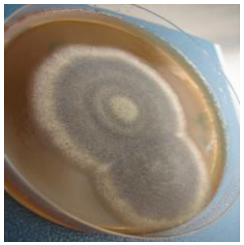
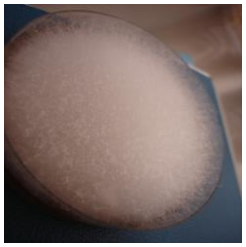
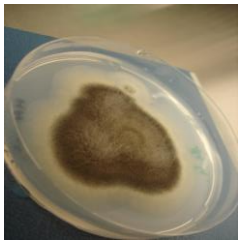
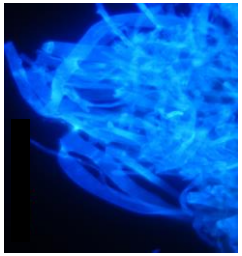






How to make a fast diagnosis

8th Advances Against Aspergillosis
1 – 3 February 2018
Lisbon, Portugal

Cornelia Lass-Flörl
Division of Hygiene and Medical Microbiology
Innsbruck Medical University

Division of Hygiene & Medical Microbiology, Medical University of Innsbruck, Austria

Medical University Hospital, 2400 beds

(covers most important medical disciplines)

Hard facts

endogenous microflora

fungi can be both colonizers and pathogens

organisms from sputum or GI do not necessarily indicate infection

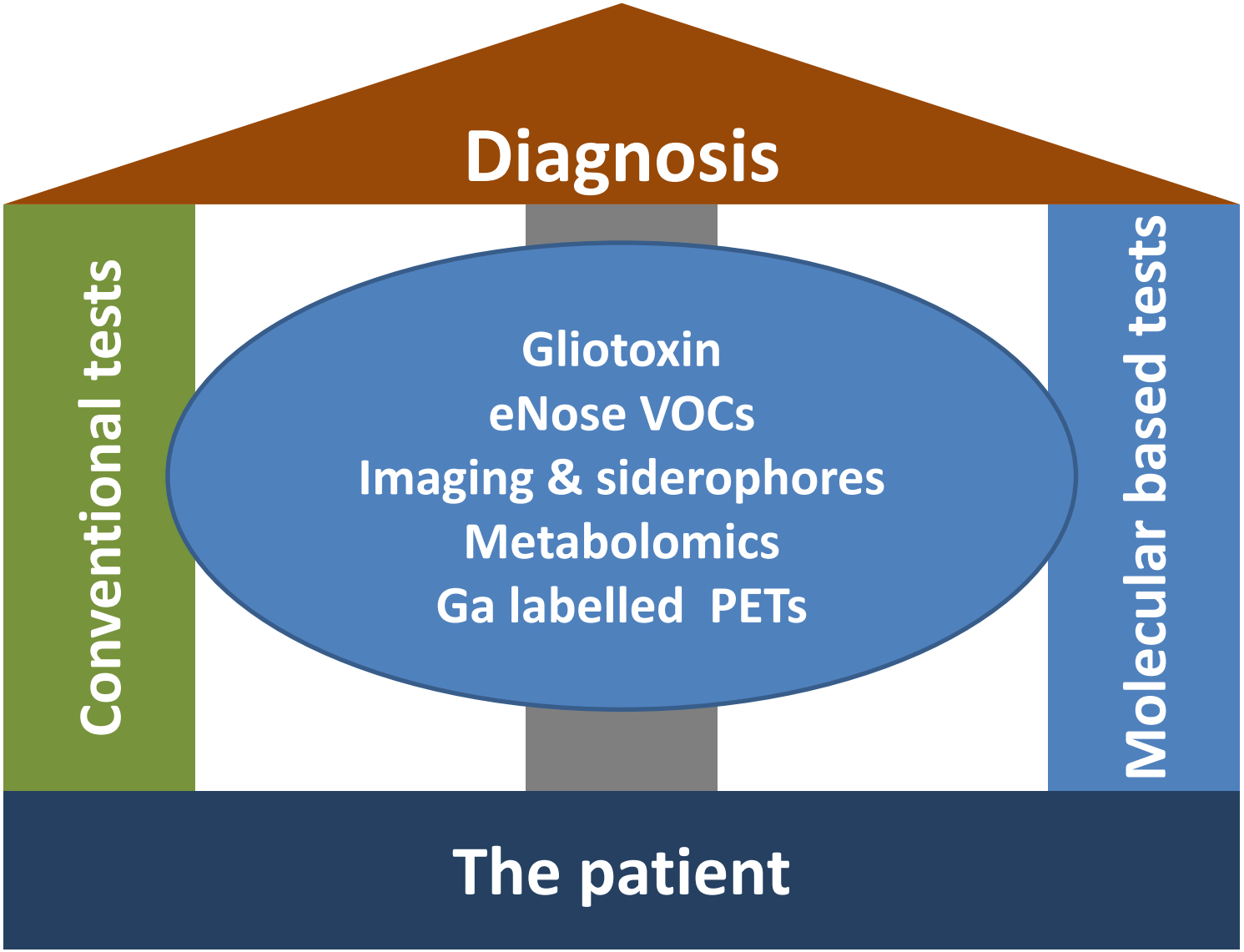
clinical manifestations are non-specific

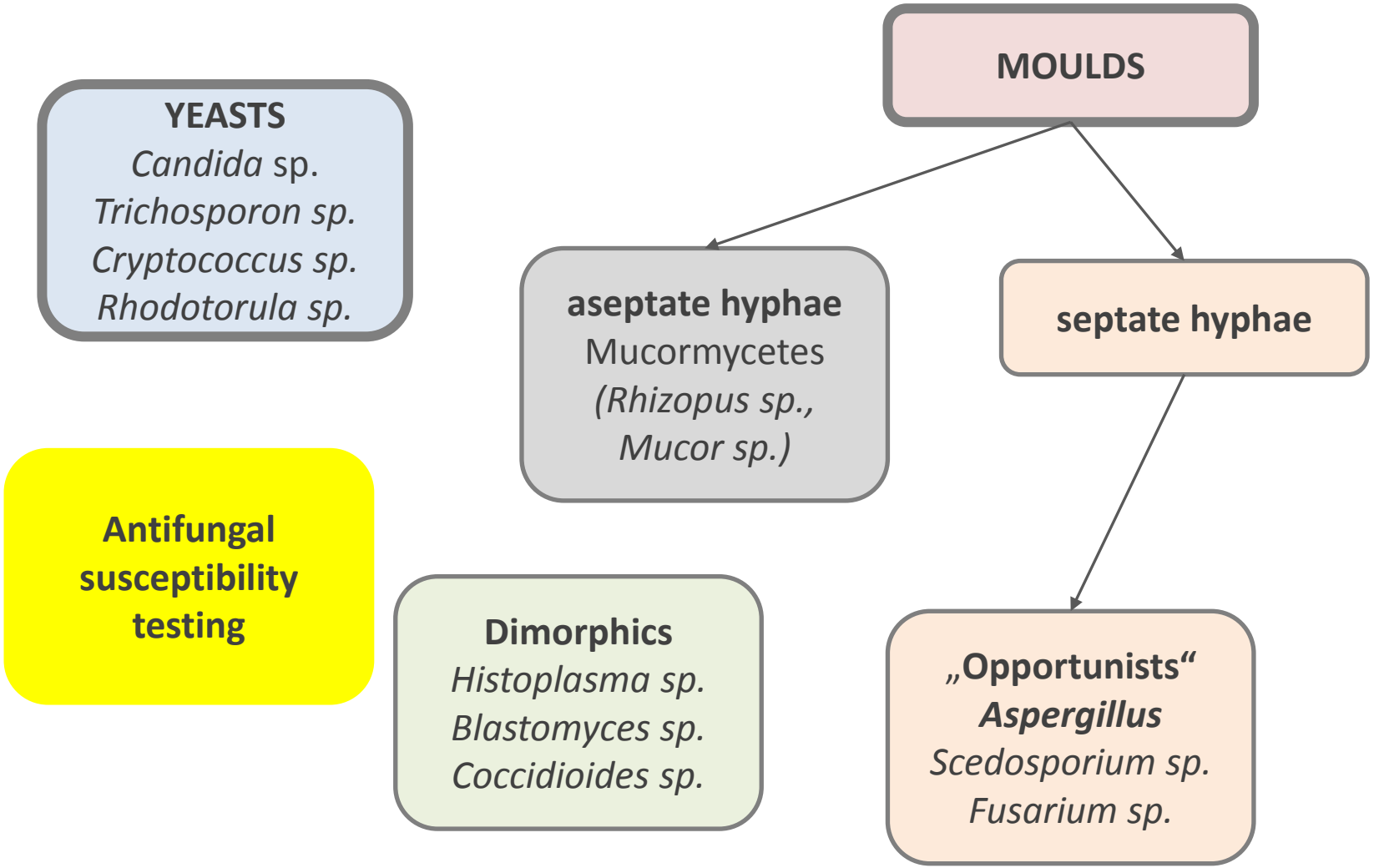
direct examination or cultures from sterile sites are the golden standard

conventional diagnostic tests are insensitive, positive late

patients with disseminated infections may have negative blood cultures

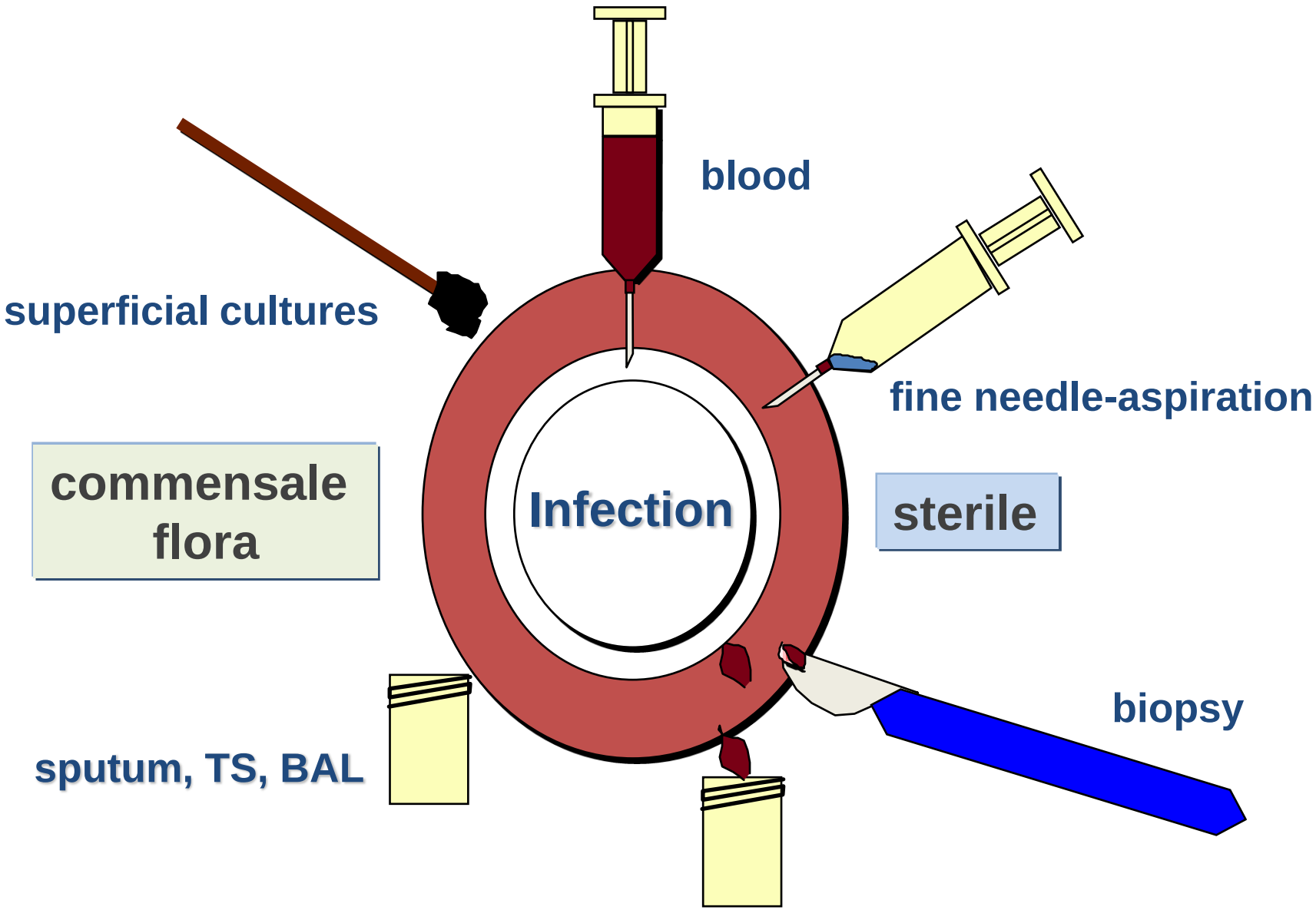
vigilance is required in the interpretation of superficial cultures, antigen tests, PCR screening, presence of antibodies and/or metabolites


Diagnosis
Conventional tests
Gliotoxin
eNose VOCs
Imaging & siderophores
Metabolomics
Ga labelled PETs
Molecular based tests
The patient

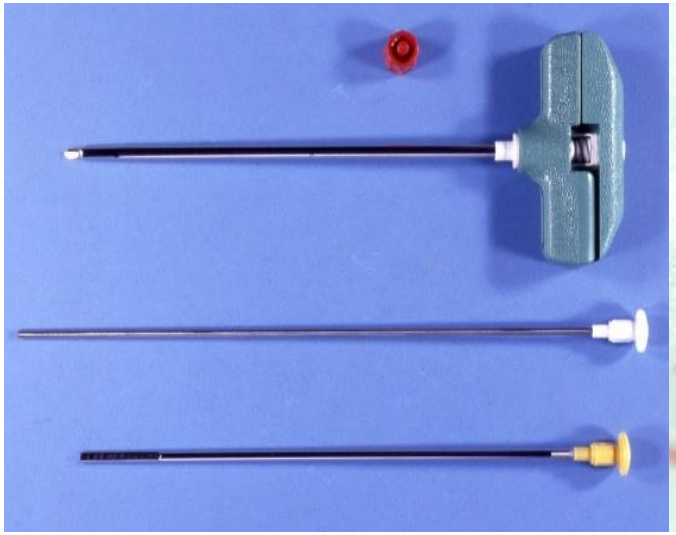


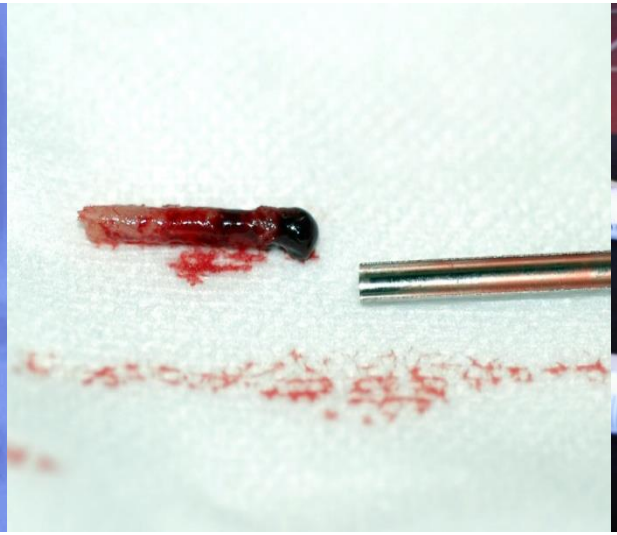
What is the need?

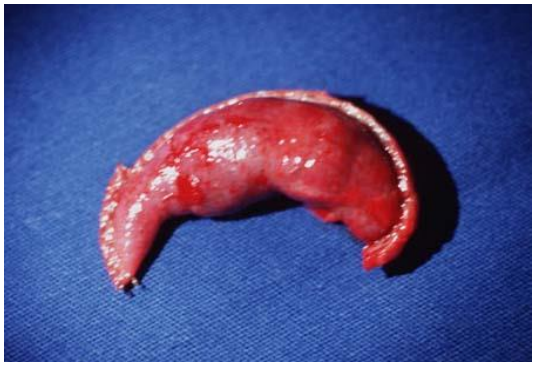
The human specimen

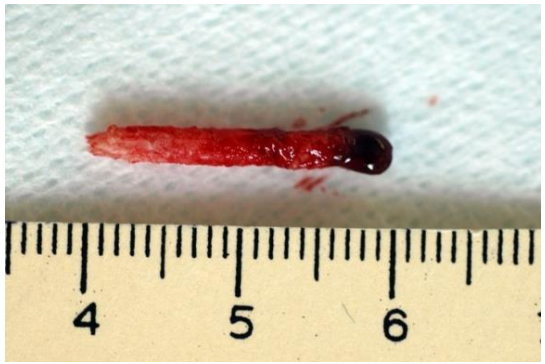


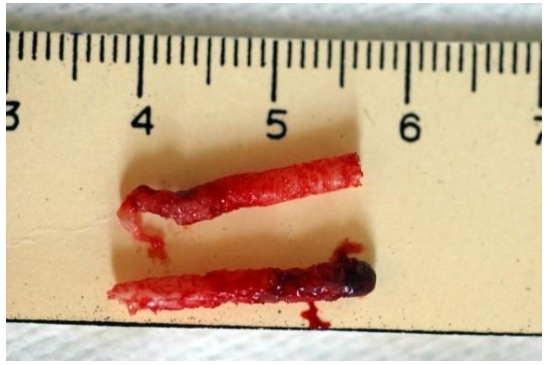
Big trocar, big sample

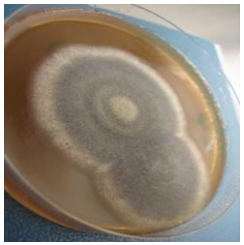


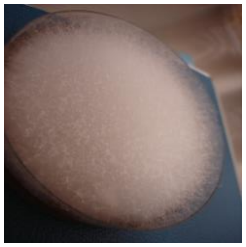


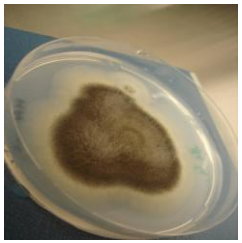


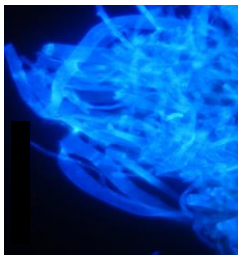












Microscopy & Culture „must have“

Sputum

Digest to remove debris

Negative stain for capsule

KOH mount (wet mount)

Special stains brighteners

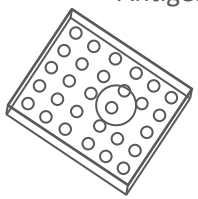
Culturing: incubation for up to 8 weeks

Selective agar
Room temperature
25°C or 30°C

Demonstrate dimorphism

Blood agar

- Differential media
- Biochemical tests
- Antigen tests



Blood, Cerebrospinal Fluid

Pus, Vaginal Secretions

KOH mount

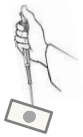
Negative stain for capsule

Direct stains of specimen

Isolation

25°C or 30°C;
Selective media

Stain colonies



Macroscopic morphology

37°C
BHI/Blood agar

Observe macroscopic morphology

- Differential media
- Biochemical tests
- Antigen tests
- Germ tube test
- Genetic probes
- Serological tests

Hair, Skin, Nails

Use of wood's light on hairs

KOH
wet mount

Highlight with brighteners; observe microscopically

Implant specimen on selective media; incubate for 4 weeks

Stain with periodic acid-Schiff (PAS)

Observe macroscopically for pigment texture

Perform hair infection test by inoculating sterile hair with culture isolates

Observe microscopically for conidial and hyphal morphology

Tissue Biopsies, Punches

Section

Grind

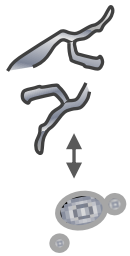
Digest

Perform histological stains

Implant onto media selective and differential for fungi

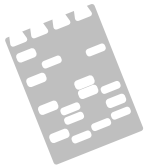
25°C - 37°C

Test for dimorphism

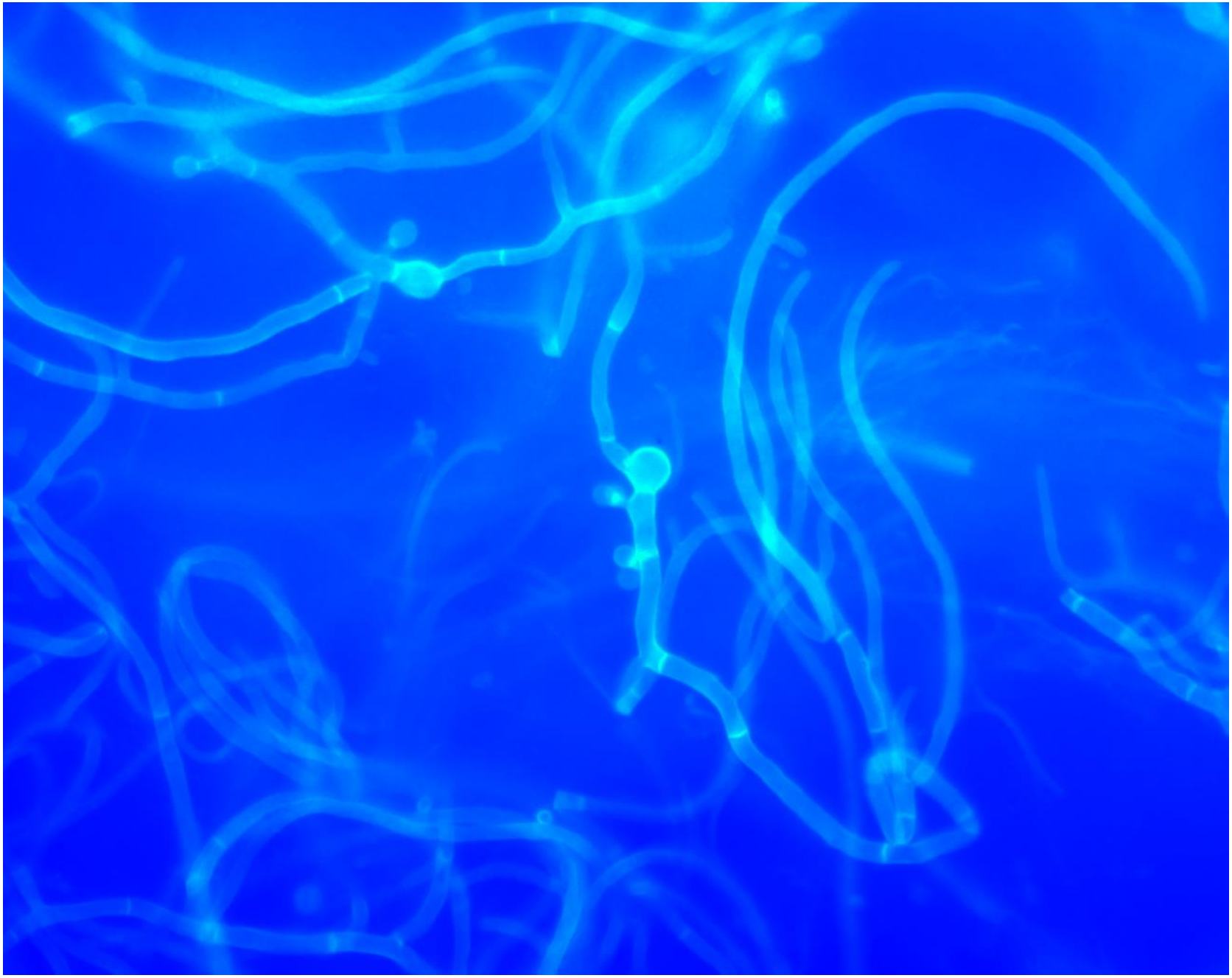


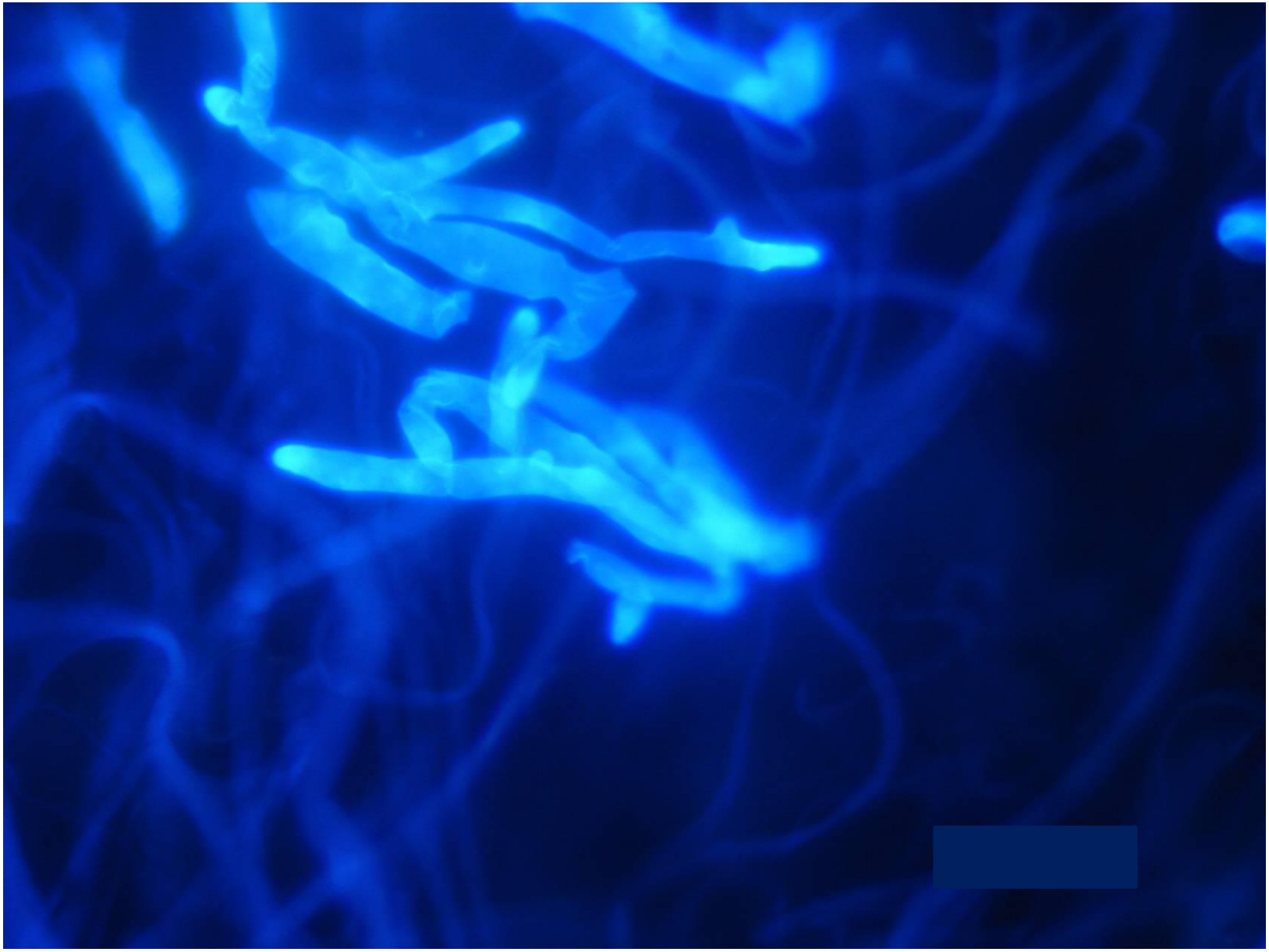
37°C

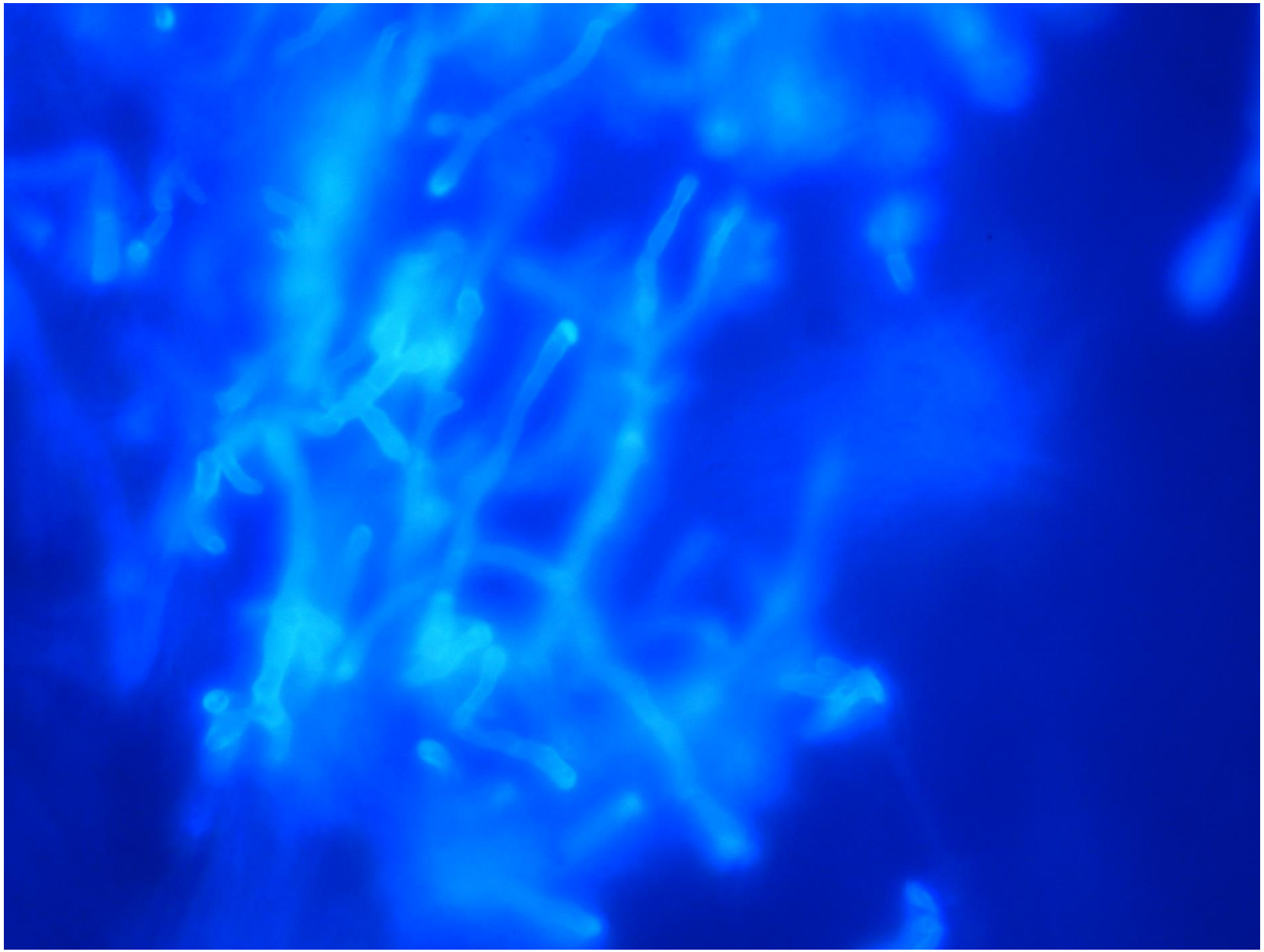
Inspect microscopic morphology after staining

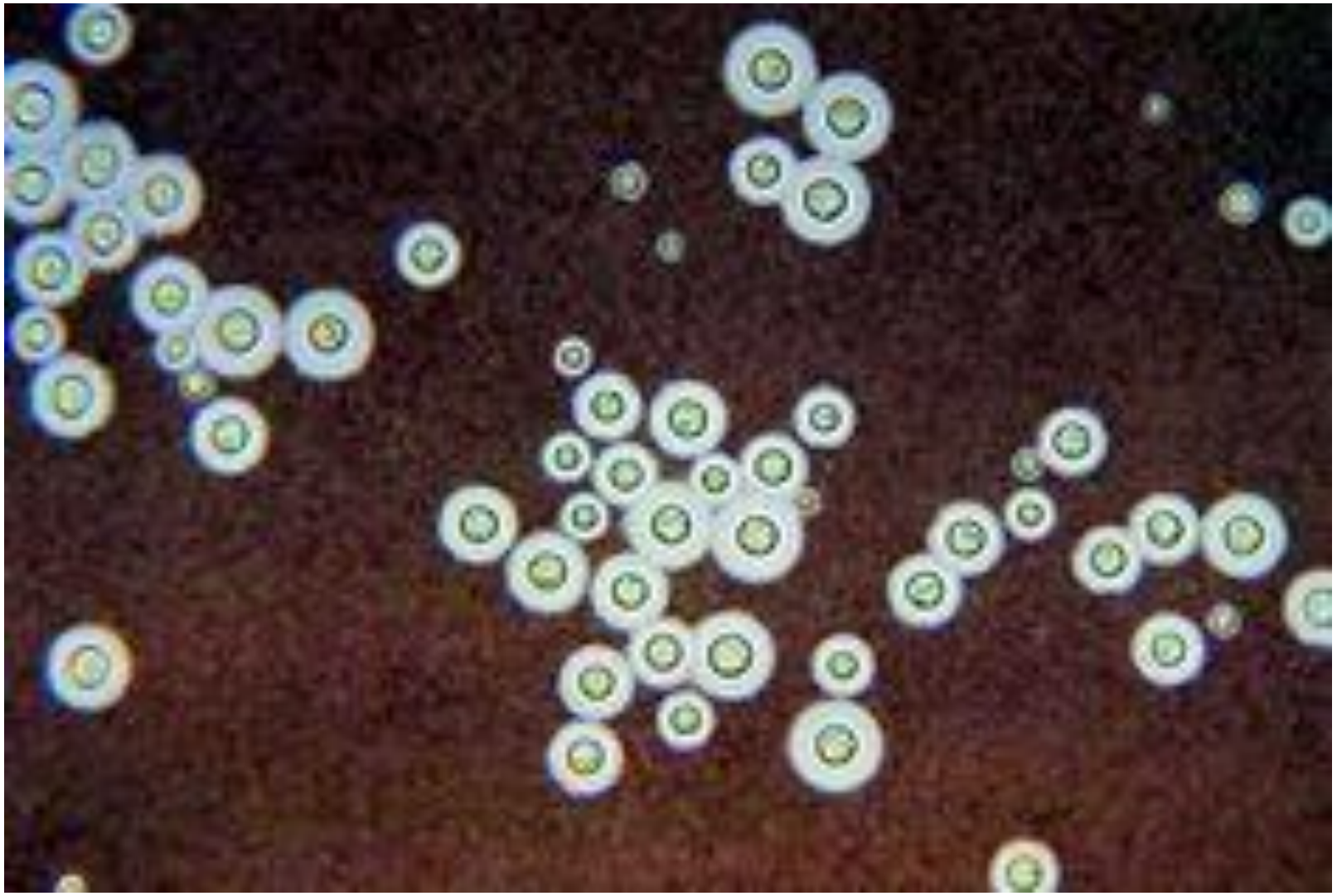


- Specific nucleic acid probe
- Selective, differential media
- Biochemical tests









Microbiology best practice recommendations

Microscopy and stains

- Fluids from usually sterile sites and bronchoalveolar lavage (BAL) from patients with suspected infection should be examined by direct microscopy with suitable methods for fungal detection.
- Adequate tissue for histology (microscopy) and culture should be ensured before processing the rest of the sample.

✓ **Optical brighteners are recommended for microscopy on all samples from immunocompromised patients.**

✓ **Direct fluorescent-antibody staining, PCR, or both is recommended for patients with suspected pneumocystis infection.**

✓ **India ink staining of cerebrospinal fluid samples from immunocompromised patients is recommended in addition to Gram staining if *Cryptococcus* capsule antigen (CRAG) testing is not available on site.**

BSMM standards, Lancet Infect Dis 2015 15:461-74

Histopathology best practice recommendations

Specialised stains

Specialised stains should be done in parallel with standard stains if mycosis or another infection is to be assessed or excluded

- Standard stain: haematoxylin and eosin (H&E) on histopathology slides; Giemsa or Papanicolaou on smears.
- Triple set of stains: Ziehl-Neelsen stain for acid-fast organisms; Gram stain for bacteria, fungi, and others; Grocott silver stain, or periodic acid-Schiff, to highlight fungi.

BSMM standards, Lancet Infect Dis 2015 15:461-74

Histopathology best practice recommendations

Reporting of results

Report fungal morphology (yeast or hyphae), including the following:

- Whether a yeast is small, medium, or large.
- Whether a yeast has cross walls or septa (ie, is splitting rather than budding).
- Whether a hyphal form has usual width, or has a dilated, bizarre shape.
- Whether H&E stained fungi are pigmented and brown, or are unpigmented and colorless or pale blue.

Positive results should be telephoned to clinicians immediately.

BSMM standards, Lancet Infect Dis 2015 15:461-74

Microbiology best practice recommendations

Culture and identification

- Bronchoscopy fluids should be cultured in suitable media to support fungal growth.
- Yeasts cultured from urine samples should be identified to specie level and reported for all critical care and immunocompromised patients.

✓ **All clinical isolates of *Aspergillus* from patients who will receive antifungal treatment should be identified to species complex level, by referral to a specialist laboratory if necessary.**

✓ **All fungi (yeasts and moulds) obtained from sterile sites, including blood and continuous ambulatory peritoneal dialysis fluids, and intravenous line tips should be identified to species complex level by referral to a specialist laboratory if necessary.**

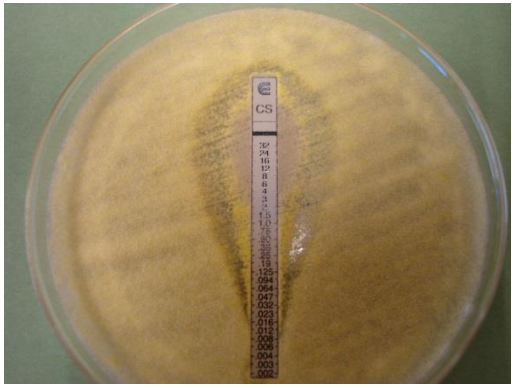
✓ **Bronchoscopy fluid and paranasal sinus material is regarded as sterile in this context for all fungi except *Candida* spp.**

BSMM standards, Lancet Infect Dis 2015 15:461-74

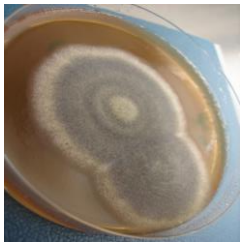
Microbiology best practice recommendations

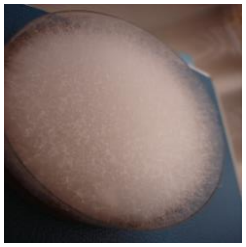
Antifungal drug-susceptibility testing

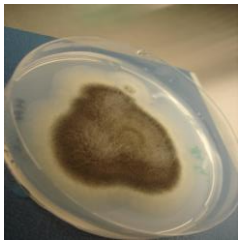
- ✓ **Isolates of *Candida* spp from sterile sites, or from patients not responding to therapy at a minimum should have their susceptibility tested against fluconazole.**
- Isolates of *Aspergillus fumigatus* should have their susceptibility tested against antifungal agents used locally for treatment (eg, itraconazole and voriconazole) if antifungal treatment is given.
- ✓ **Antifungal susceptibility testing of *Aspergillus* isolates should be performed in patients who are unresponsive to antifungal treatment, for epidemiological purposes or in patients who are clinically suspected suffering from an azole-resistant isolate or in regions with a high prevalence of azole resistance.**

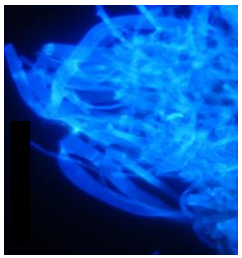


BSMM standards, Lancet Infect Dis 2015 15:461-74
ESCMID Aspergillus Guideline, 2018, CMI in press

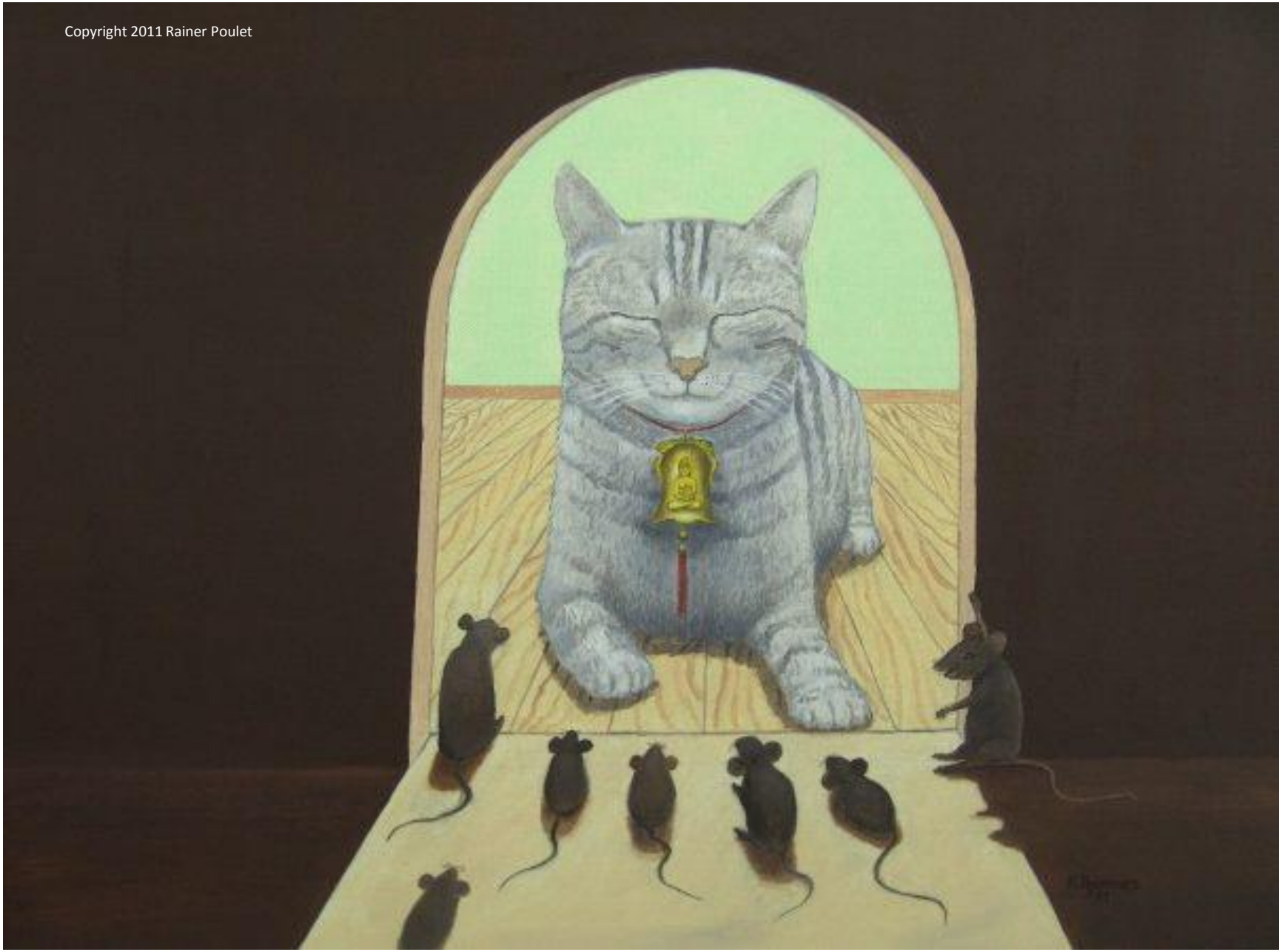








Serology and/or PCRs are „add on tests“



Serological and molecular methods in the diagnosis of invasive fungal infections

Method	Indication	Advantages	Disadvantages
Galactomannan (GM)	Early detection of invasive aspergillosis (IA) 2 serum samples/week, positive cut-off index > 0.5 1 single samle, positive cut-of index > 0.7	A screening test to accompany conventional diagnostic methods in patients at high risk of IA. In neutropenic adults In neutropenic children Serum value > 1: sign of therapeutic failure in adults and children Quantification in BAL (cut-off >1) and CSF (cut-off > 0.5) (useful in neutropenic and non-neutropenic patients)	In non-neutropenic patients: not the same diagnostic and prognostic value Mold-active antifungal drug therapy is one of the factors that may have an impact on sensitivity Persistent GM antigenemia during therapy is a poor prognostic sign and should prompt a reassessment

Lackner & Lass-Flörl, Curr Pharm Res 2013, 19: 3595-614
ESCMID Aspergillus Guideline, CMI in press, 2018
Kullberg et al. N Engl J Med 2015;373:1445

Method	Indication	Advantages	Disadvantages
B-D-glucan (BG)	Diagnosis of IFI 2 samples/week (minimum)	Pan-fungal marker in critically ill patients and in cases of *P. jiroveci* pneumonia Does not cover Mucormycetes and *Cryptococcus neoformans* A frequency of 2 tests per week seems an appropriate screening strategy 37% false positive result: 1x 80 pg/mL 23% false positive results: 2x 80 pg/mL Increases the specificity but decreases the sensitivity Site of infection may be important: patients with tissue infections failed to show a significant drop in BG levels despite successful outcomes High NPV	False - (+) results (bacteraemia) Limited experience (less widely used than GM) The threshold for positive results depends on the test that is used: Fungitell > 80 pg/mL Wako > 70 pg/mL Declines slowly in most IA, IC and PCP patients with appropriate antifungal therapy; May persists above the usual threshold for positivity long after clinical resolution of the original infection Less acurate in hematological patients

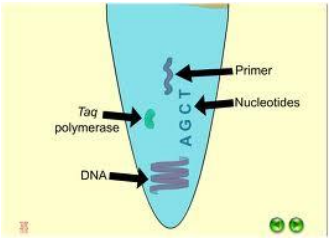
Hanson KE et al. PLoS One 2012;7(8):e42282; Koo S et al. Clin Microbiol Infect 2012;18:E122-127; Jaijakul S et al. Clin Infect Dis 2012;55:521-526; ESCMID Aspergillus Guideline, CMI in press, 2018

Serological and molecular methods in the diagnosis of invasive fungal infections

Method	Indication	Advantages	Disadvantages
Mannan plus Anti-mannan	Candidemia	Good sensitivity and specificity when combined in ICU patients Early diagnosis prior to blood culture results ESCMID Diagnostic & Management Guideline for Candida Diseases 2012 recommend this test-combination, high negative PV	Limited experience Non-mycological criterion The sensitivity and specificity were 87.5% and 85.5% for (1→3)-β-D-glucan and 89.3% and 63.0% for mannan antigen plus anti-mannan antibody *C. parapsilosis* and *C. guilliermondii* fungemias were not detected by the Platelia Candida Ag Plus assay

Mikulska et al, Crit Care Med 2010 14: R222; Held J et al, C Clin Microbiol 2013, 51(4):1158-64; Kullberg et al. N Engl J Med 2015;373:1445

Serological and molecular methods in the diagnosis of invasive fungal infections

Method	Indication	Advantages	Disadvantages
Molecular methods (polymerase chain reaction; PCR) Most experience of in-house tests 	DNA detection mainly of Aspergillus less experience for Candida Blood, BAL, …..	Early diagnosis (rapid techniques), high NPV High sensitivity (multicopy genes), capacity for rapid speciation and ability to quantitate fungal burden Low burden of organisms during bloodstream infections: <10 CFU/mL (in 25% <1 CFU/mL) and intermittent nature of candidaemia due to hepatic clearance of fungal cells and/or periodic release of cells from deep organ sites into circulation	Additional techniques Non-mycological criterion (they are still in development) Limited to reference laboratories (low availability) High costs, improve technical equipment Technical difficulties of efficient fungal DNA extraction from complex clinical samples

Khot PD and Fredericks DN, Expert Rev Anti Infect Ther 2009; 7:1201-21; Clancy CJ and Nguyen MH, Curr Fungal Infect Rep 2011; 5: 135-140

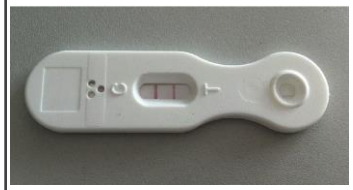
Examples on commercially available DNA-detecting methods for clinical specimens

Assay	Methods	Fungi	Sensitivity (%)	Specificity (%)	Detection limit	Processing time	Specimens
ePlex-BCID-FP GenMark DX „Bedside test“	Ready to use (DNA hybridization and electrochemical detection)	16 fungal targets: *C. albicans* *C. dubliniensis* *C. famata* *C. glabrata* *C. guilliermondii* *C. kefyr* *C. krusei* *C. Lusitaniae* *C. Parapsilosis* *C. tropicalis*	-	-	-	1.5 h	Positive blood cultures
FilmArray® BCID Panel Biomerieux	Ready to use Multiplex PCR	*C. albicans* *C. glabrata* *C. krusei* *C. parapsilosis* *C. tropicalis*	100	99.8-100	-	1 h	Positive blood cultures
T2 Candida Panel T2 Biosystems	Ready to use (magnetic resonance assay)	*C. albicans* *C. Tropicalis* *C. parapsilosis* *C. krusei* *C. glabrata*	91.1	99.4	1 cfu/mL	4.5 h	Whole blood
IRIDICA BAC BSI Abbott Diagnostics	PCR & mass spectrometry	Panmicrobial	81	84	8 cfu/mL	6 h	Whole blood, sterile fluids, tissue, BAL, endotracheal aspirate

Tests vary in the target, sen., spec., turnaround time and specimen application!

Lackner M and Lass-Flörl C, Methods Mol Biol. 2017;1508:85

BAL Lateral Flow Device Test for IA

Method	Indication	Advantages	Disadvantages
Laterial Flow Device Test for IA 	Point-of-care test for invasive aspergillosis Detects an extracellular glycoprotein secreted during active growth of Aspergillus via mAB JF5	Simple, rapid (15 min), single-use test. Can be performed in rudimentary facilities using BAL or serum specimens Sensitivities for LFD, GM, BDG, PCR were between 70 and 88%. Combined GM (cut off >1.0 OD) with LFD increased the sensitivity to 94%, while combined GM (cut off >1.0 OD) with PCR resulted in 100% sensitivity (specificity for probable/proven IPA 95-98%).	Sensitivity and specificity of BAL FD tests for probable IPA were 100% and 81% (PPV 71%, NPV 100%), Only few proven patients

ESCMID Aspergillus Guideline, CMI in press, 2018

Non-molecular tests used for diagnosis of the most common invasive fungal infections[a]

Microorganism	Diagnostic test	Optimal specimen type	Sensitivity (%)	Specificity (%)	Reasons for false-positive results	Reasons for false-negative results	Comments
Cryptococcus spp.	Cultures	CSF	>95	100	Uncommon	Uncommon	Gold standard, but takes 3–7 days for a positive result.
	Histopathology	Mostly CSF	75	100	Uncommon	Low levels of microorganism	India ink stain often used as a screening test.
	Cryptococcal antigen test (LA, EIA, or LFD)	CSF or serum	97 for CSF, 87 for serum	93–100	Trichosporon sp., Capnocytophaga sp., or Stomatococcus sp. invasive infections	Uncommon	Most accurate test when performed on CSF. The three methods are comparable, although LA gives more false-positive results. LFD is best for rapid point-of-care diagnosis.

[a]BAL, bronchoalveolar lavage; CAGTA, Candida albicans germ tube antibody; CF, complement fixation; CSF, cerebrospinal fluid; EIA, enzyme immunoassay; GM, galactomannan; ID, immunodiffusion; LA, latex agglutination; LFD, lateral-flow device; PPV, positive predictive value; TP, tube precipitin.

Arvanitis M et al. Clin Microbiol Rev. 2014, 27: 490

Microbiology best practice recommendations

Fungal serological and molecular testing

- Serum samples from immunocompromised patients with presentations consistent with cryptococcal meningitis for whom a CSF specimen is not available (eg, cases in which lumbar puncture is contraindicated) should be tested for *Cryptococcus* spp antigen (CRAG).
- ✓ **Galactomannan screening of serum (two times per week) from patients with haematological malignancies at high risk of invasive aspergillosis should be considered in those not receiving mould-active prophylaxis; optical density (OD) index threshold of 0.5 has a high negative predictive value, enabling invasive aspergillosis to be excluded.**

BSMM standards, Lancet Infect Dis 2015 15:461-74

Microbiology best practice recommendations

Fungal serological and molecular testing

- ✓ **Galactomannan testing of BAL from patients at high risk of invasive aspergillosis should be considered, although the current OD index cutoff of 0.5 might change.**
- ✓ **β-D-glucan screening of serum from patients at high risk of invasive fungal disease should be considered; a negative result has a high negative predictive value, enabling invasive fungal disease to be excluded.**
- ✓ **PCR screening of serum for *Aspergillus* from patients at high risk of invasive fungal disease should be considered; a negative result has a high negative predictive value, enabling invasive fungal disease to be excluded.**

BSMM standards, Lancet Infect Dis 2015 15:461-74

Microbiology best practice recommendations

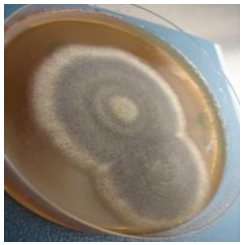
Therapeutic drug monitoring

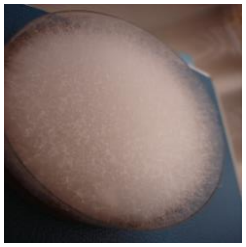
- ✓ **No indications for therapeutic drug monitoring of amphotericin B or the echinocandins; measurement of fluconazole concentrations is rarely necessary.**

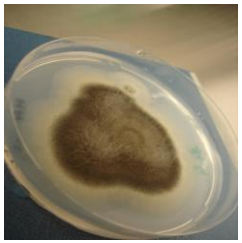
- ✓ **Therapeutic drug monitoring of itraconazole, voriconazole, and posaconazole is usually needed. Specifically, voriconazole monitoring is needed in most patients, and certainly in children, including repeat monitoring after dose changes and shift from intravenous to oral treatment; dose optimisation during long-term therapy needs such monitoring.**

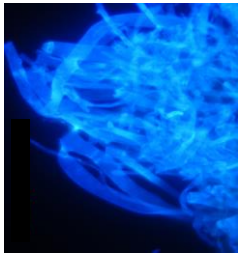
- ✓ **Flucytosine monitoring is recommended for all patients receiving treatment.**

BSMM standards, Lancet Infect Dis 2015 15:461-74









Fungal diagnosis: limitations

Clinical manifestations are non-specific

Conventional diagnostic tests insensitive, positive late

Fungi can be both, colonizers and pathogens, hence vigilance is required in the interpretation of:

- superficial cultures

- antigen tests, PCR screening, presence of antibodies and/or metabolites

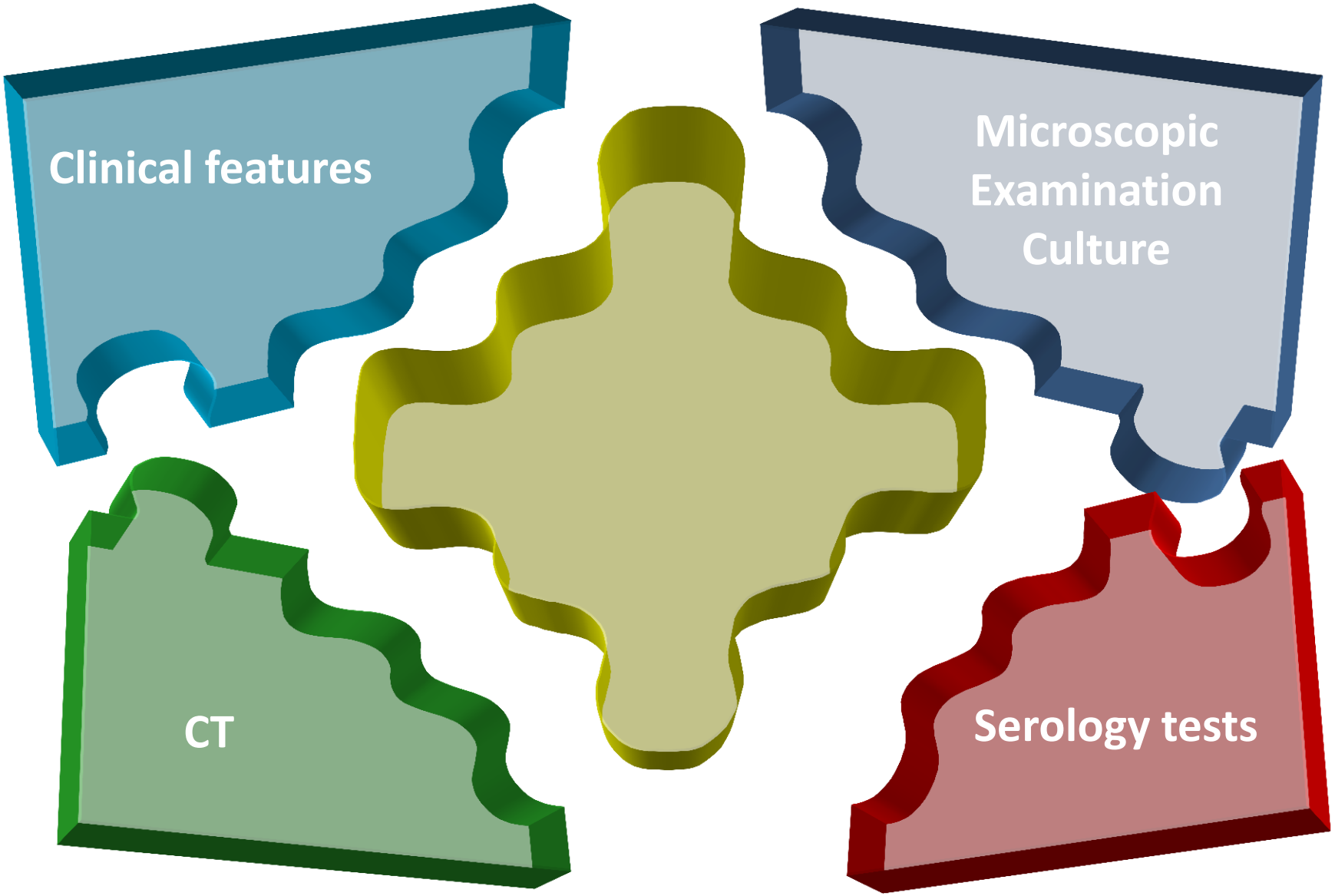
Lass-Flörl 2016, Walsh 2012, Kontoyiannis 2008, Kullberg et al. N Engl J Med 2015;373:1445

Variable contribution of diagnostic tools according to the disease

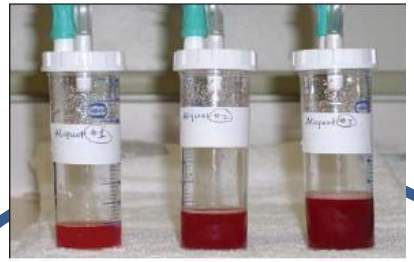
	Culture Microscopy	Anti-*Aspergillus* antibodies	*Aspergillus* antigens	PCR	Imaging
Chronic aspergillosis	+	++	-	+	Radiography
Invasive aspergillosis	++	-	++	++	CT scan
Allergic aspergillosis	+/-	+	-	-/+	Radiography

ESCMID Aspergillus Guideline, CMI in press, 2018

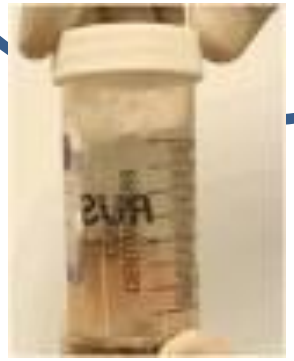
„Puzzle diagnosis“



Based on the patient population targeted decide what to do when and how!

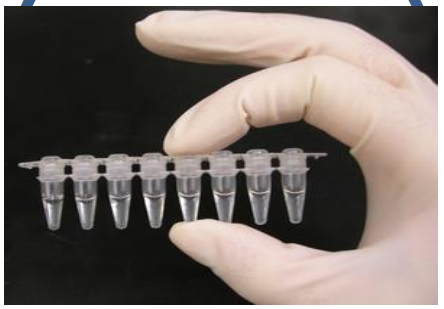


Specimens



Patients

Tests



Important rules

1. Educate your doctors to give you the „best clinical specimens“
2. Choose tests according your „local epidemiology“ and „patients‘ symptoms & history“
3. Culture and microscopic examinations: must have!
4. Define indirect tests as an „add on“ and have „assay variabilities“ in mind
6. Be aware of the pro & cons
7. No test covers all fungi!

YEAST INFECTIONS

Self-Test* for a Candida Infection

1. Do you feel tired most of the time or have muscle aches with normal activity?
2. Do you suffer from intestinal discomfort—bloating, constipation and/or diarrhea?
3. Do you crave sugar, breads, beer or other alcoholic beverages?
4. Do you suffer from mood swings, irritability, anxiety or depression?
5. Are you ever dizzy, light-headed or have trouble concentrating or thinking clearly?
6. Have you ever used antibiotics, birth control pills or steroid drugs?

Three or more "yes" answers indicate a high to very high probability that you have a yeast infection.
Cantrol helps combat that infection.

ECMM EXCELLENCE CENTERS
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MANAGEMENT AND EVALUATION
Any centre which fulfills the ECMM related requirements (please check at www.ecmm.info) is encouraged to apply for a designation. At the end of each twelve-month period, an ECMM EC must submit a report on the implementation of activities with the concerned program in ECMM. This report should reflect progress achieved in respect of the work plan, underlining possible difficulties and formulating suggestions for improvements for future course of cooperation. A final evaluation takes place at the end of the four-year designation period.

APPLICATION
Applications should be submitted electronically to president@ecmm.info where an ECMM reference number is issued for further correspondence via the ECMM office. All proposals for designation are processed electronically. The ECMM office initiates the procedure of designation by notifying the ECMM Subcommittee. Submissions are welcome anytime. The ECMM website will give updated information as appropriate. ECMM EC application must contain the following information:

1. Person in charge
2. Title
3. Name of the head of the applying ECMM EC
4. Name and contact details of the institution
5. Date of original designation, date of last re-designation (if any) and date of expiry
6. Terms of reference, i.e. application for laboratory or clinical mycology or both
7. Types of activity

FEES & CHARGES
The applicant bears the cost of the audit process, i.e. travel and accommodation of the two ECMM inspectors. ECMM covers administrative costs with their organization. Fees are due upon application and at the beginning of each subsequent year. Fees orient along the World Bank data on gross national income (GNI) per capita converted to international dollars using purchasing power parity (PPP) rates. Source data derived from http://data.worldbank.org/indicator/NY.GNP.PCAP.PP.CD

Initial Fee is 1/20 of the current (or latest) GNI PPP in Euros.
Annual Maintenance Fee is 1/4 of the initial fee.

ECMM EC AUDIT PLAN
The ECMM Subcommittee runs the evaluation according to the ECMM EC audit plan and initiates a site visit. Site visits last one full day and include an inspection consisting of at least two experts in the field. Experts named by the Subcommittee will be disclosed to the applicants prior to the visit. Applicants are encouraged to nominate national or international experts. The experts should submit a full report to the Subcommittee within two months after the site visit. In turn, the Subcommittee recommends to the ECMM Board, which decides whether an institution will be designated as ECMM EC.

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The European Confederation of Medical Mycology (ECMM) is the overarching organisation of the National Medical Mycology Societies in Europe, whose purpose is the rendering of support to science and research, the international coordination of scientific and clinical activities, the organisation of mycological conventions, and of training programmes.

The ECMM works towards the unification of all European scientists interested medical issues related to mycology.

www.ecmm.info

Thank you for your attention!