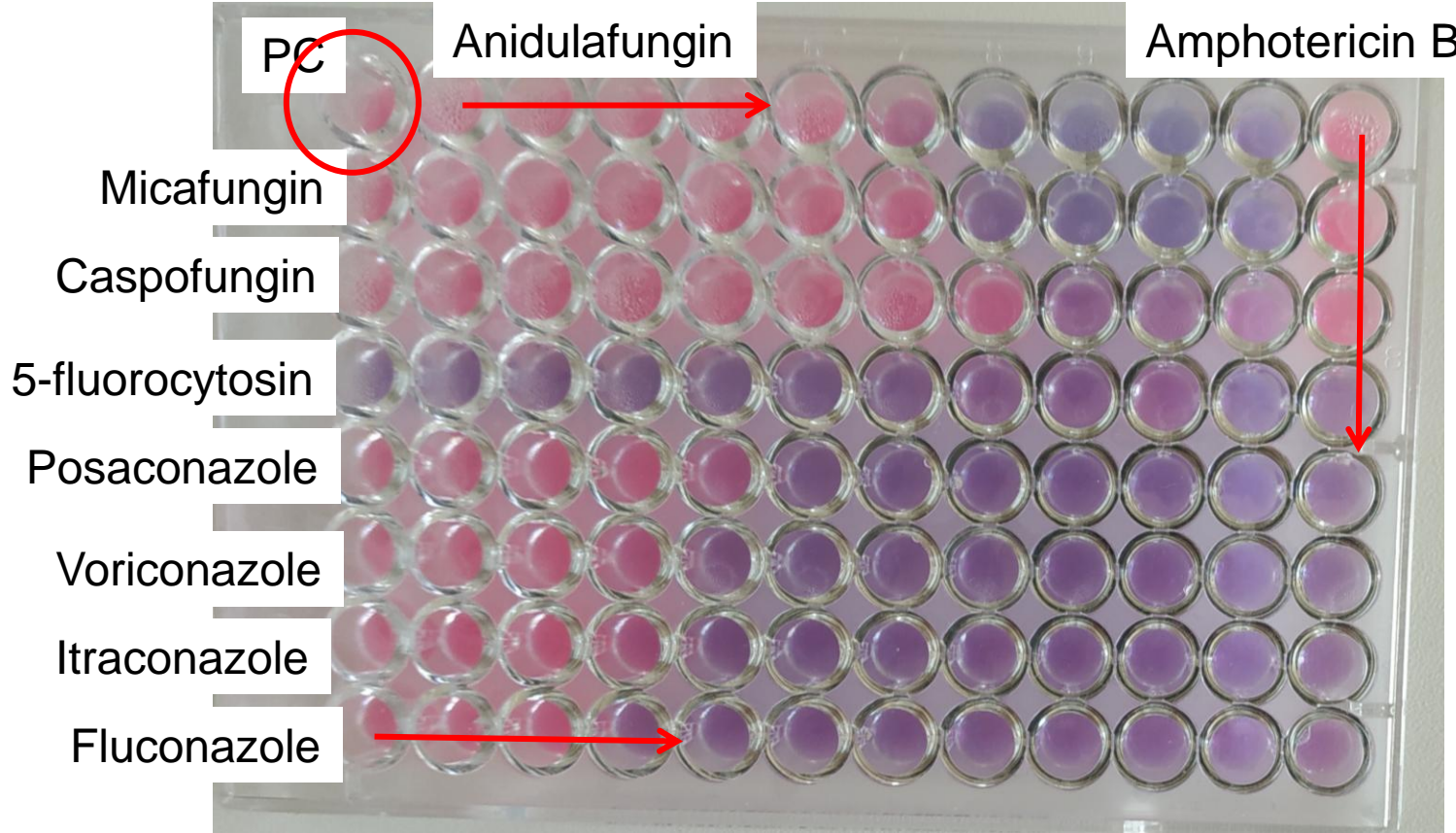
- Mueller-Hinton agar supplemented with glucose and methylene blue
- Incubation 24 h, 35 °C



***Candida parapsilosis*, microdilution method**

Sensititre Yeast One

RPMI 1640 medium supplemented with glucose, 35 °C, ambient atmosphere, 24 – 48 h

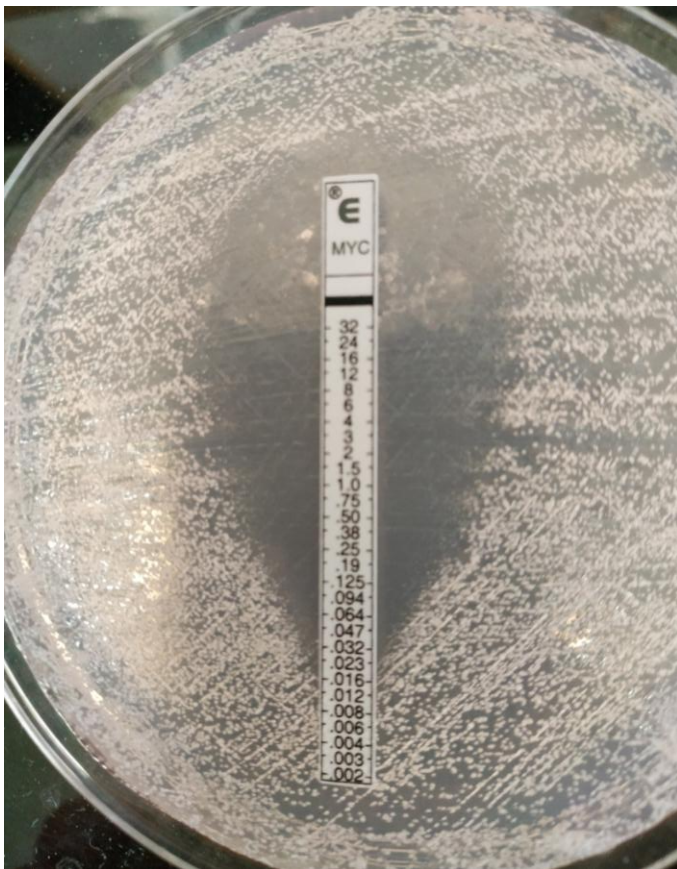


Candida albicans, gradient diffusion method – E-test

RPMI 1640 agar supplemented with glucose

Incubation 35 °C, ambient atmosphere, 24 – 48 h

Micafungin



Fluconazole



***Cryptococcus neoformans* – microbiological diagnosis**

Opportunistic pathogen, acquired from environment by inhalation, **central nervous system and less frequently lungs affected**

Biological material for testing – **cerebrospinal fluid (CSF)**, blood culture, lower respiratory tract liquid samples, serum and urine for antigen

Microscopy

- **India ink negative capsule staining** – blastoconidia can be stained with crystal violet
- Atypical blastoconidia morphology in Gram stain

Specific antigen (glukuronoxylomannan) detection in CSF, serum, urine, BALF

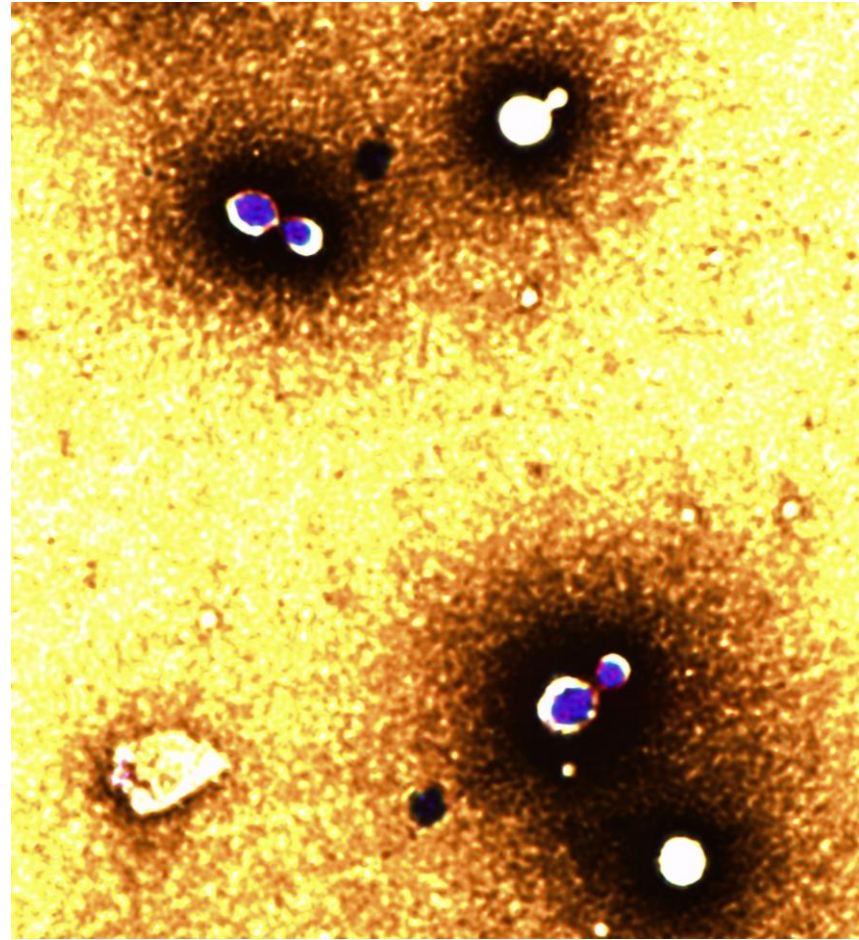
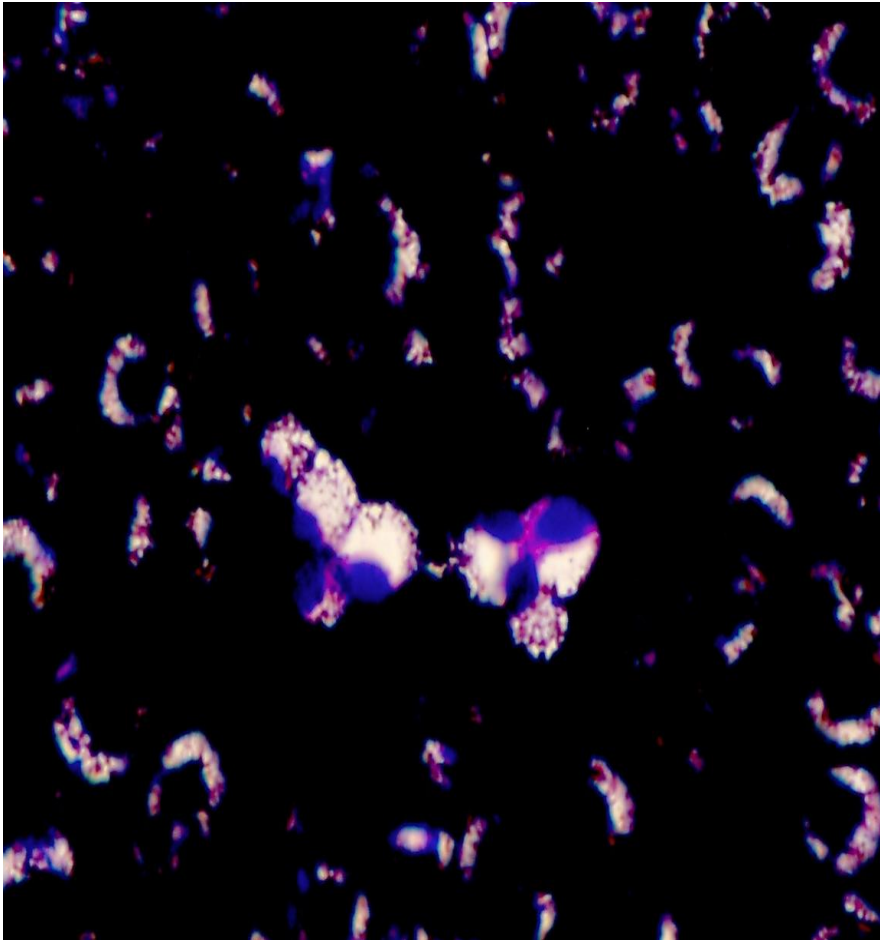
- Latex agglutination
- Immunochromatographic test (lateral flow assay)

Culture

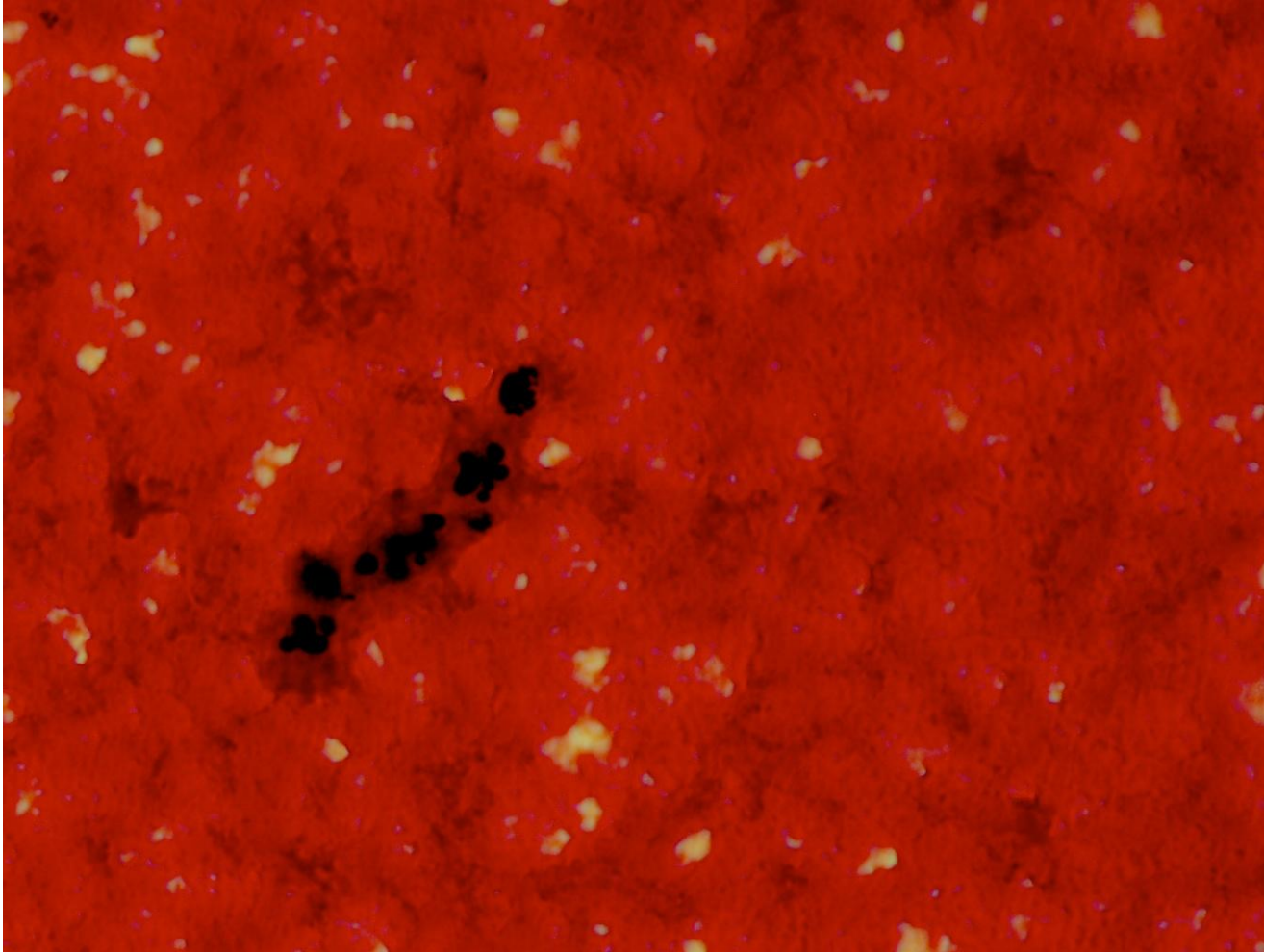
Chocolate or Sabouraud agar, 37 °C, ambient atmosphere, **prolonged for up to 4 weeks**, susceptibility testing not different from other yeasts

PCR detection in CSF

India ink– *Cryptococcus* sp., blood culture



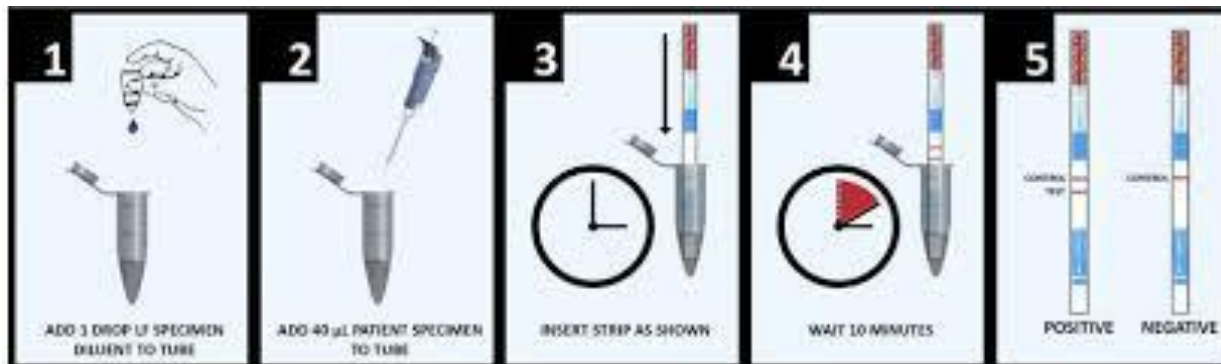
Cryptococcus neoformans, Gram stain, blood culture



Cryptococcus neoformans – antigen detection (glucuronoxylomannan)



Latex agglutination



Imunochromatografic detection (Lateral flow assay)

Filamentous fungi – invasive infections

Microscopy

Primarily sterile specimens – proof of infection

Does not allow identification

- Wet mount with 10% KOH
- Fluorescence microscopy – optical brightener

Culture

Sabouraud agar with 2 % glucose, Sabouraud broth

20 – 25 °C up to 4 weeks

Malt extract agar - culture isolation and identification

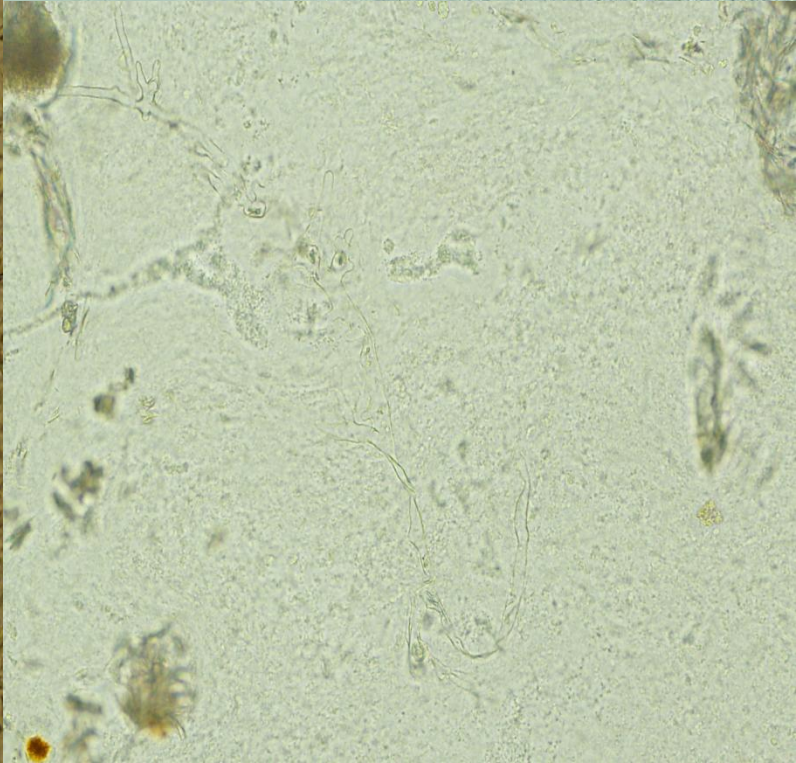
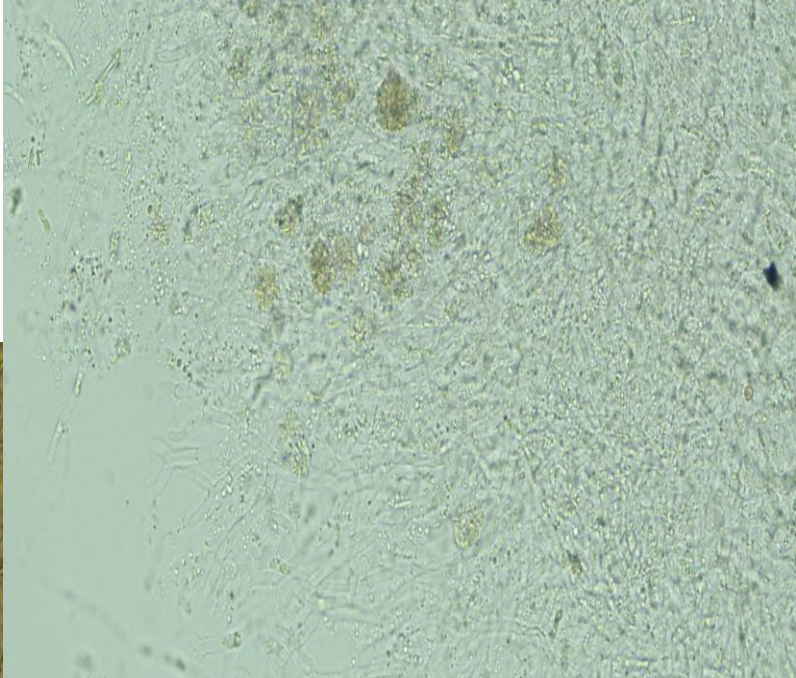
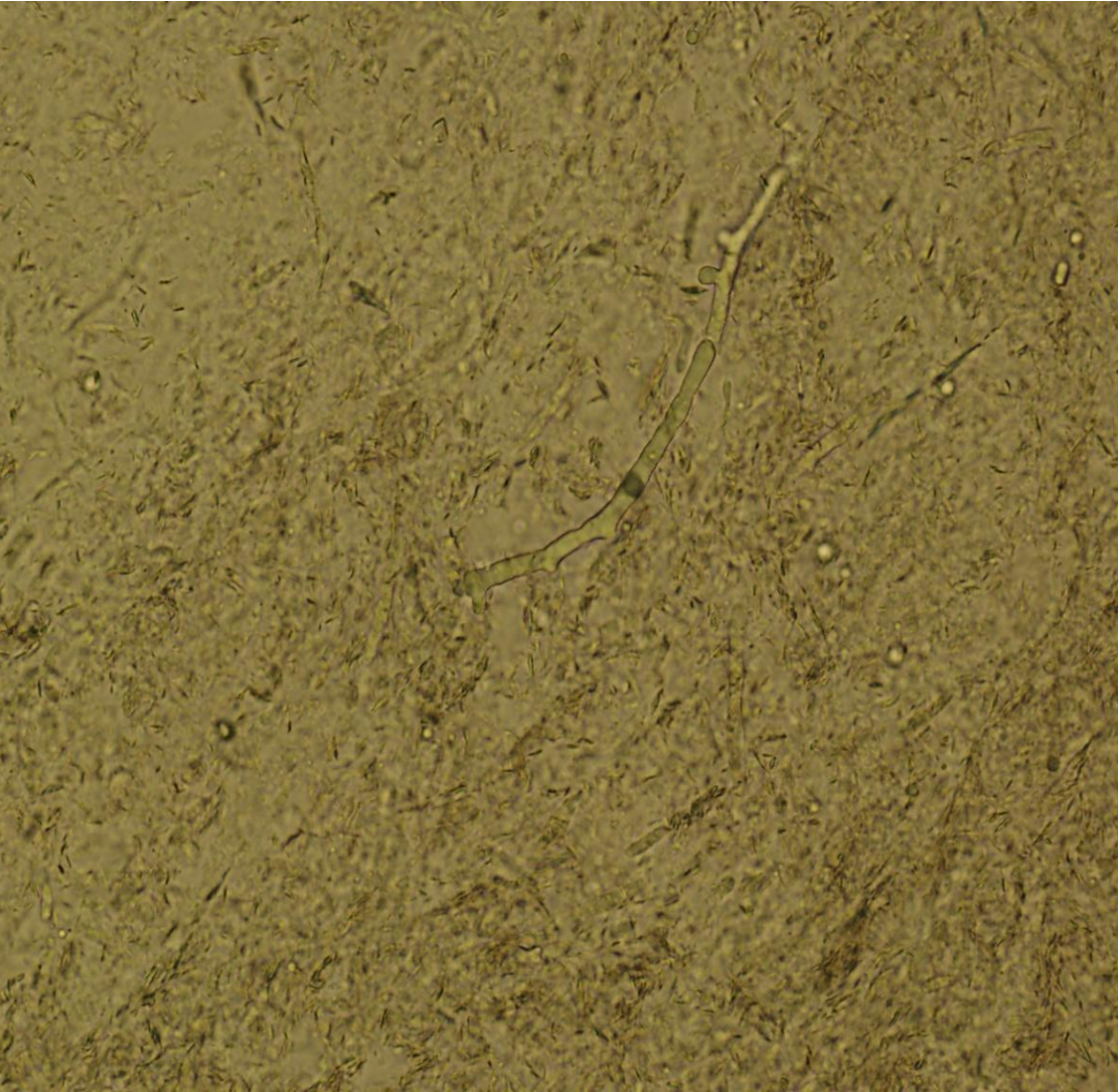
Identification

- Macromorphology – aerial mycelium, colony appearance (upper/bottom side), time of growth, optimal temperature
- Micromorphology - transparent tape and lactophenol blue technique
- Slide culture**
- Genome sequencing**

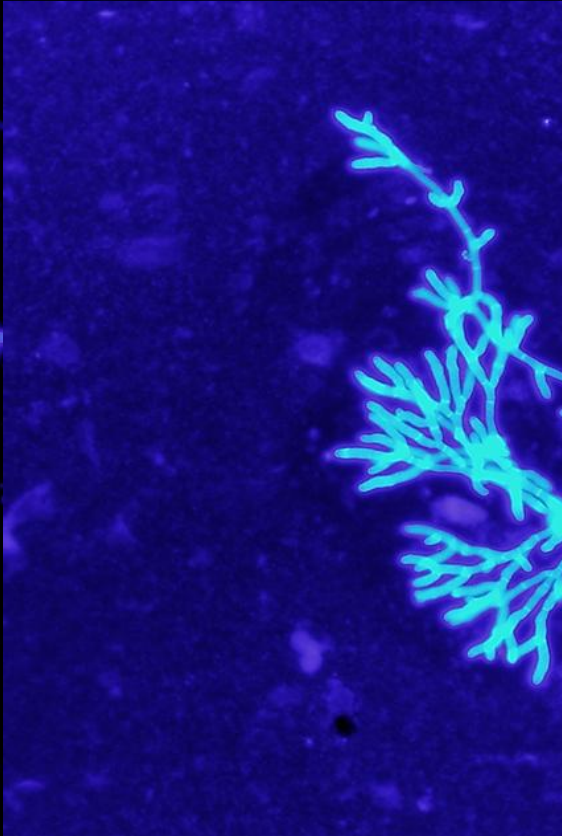
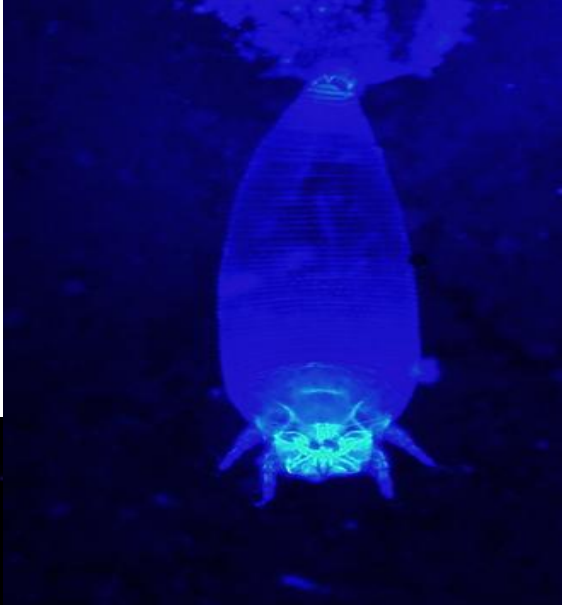
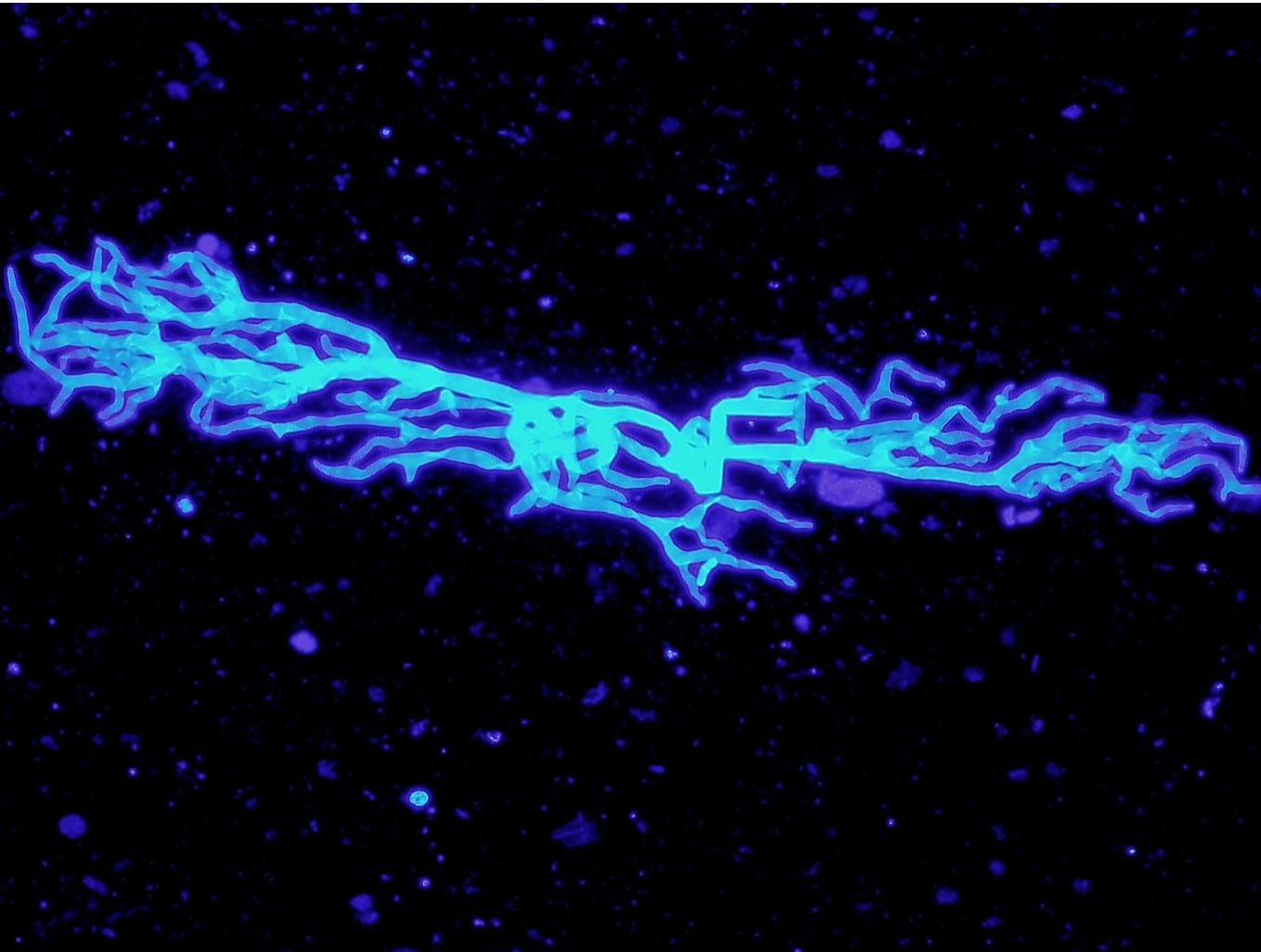
Antifungal susceptibility testing – breakpoints available in common *Aspergillus* species

Biomarkers – Antigens (galactomannan, beta-D-glucan), **DNA – PCR** (standardized in *Aspergillus* sp. only)

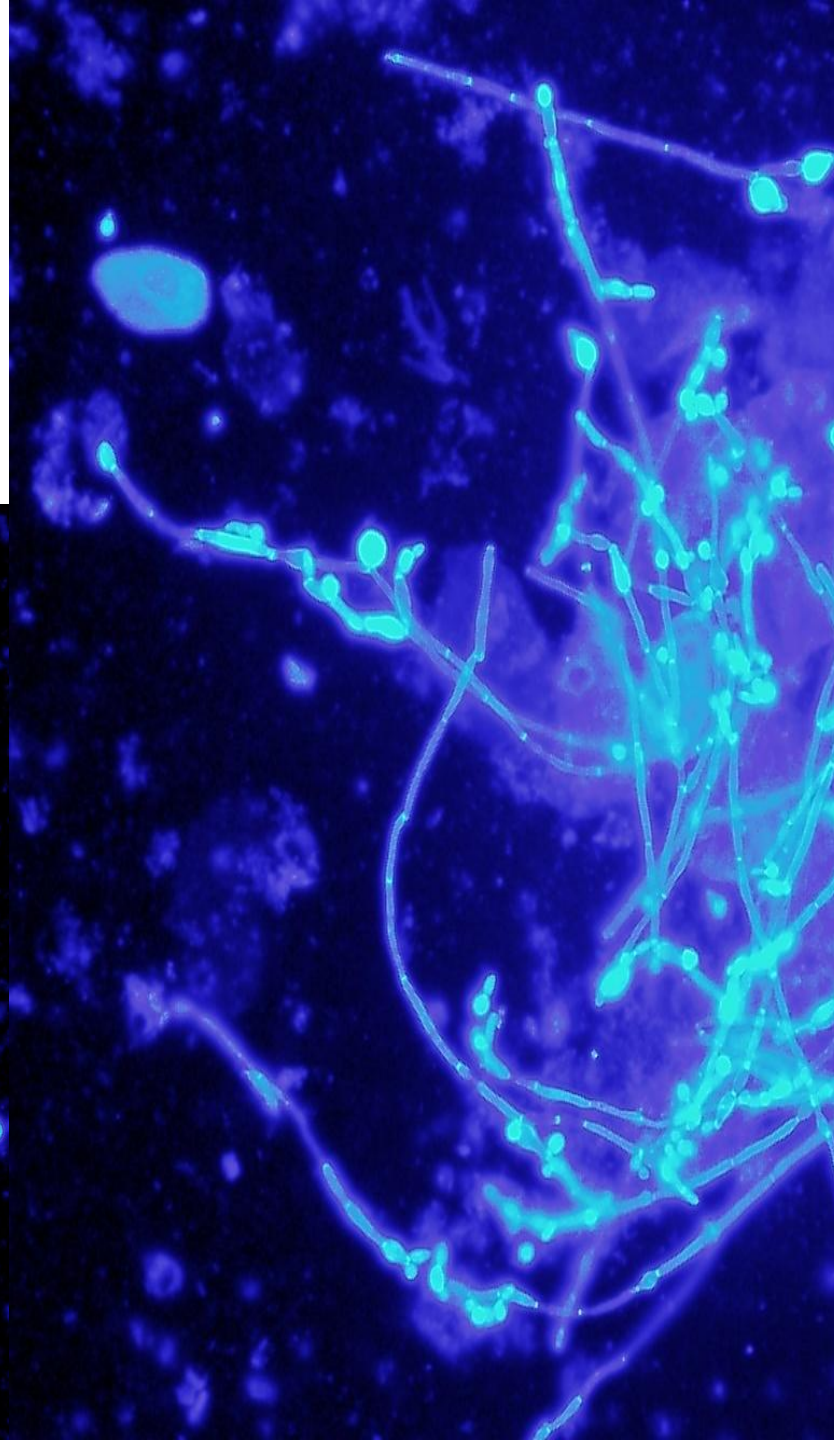
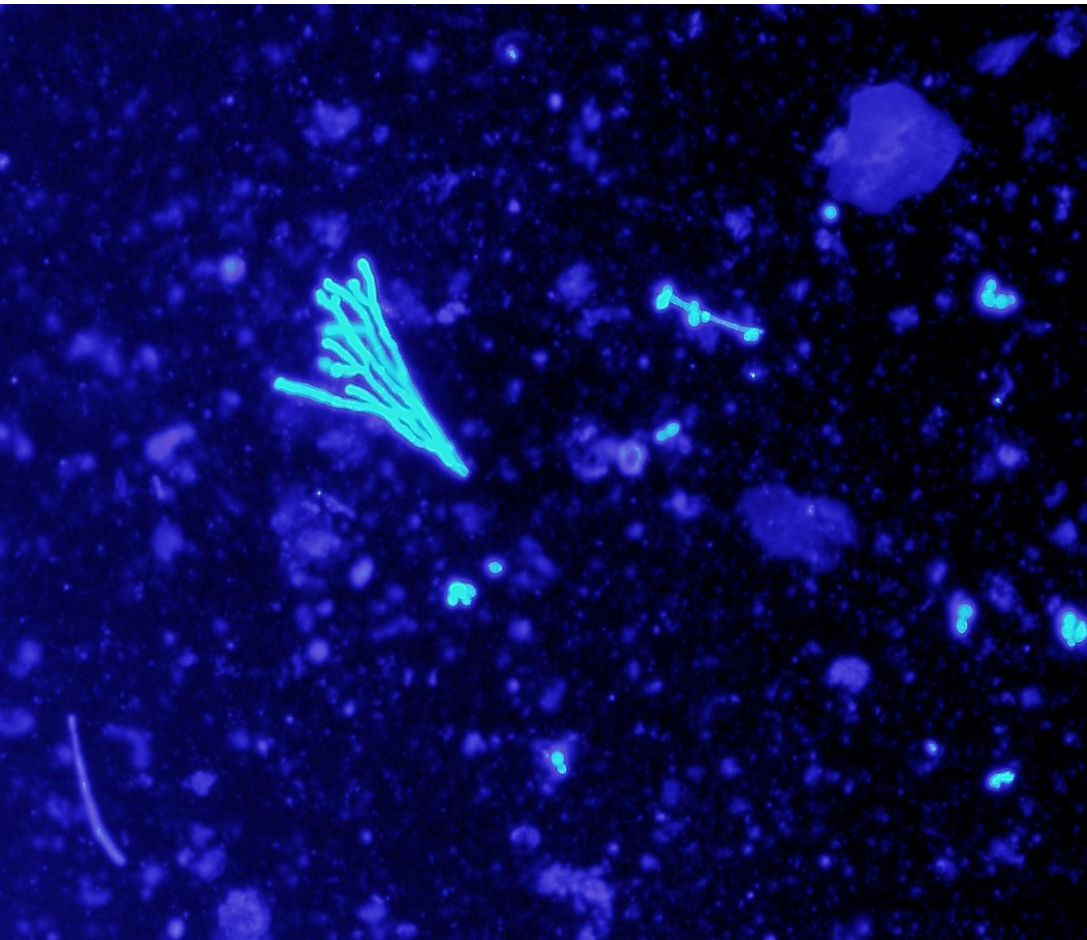
Wet mount, KOH, tissues and liquids



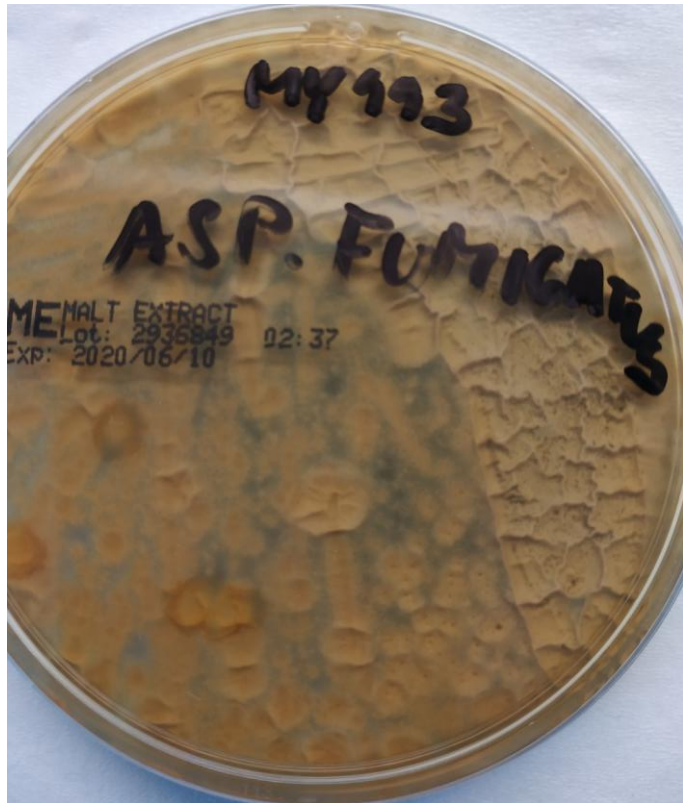
Calcofluor white, KOH



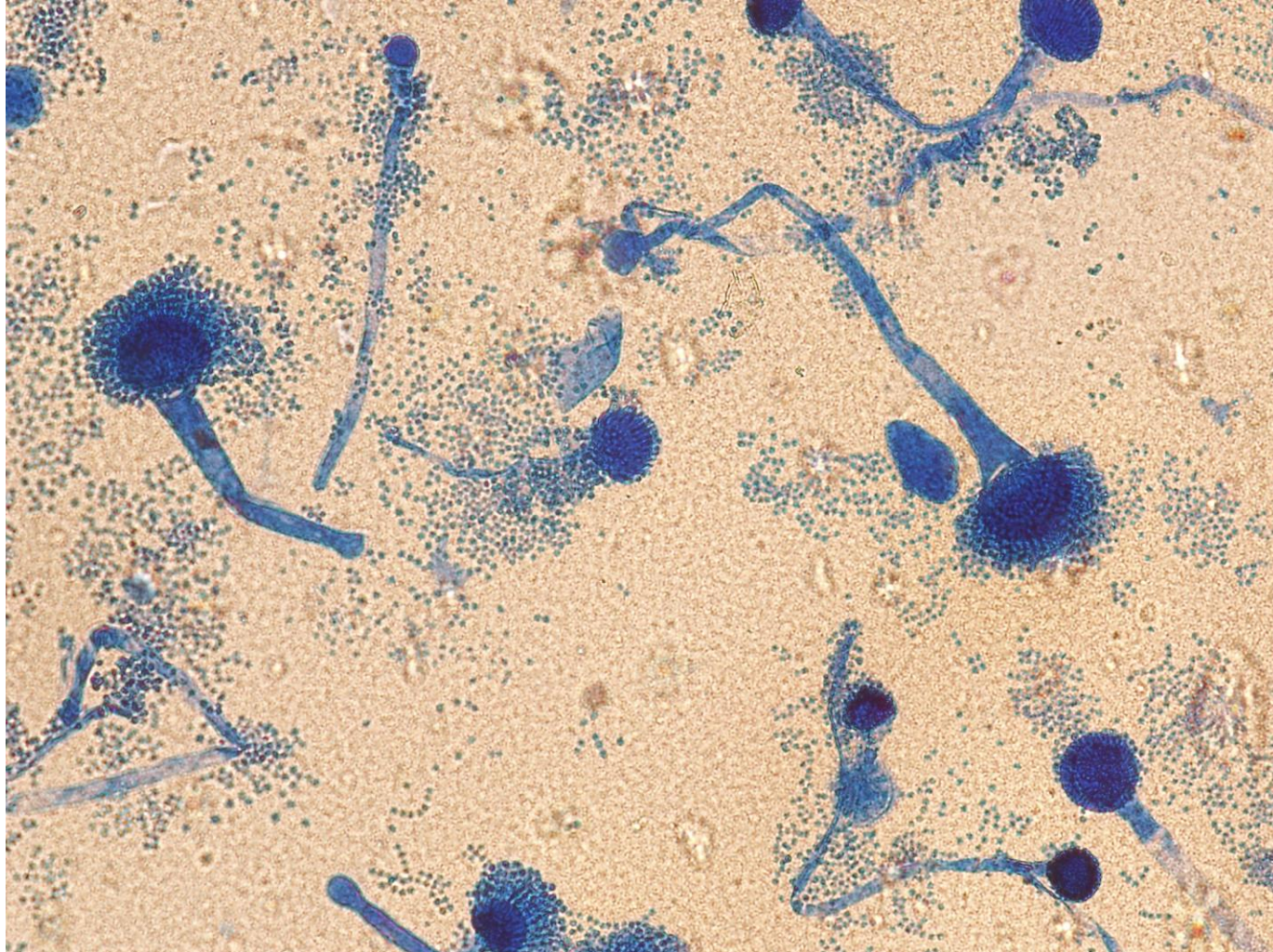
Calcofluor white, KOH



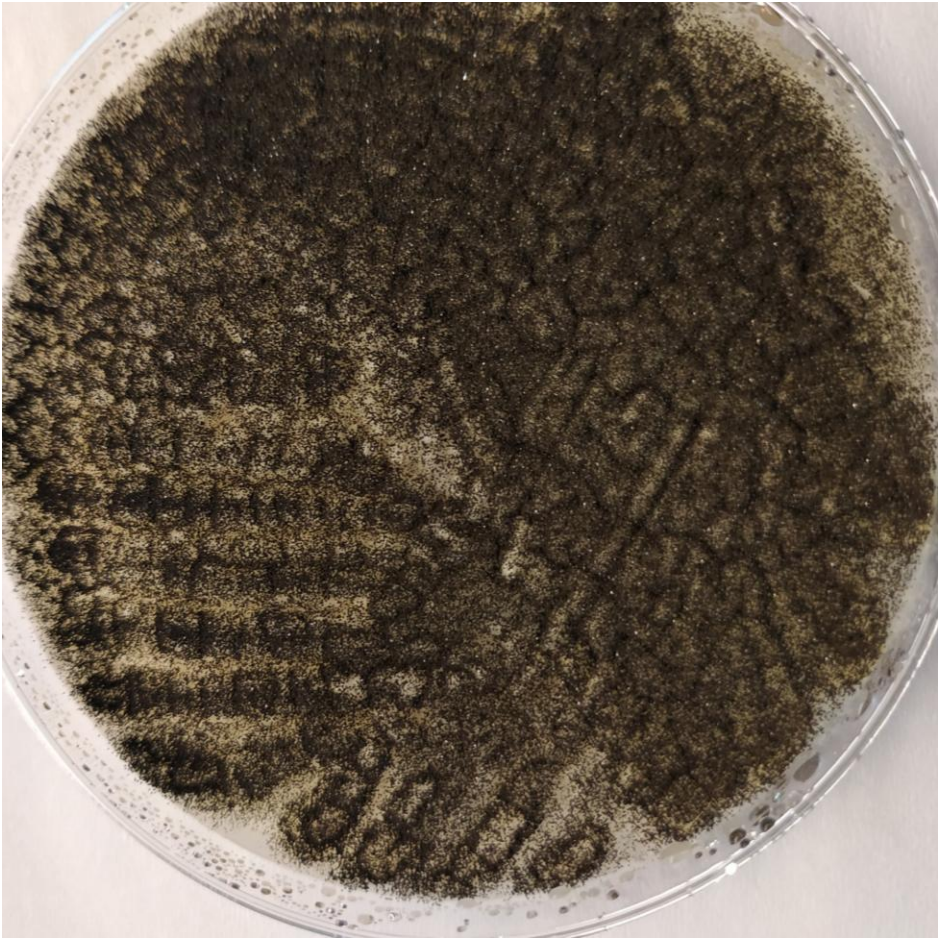
Aspergillus fumigatus, Malt extract agar, 25 °C, 3 days



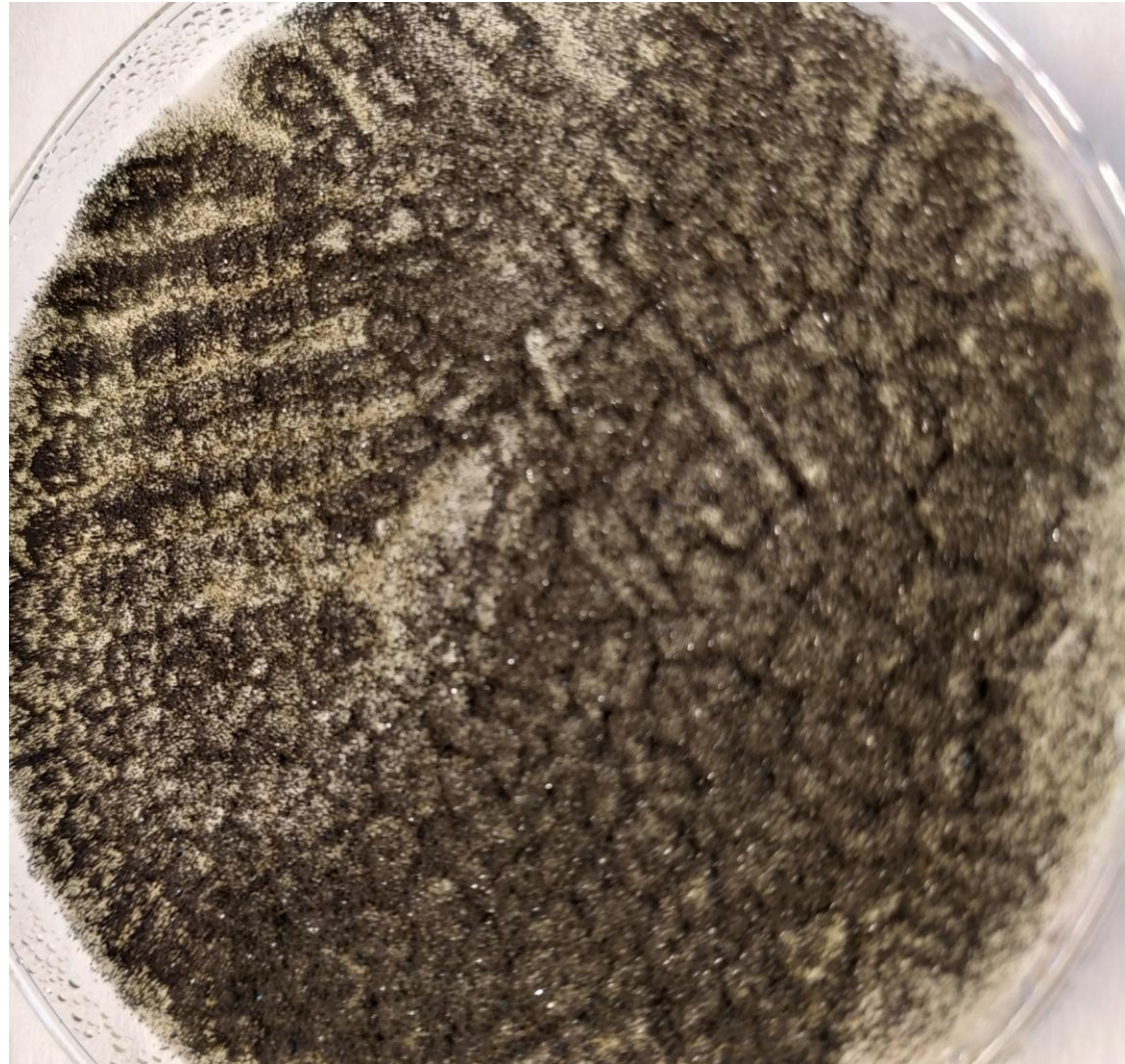
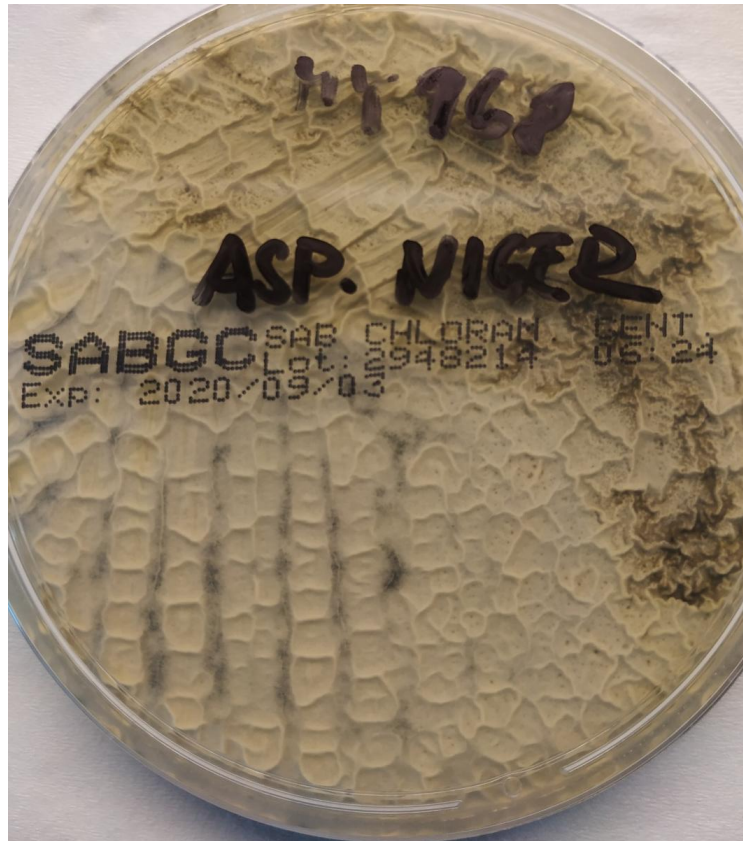
***Aspergillus fumigatus*, Lactophenol blue, 400x**



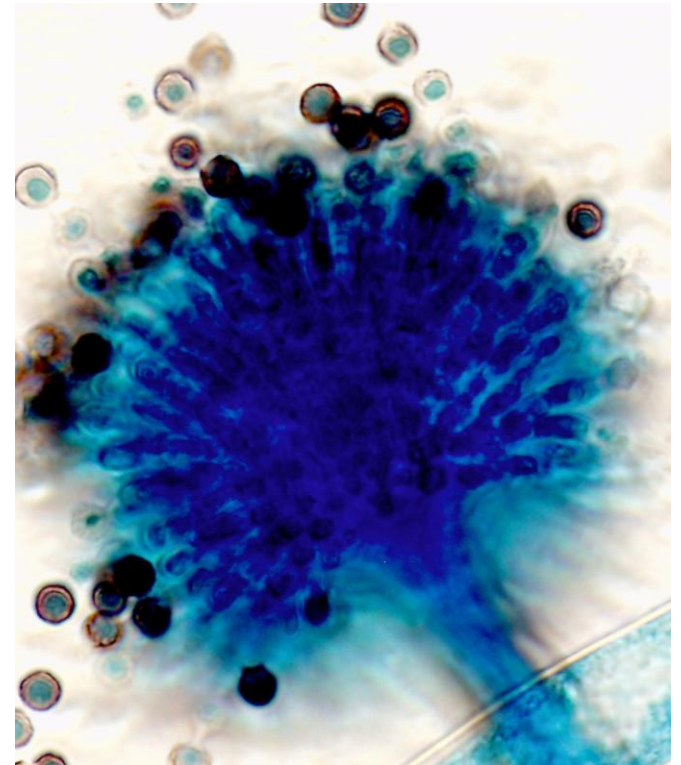
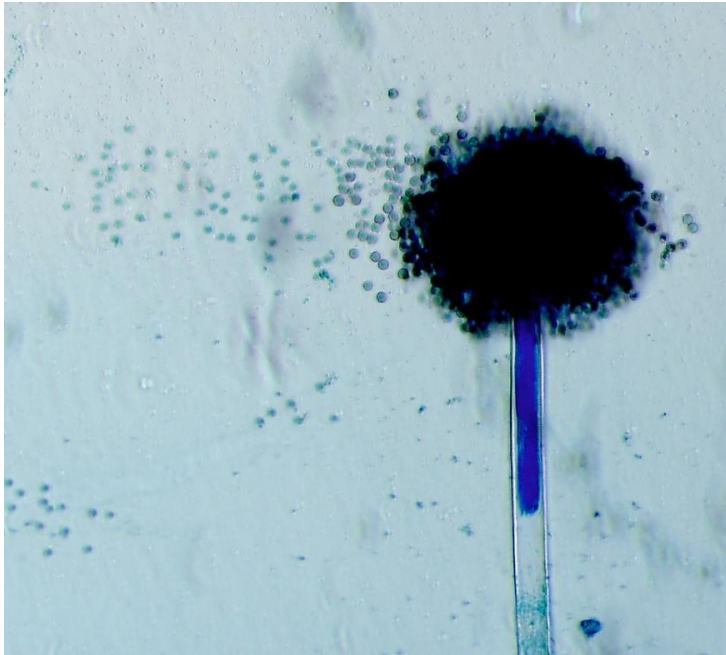
Aspergillus niger, Malt extract agar, 25 °C, 3 days



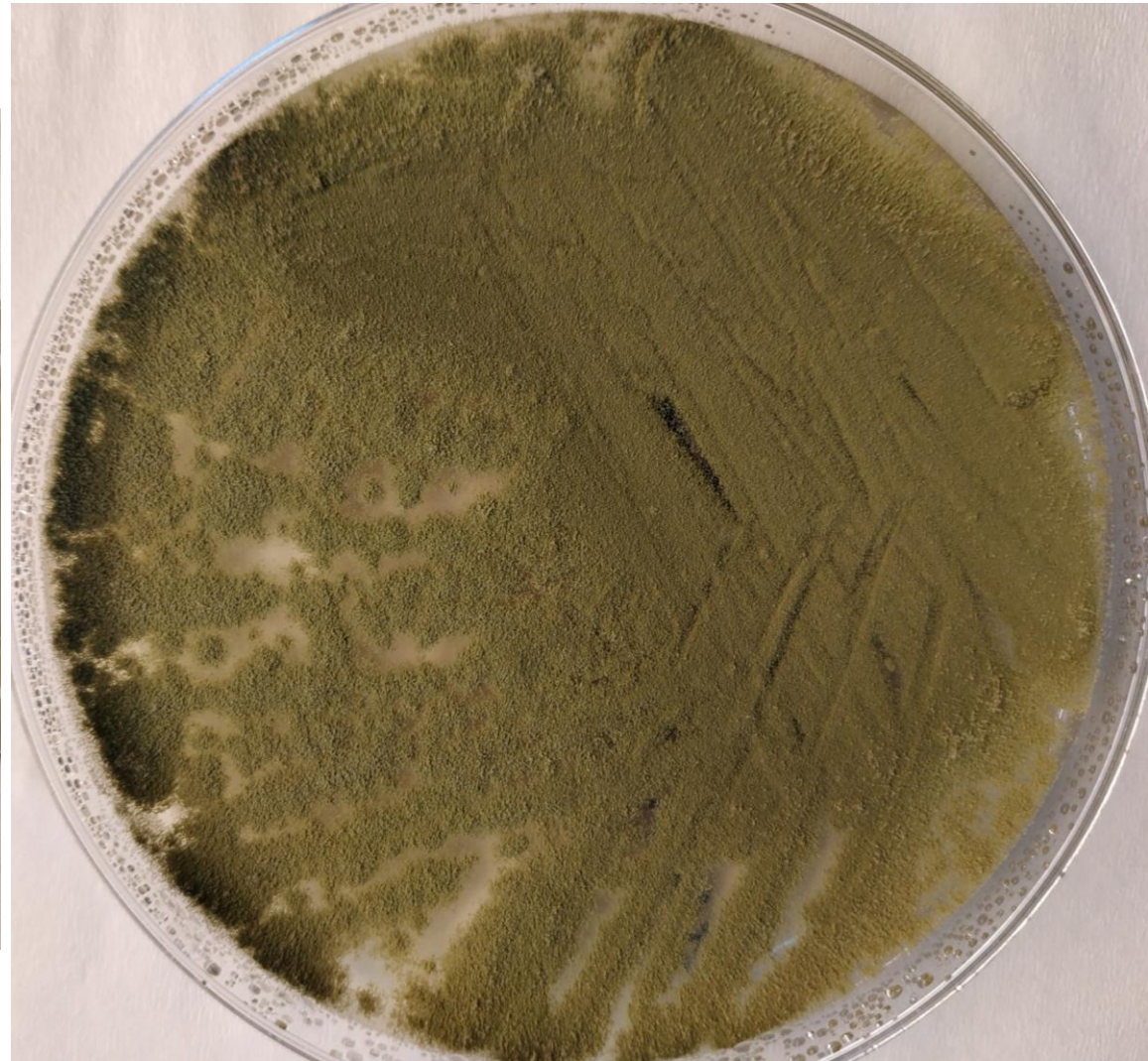
***Aspergillus niger*, Sabouraud agar, 25 °C, 3 days**



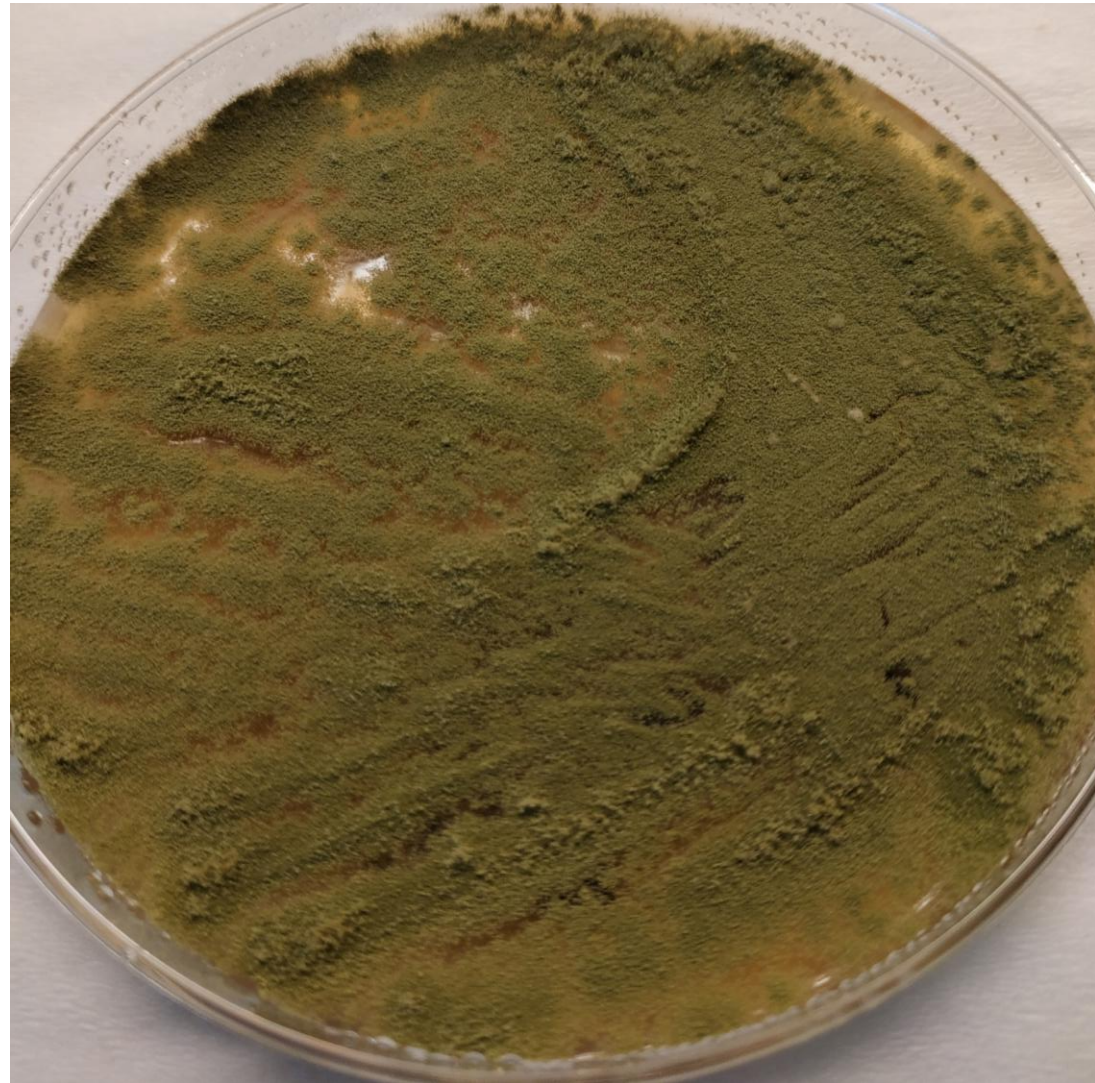
A. niger, Lactophenol blue



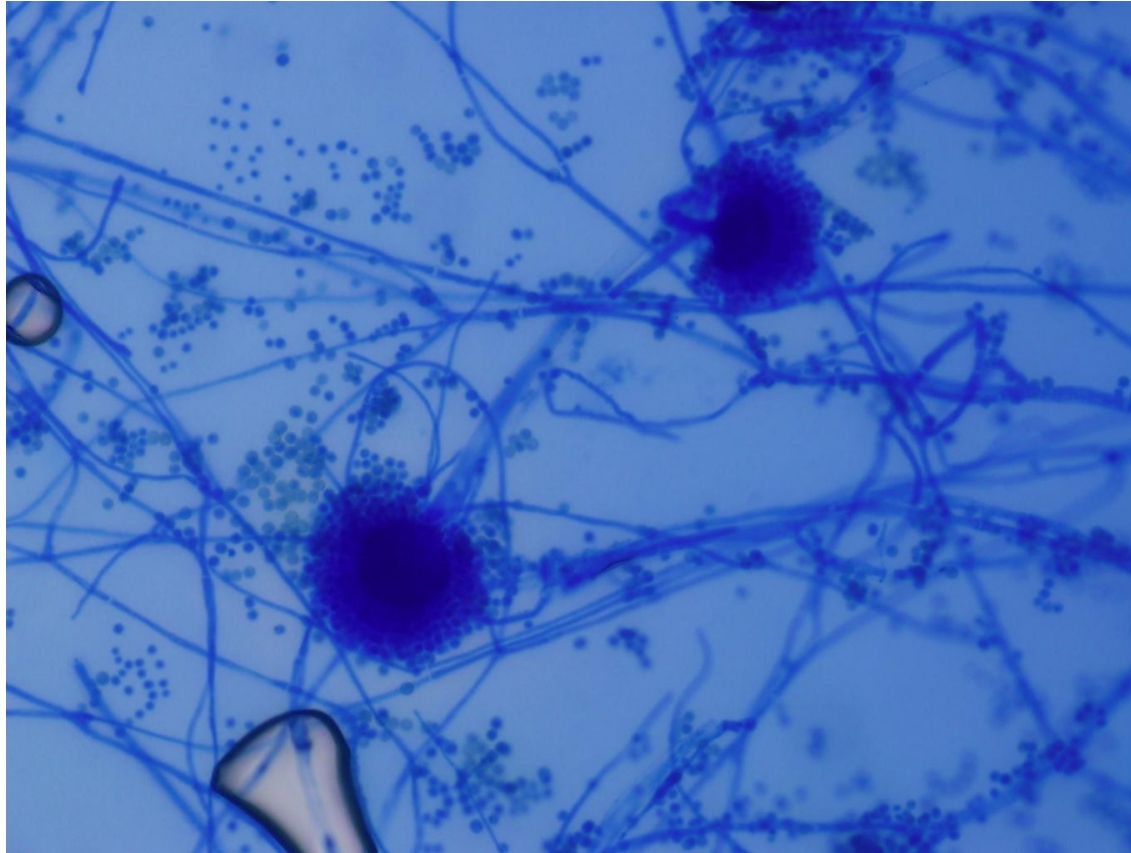
***Aspergillus flavus*, Sabouraud agar, 25 °C, 3 days**



***Aspergillus flavus*, Malt extract agar, 25 °C, 3 days**



***Aspergillus flavus*, Lactophenol blue, 400x**

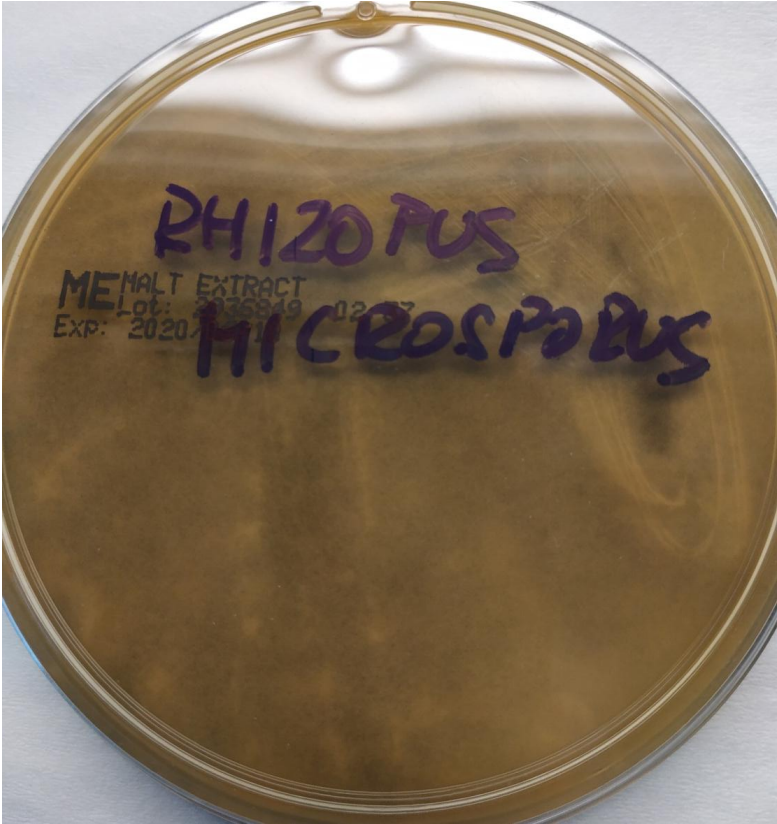


Rhizopus microsporus, Sabouraud agar

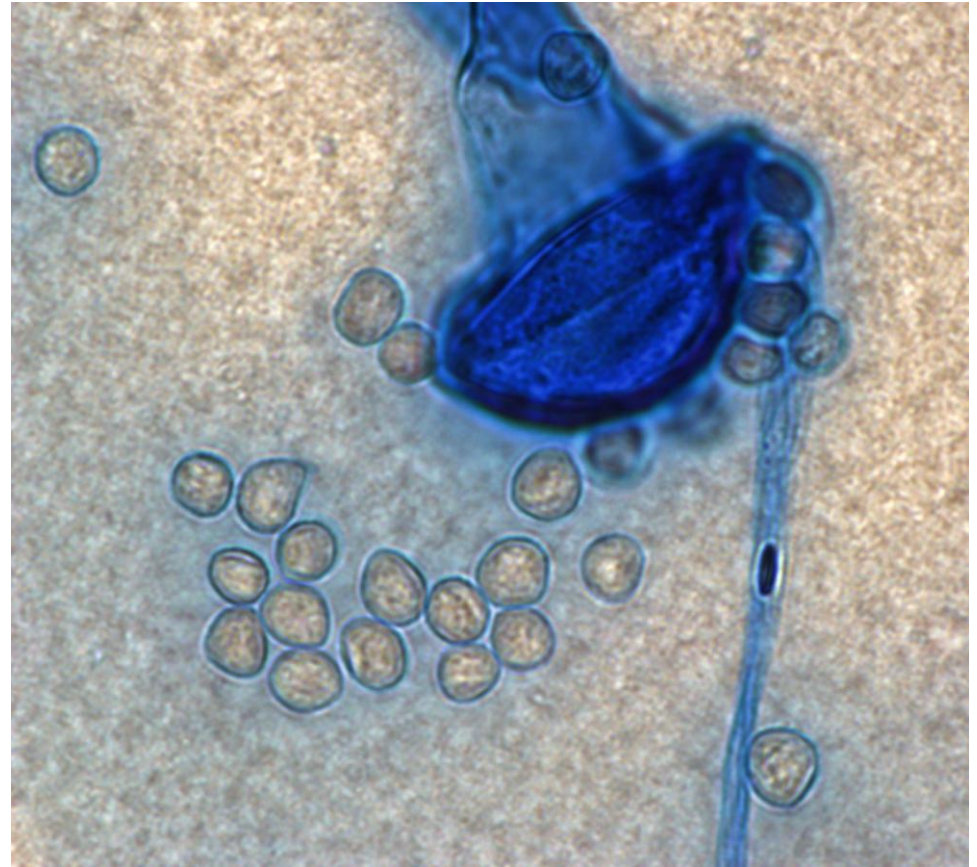
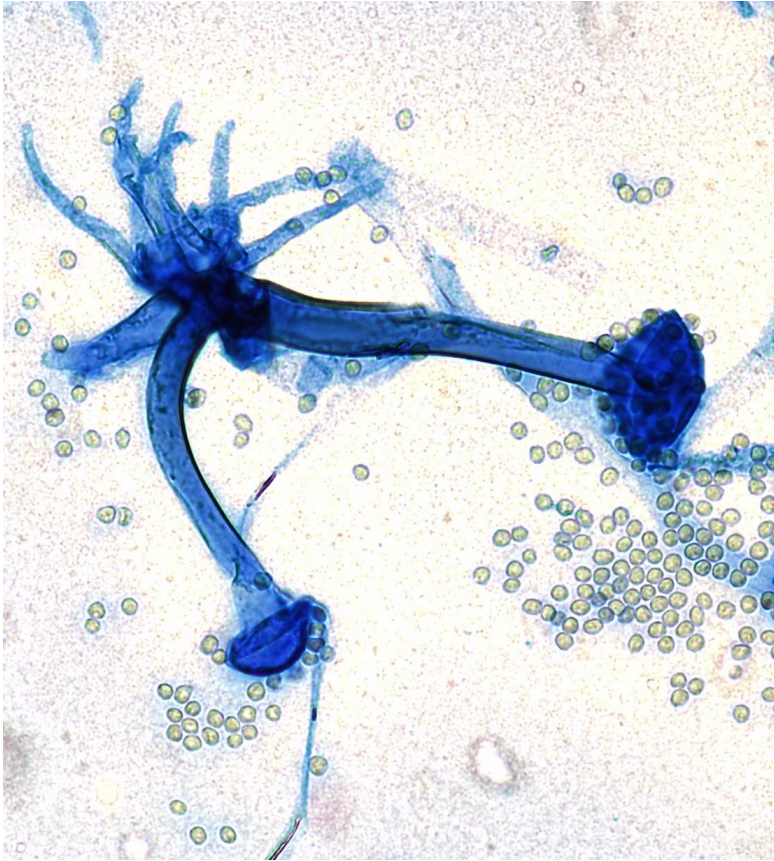


Rhizopus microsporus

Malt extract agar, 25 °C, 48 h



Rhizopus microsporus, lactophenol blue



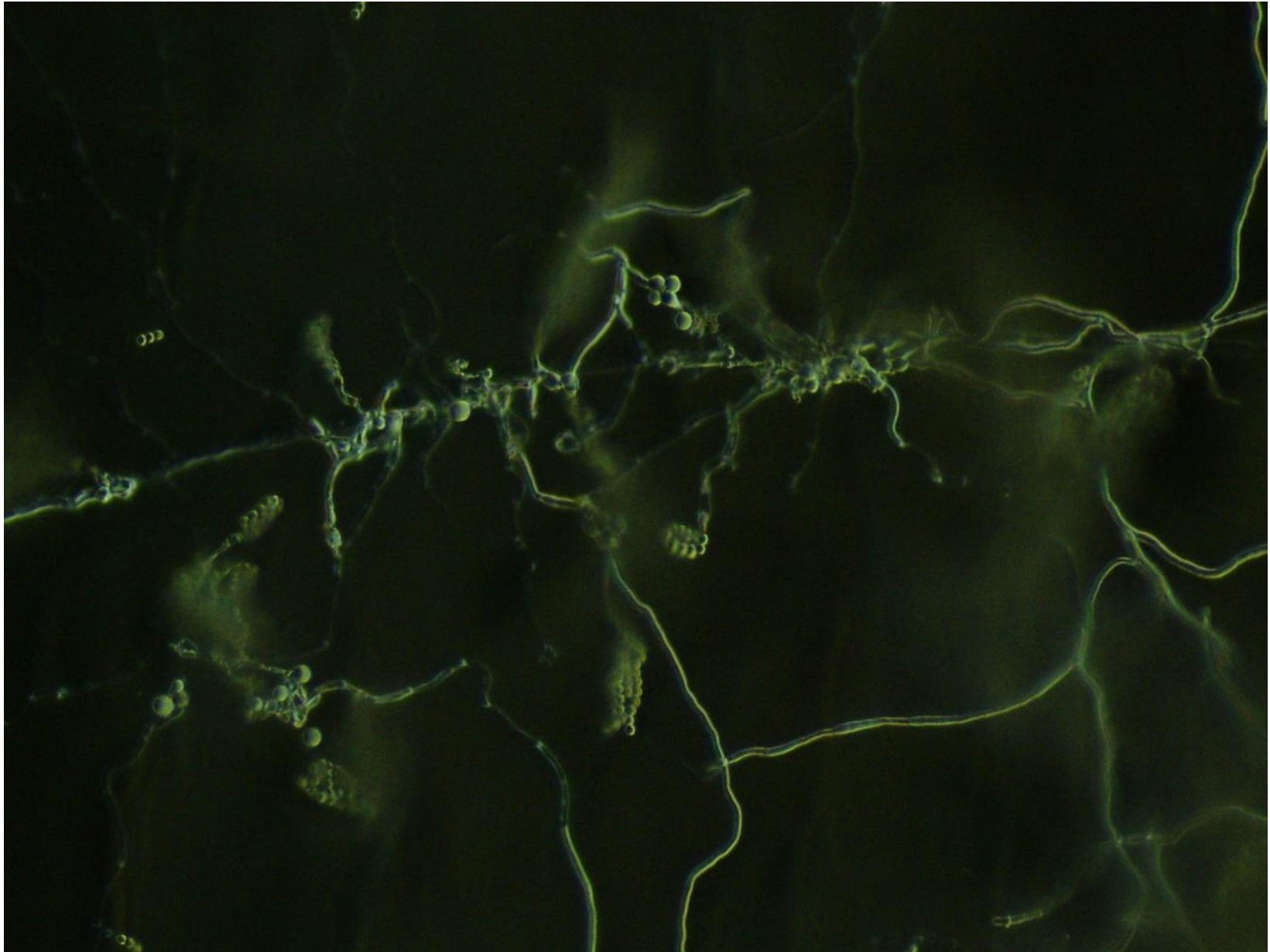
Filamentous fungi – slide culture

Small piece of agar with
inoculated fungus is
placed between slides
and incubated in wet
chamber

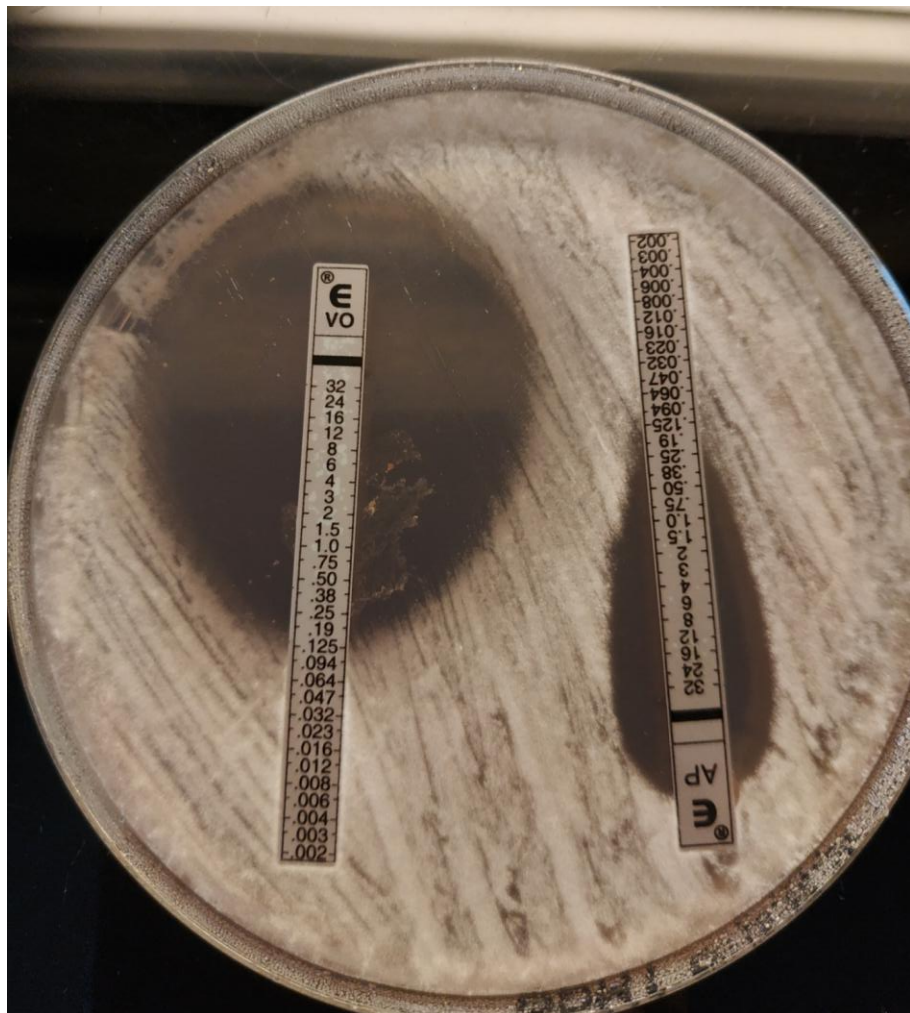
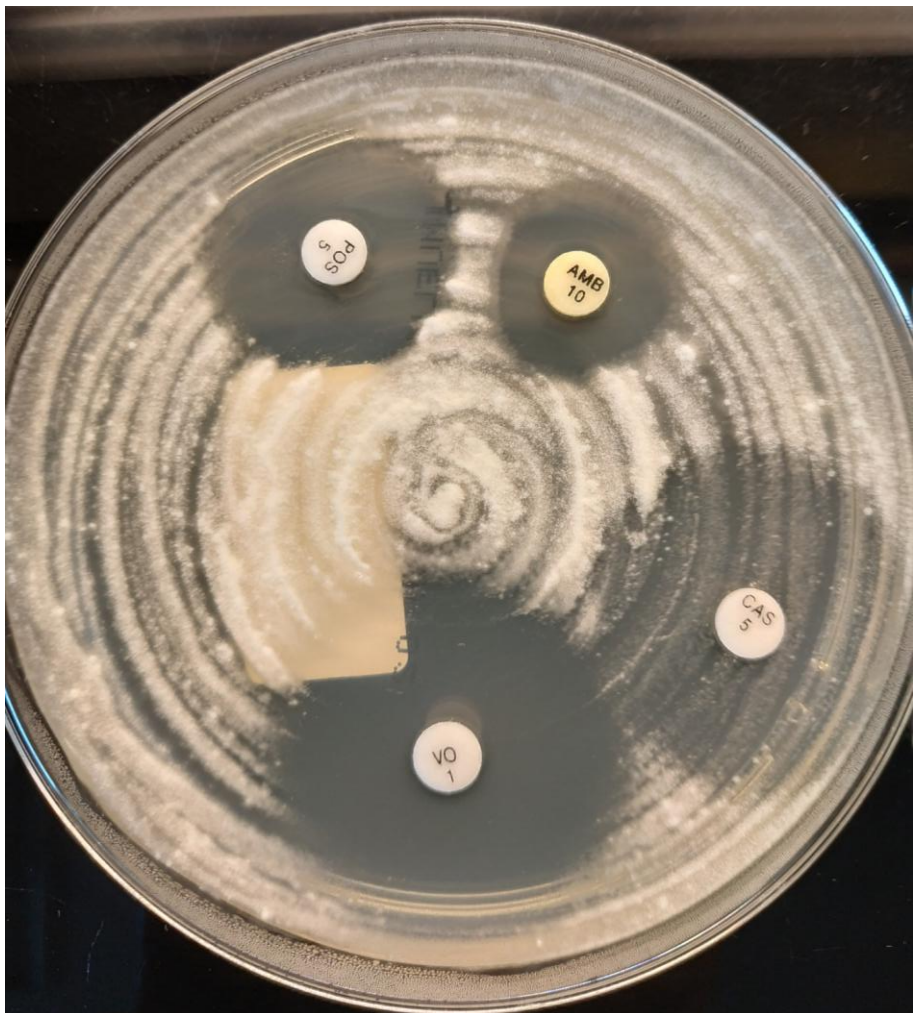
Culture is subsequently
observed under
microscope



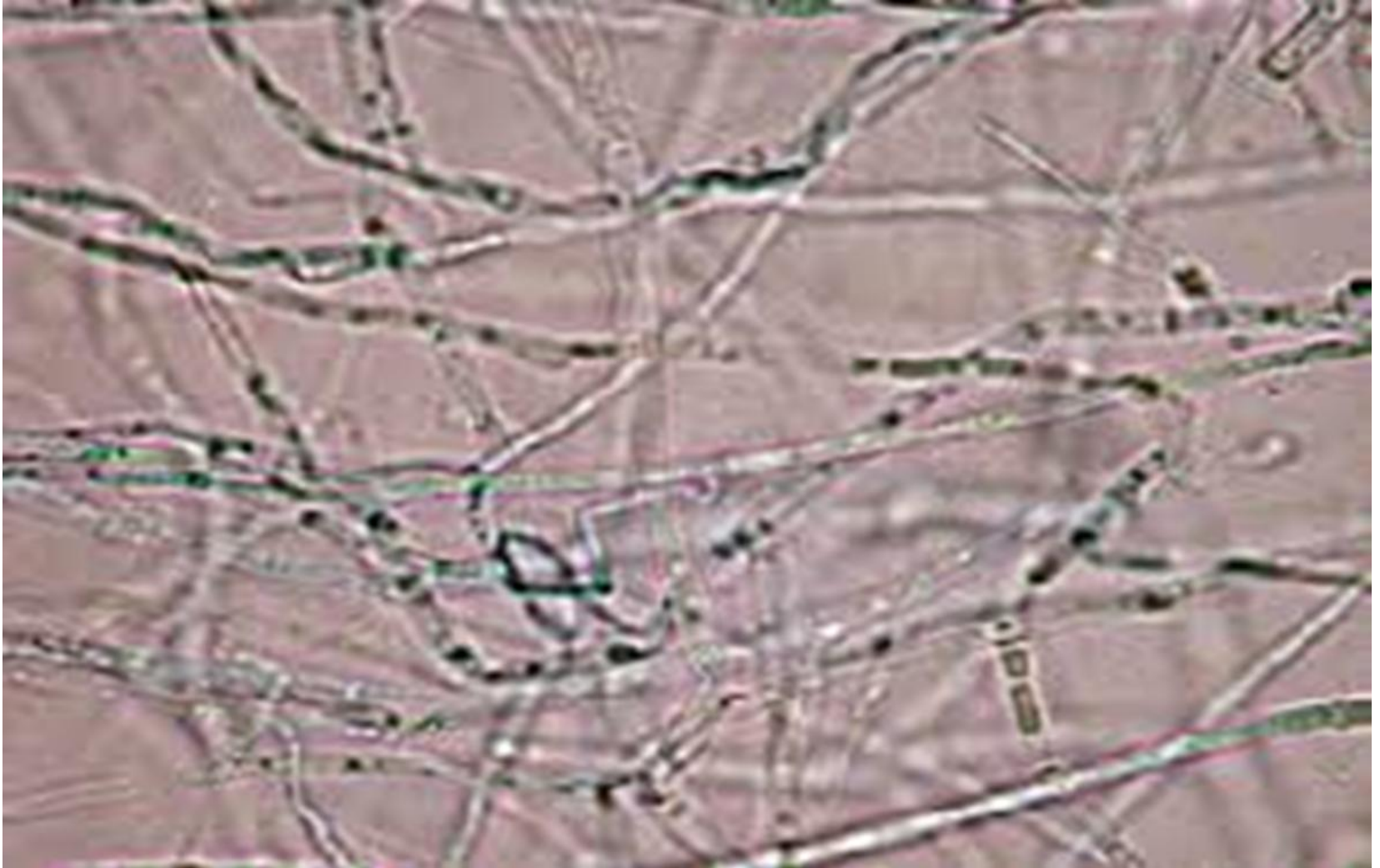
Slide culture, *Aspergillus* sp.



Antifungal susceptibility testing – *Aspergillus fumigatus*



Wet mount, KOH, dermatophytes



Dermatophytes

Microscopy

Wet mount with KOH (+- Myco-ink)

Culture

Sabouraud agar with 2 % glucose, cycloheximide for selectivity, up to 6 weeks, 28 °C

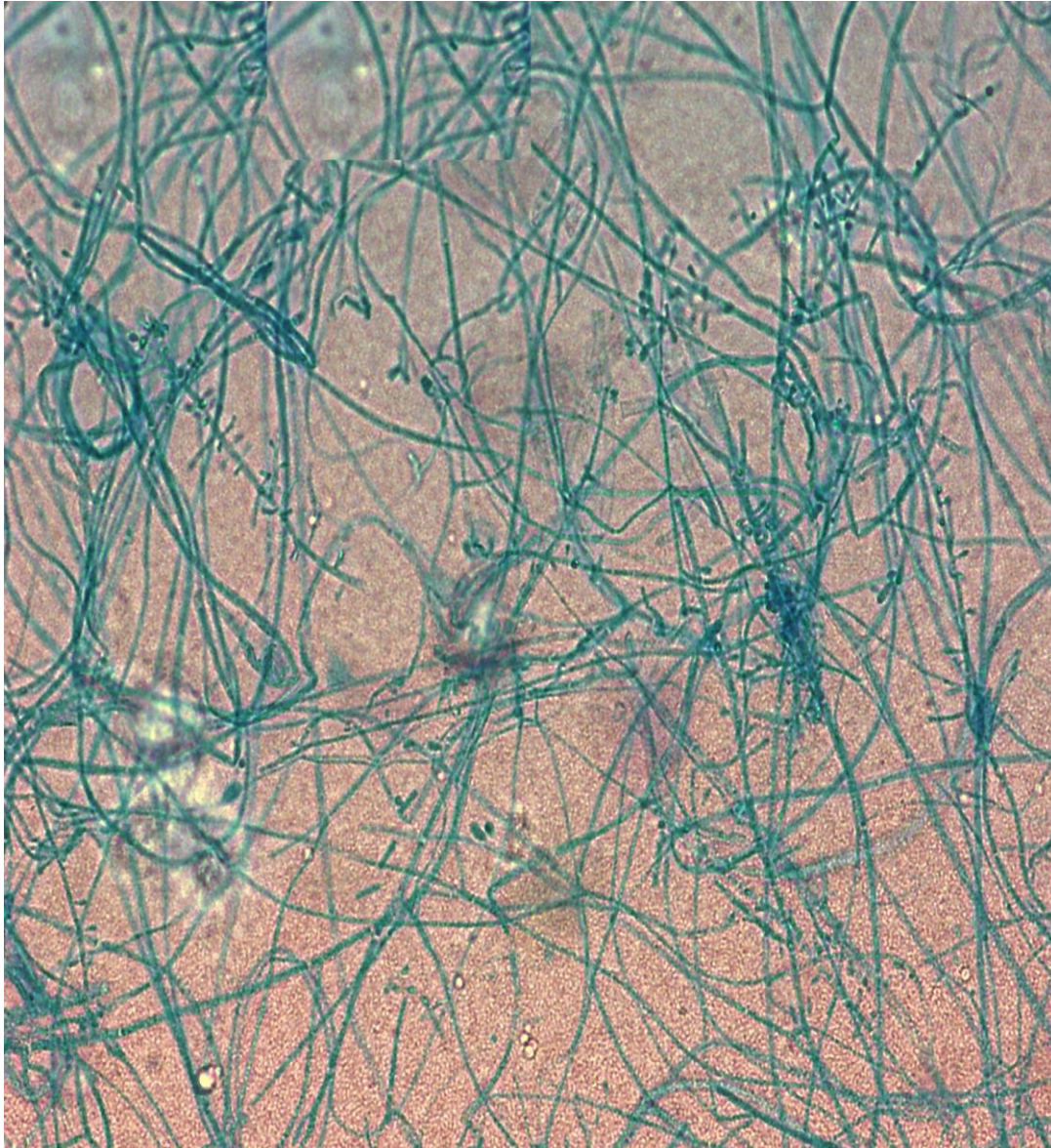
Antifungal susceptibility testing – standardized procedure available

PCR – multiplex, nails/skin/hair, not generally recommended

Trichophyton rubrum, Sabouraud agar



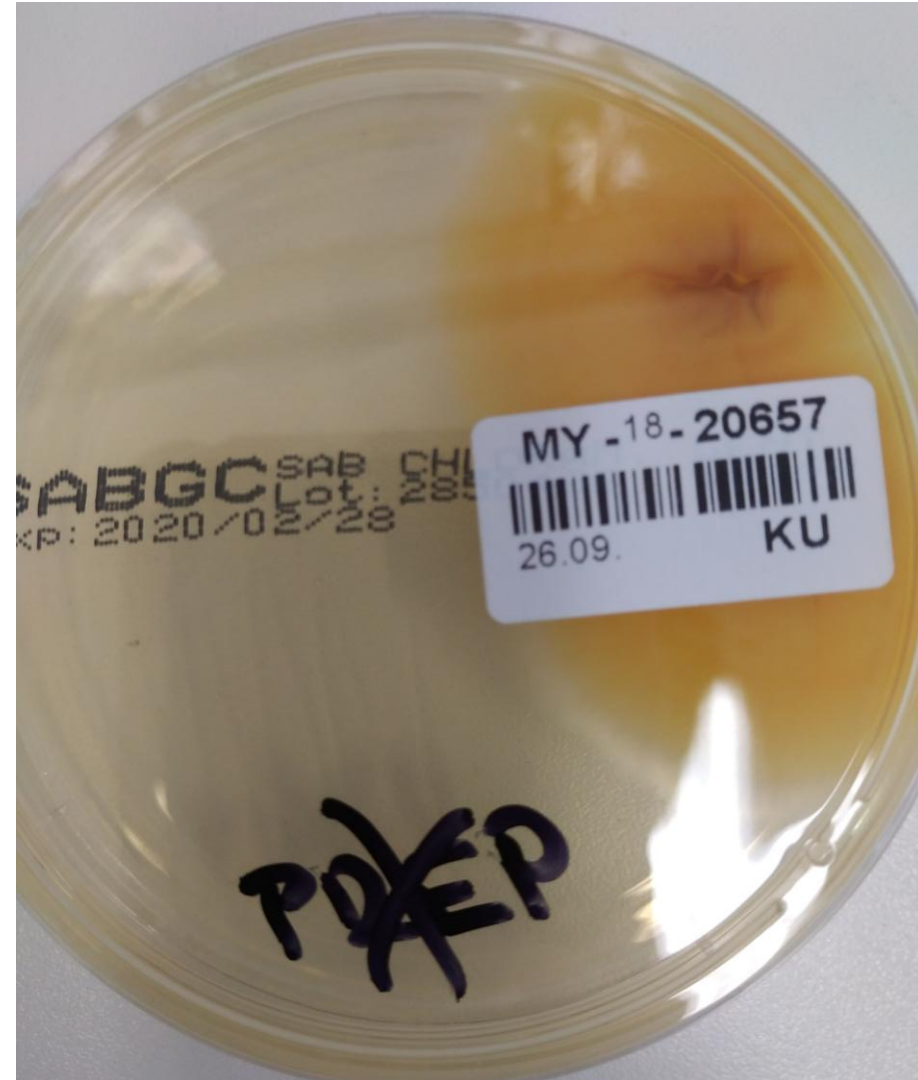
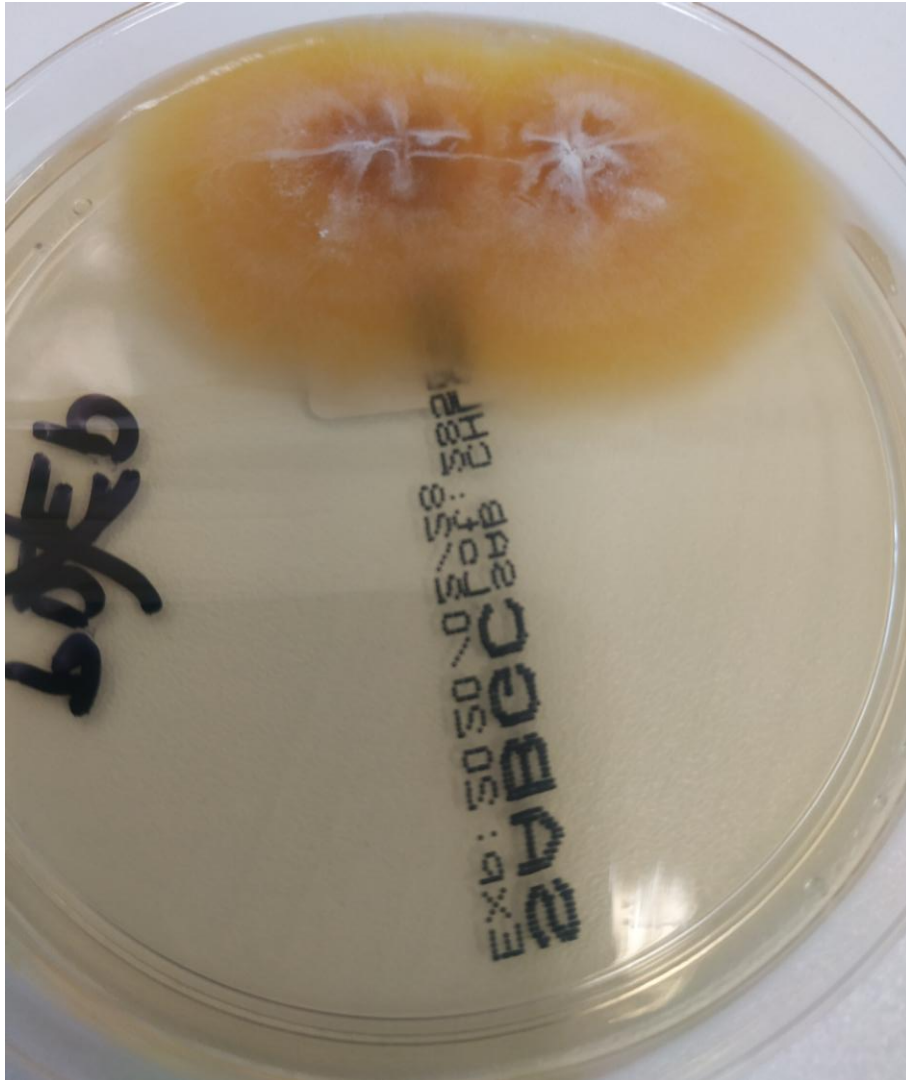
Trichophyton rubrum, latophenol blue



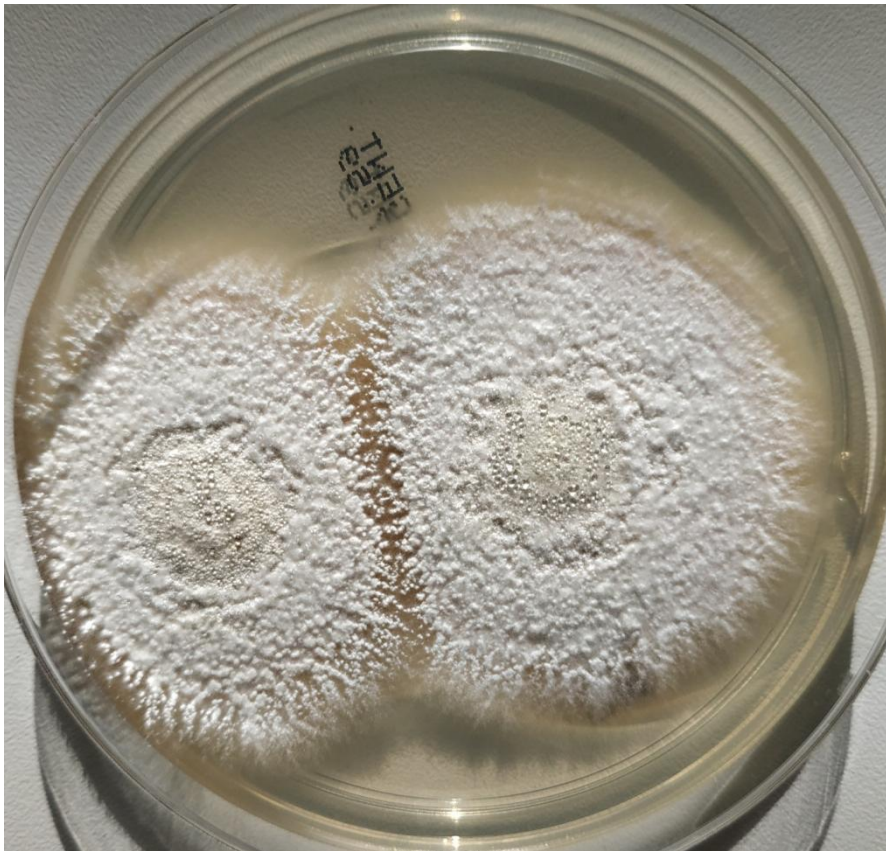
Trichophyton rubrum, microconidia



Trichophyton benhamiae, Sabouraud agar



Trichophyton mentagrophytes, Sabouraud agar



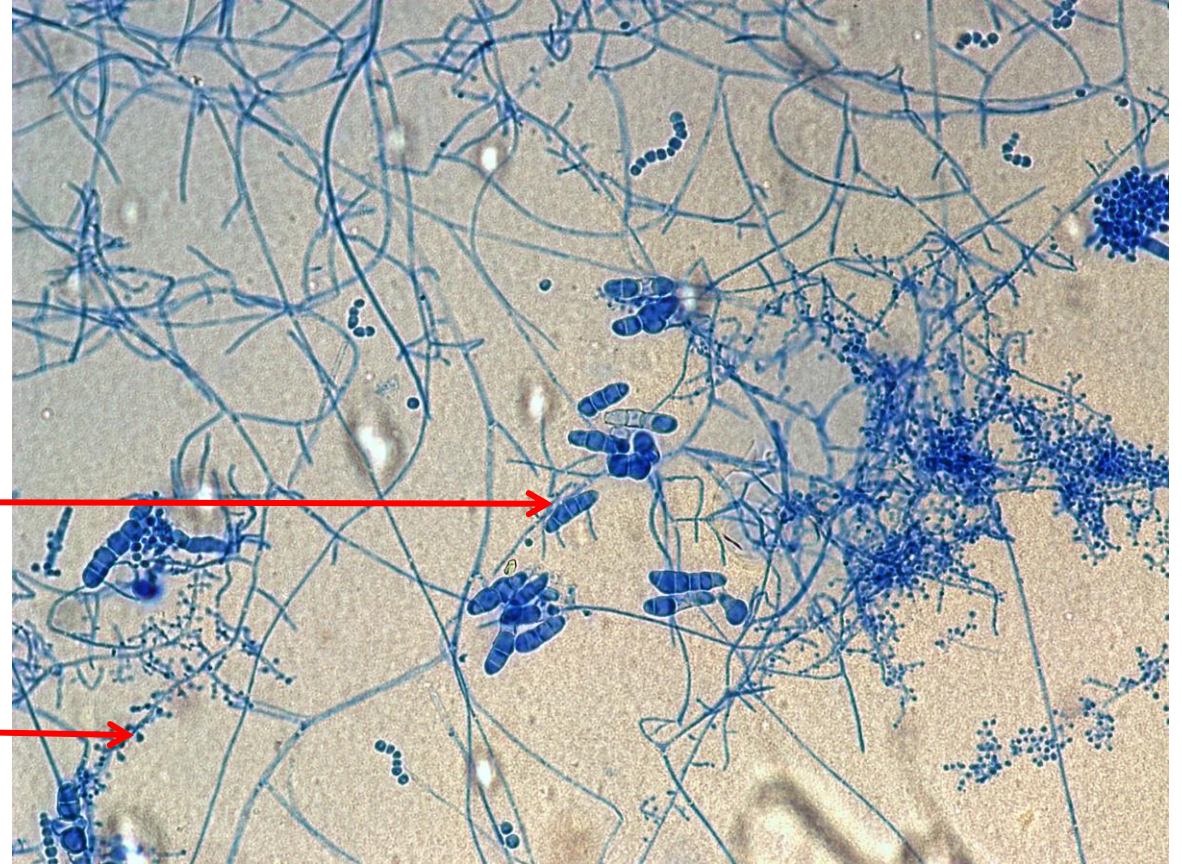
*Trichophyton
mentagrophytes*

Lactophenol blue,
400x

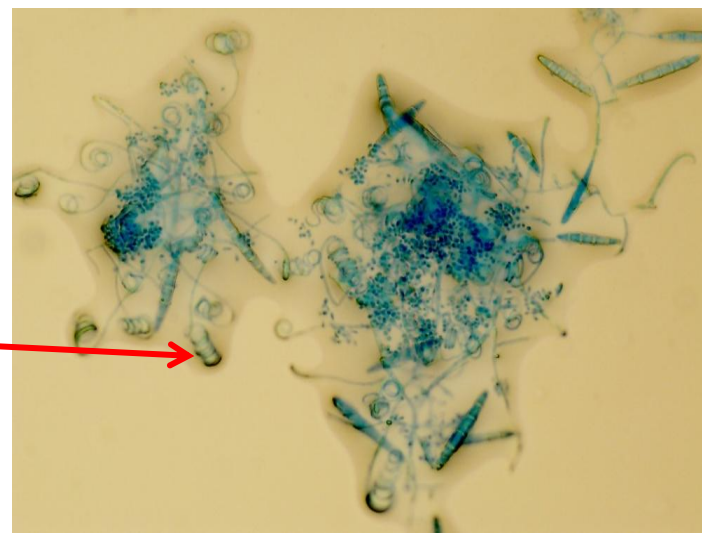
macroconidia



microconidia



Spiral hyphae



Pneumocystis jirovecii

Opportunistic pathogen transmitted from humans

Interstitial pneumonia, respiratory failure, low oxygen saturation

Specimen: Lower respiratory tract secretions/aspirate – ideal
bronchoalveolar lavage fluid, 4th portion

Culture impossible

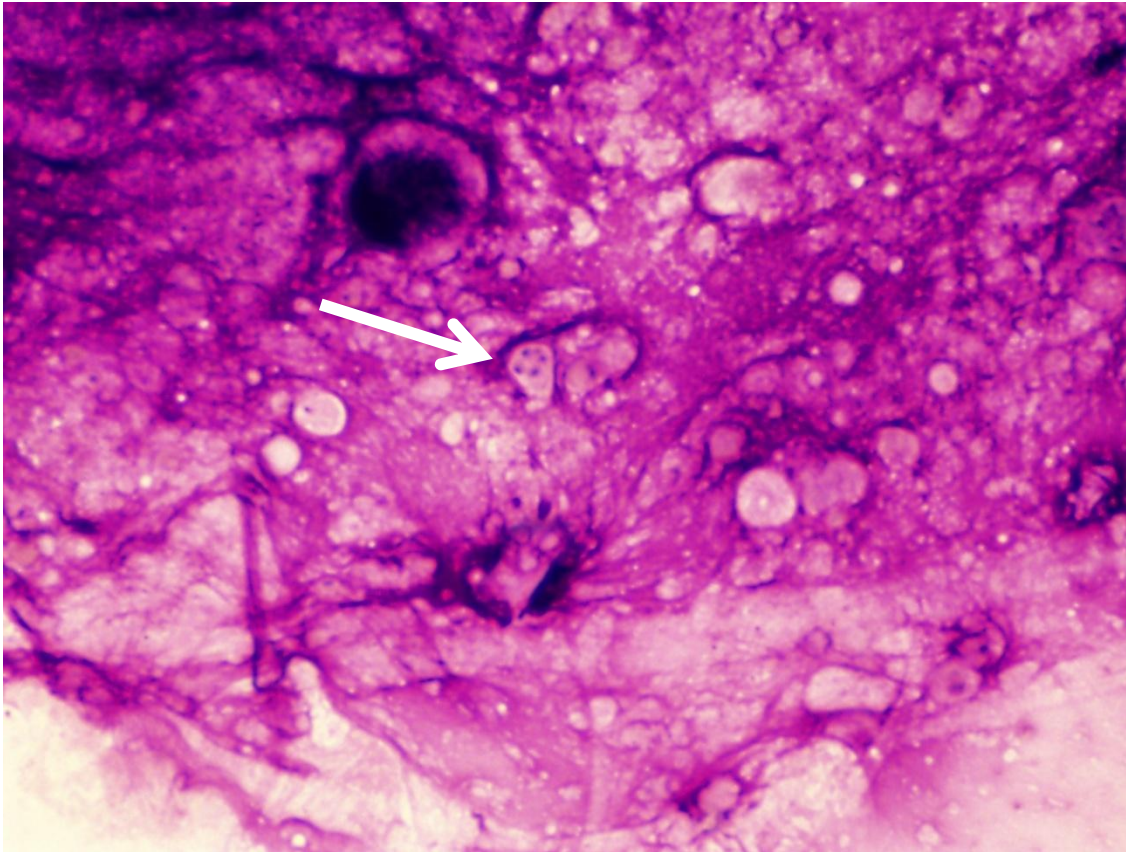
Microscopy of clinical material:

- Giemsa stain** (trophozoites, precysts, cysts)

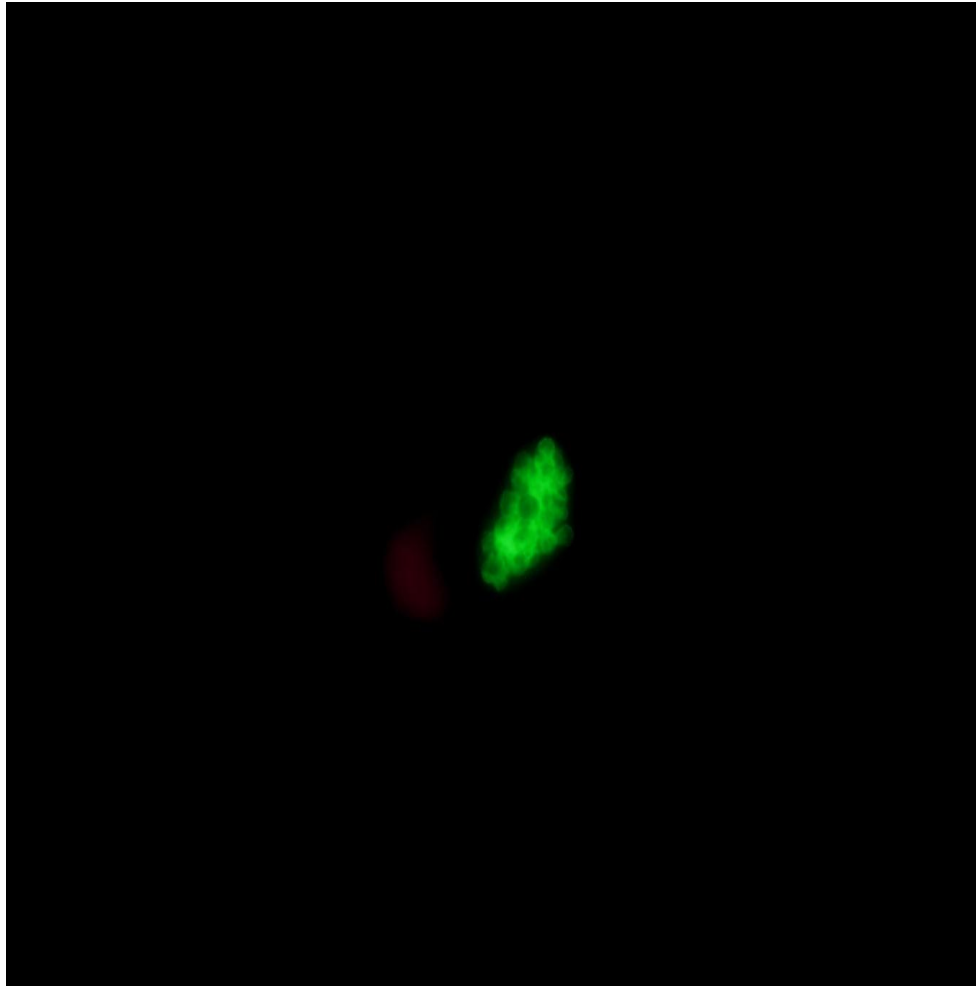
- Immunofluorescence** – cysts, trophozoites and intercellular matrix detected by fluorescein-conjugated monoclonal antibody

Targeted PCR – also performed from nasopharyngeal swab, high sensitivity, difficult to distinguish between colonization and infection (beta-D-glucan discriminatory)

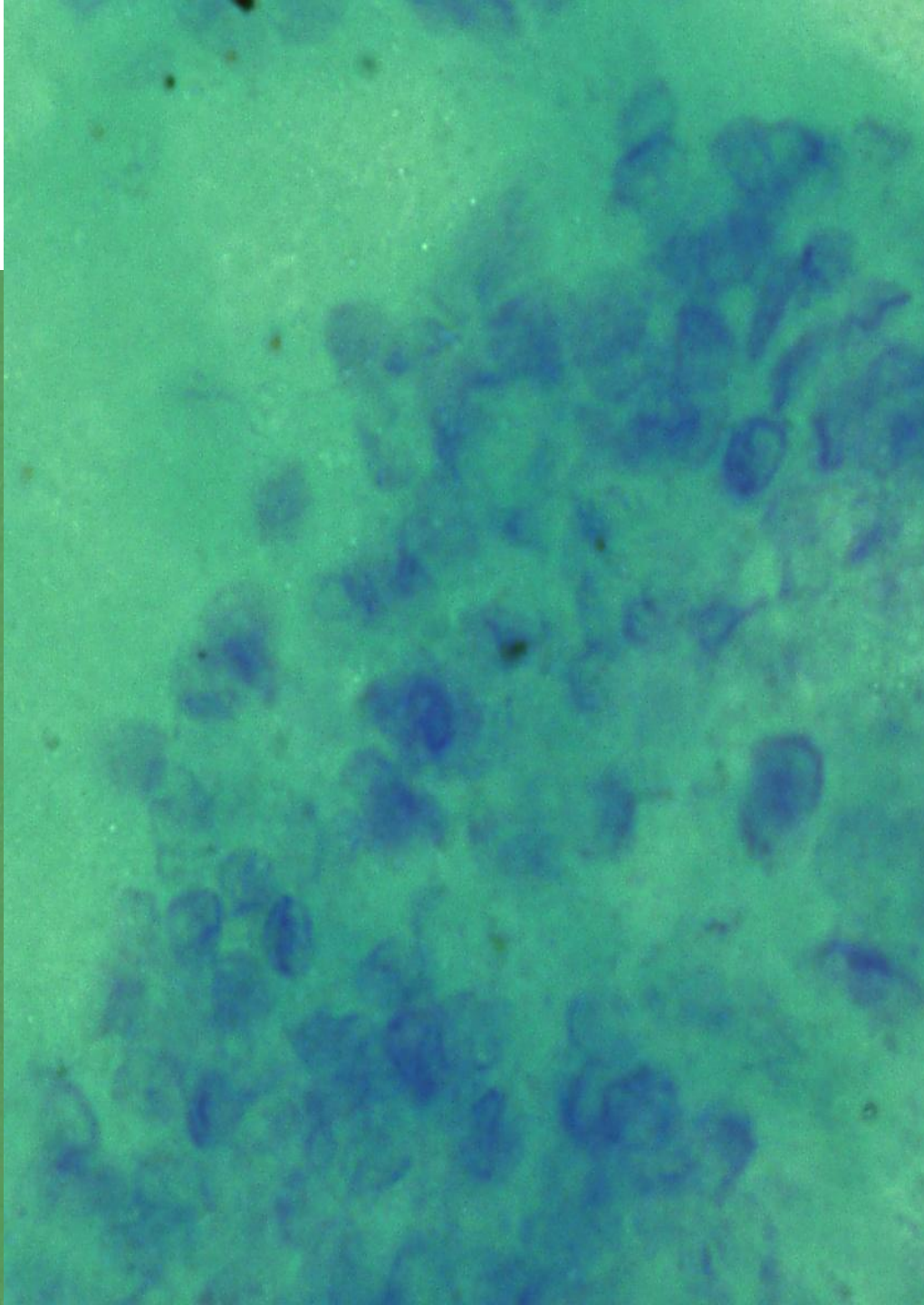
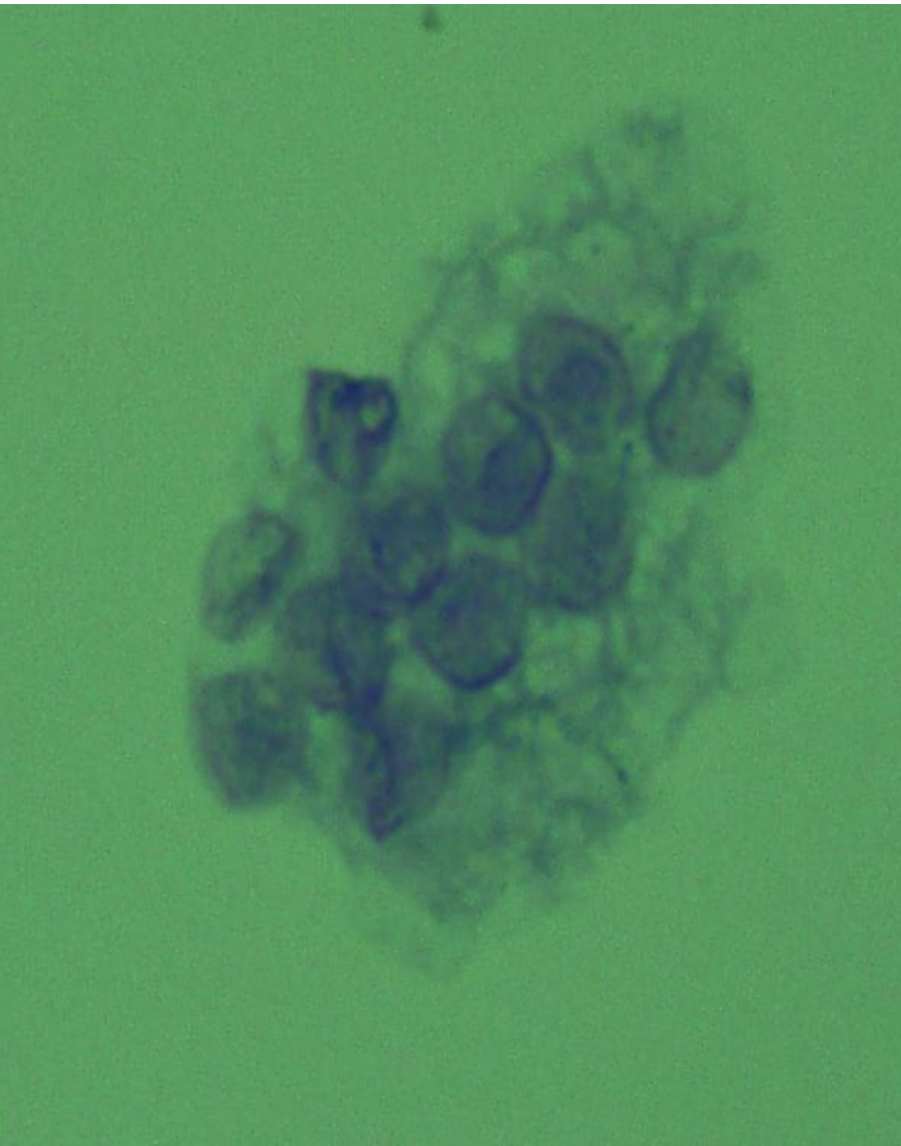
Giemsa stain, *Pneumocystis jirovecii*



Pneumocystis jirovecii, immunofluorescence, BALF



Toluidine blue, *P. jirovecii*, BALF



Biomarkers in medical mycology – overview

Antigens

Glucuronoxylomannan - *Cryptococcus* sp.

CSF, serum, urine

Standardized, high sensitivity and specificity, recommended in diagnosis

LFA, latex agglutination

Galactomannan – *Aspergillus* sp.

Serum – for monitoring of neutropenic patients without antifungal prophylaxis

BALF – suspected pulmonary aspergillosis, PCR as additional marker - combination

CSF – Suspected central nervous system aspergillosis

ELISA, LFA

Beta-D-glucan - panfungal“ antigen

- serum

- negative in cryptococcosis and mucormycosis

- Disseminated candidiasis with negative blood culture

- Pneumocystis jirovecii* pneumonia



Mannan/antimannan – *Candida* sp., may support dg of disseminated candidiasis

PCR – tissue (paraffin embedded, too), BALF, serum, CSF, plasma...

Cryptococcus, *Pneumocystis jirovecii*, aspergilli– standardized

Useful in rare mycoses, too – *Mucorales*, panfungal assays

Thank you