UZ LEUVEN

# State of the art mycologische diagnostiek

Katrien Lagrou

# Case: women, 55 years

- Sept 2019: single lung transplantation for idiopathic pulmonary fibrosis – treatment with immune suppressants (tacrolimus, methylprednisolone)
- 9 Dec 2020: erythematous raised lesion on hand since 3 weeks, no history of trauma, referral to dermatologist (exclusion Kaposi sarcoma)
  - **Punch biopsy** is taken: send to histopathology lab only

    **Report pathology**: granulomatous inflammatory reaction with some eosinophils, neutrophils and histiocytes. Also impression of presence of uniform, round, clarified, micro-organisms (‘tevens indruk van aanwezigheid van uniforme, afgeronde, opgehelderde micro-ogranismen’)

    No Grocott staining performed

    Conclusion: localisation of a granulomatous cryptococcal infection
- Exclusion of haematogenous spread by PET CT, MR brain and lumbar puncture, cryptococcal antigen testing
- Initiation of fluconazole 400 mg/day

# Bacteriologie Serologie

**05-01-2021 08:00 - bloed**

| | | | |
|---|---|---|---|
| Cryptococcus Ag detectie | negatief | | |

**05-01-2021 11:12 - cerebrospinaal vocht**

| | | | |
|---|---|---|---|
| Cryptococcus Ag detectie | negatief | | |

**17-03-2021 10:39 - bronchoalveolaire lavage**

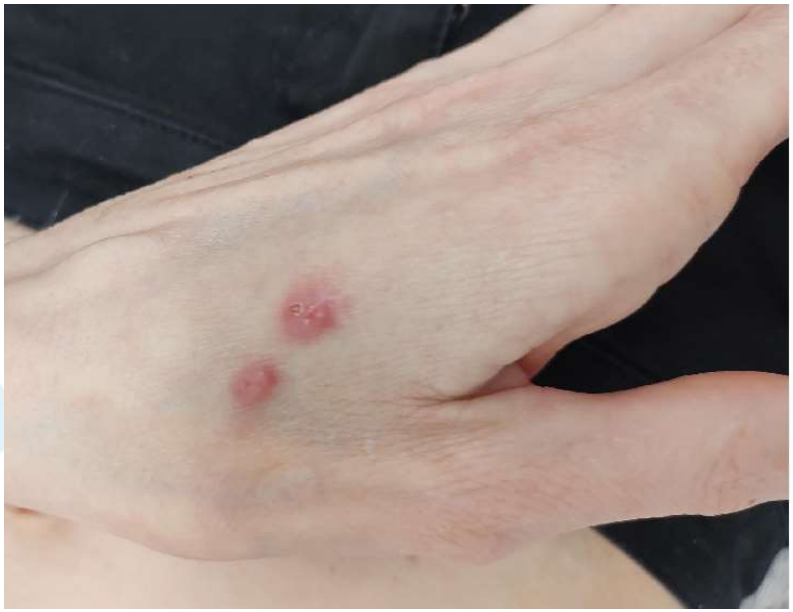
| | | | |
|---|---|---|---|
| Aspergillus Ag detectie | negatief | | |
| Aspergillus Ag detectie | | 0.1 | index |

**21-04-2021 08:00 - bloed**

| | | | |
|---|---|---|---|
| Cryptococcus Ag detectie | negatief | | |

# Case: women, 55 years

- 21 April 2021: reappearance of lesions while under fluconazole therapy
  - Pulmonologist asks for other treatment options
  - Advice to take a new biopsy sample for histopathology, panfungal PCR and culture



**28-04-2021 09:33 - biopsie**

| | |
|---|---|
| Panfungale PCR | positief |
| Type fungi | Alternaria infectoria |

**28-04-2021 09:**

Gisten/Schi

positi

Cultuur

groei

Alter

**Do not identify fungi on genus/species level on histopathology alone**

*belig aan amfotericine B,*

MIC bepaling:

| | | |
|---|---|---|
| amfotericine B | 0.25 | mg/L |
| isavuconazol | > 16.00 | mg/L |
| itraconazol | 0.25 | mg/L |
| posaconazol | 0.06 | mg/L |
| voriconazol | 8.00 | mg/L |

# Evolving risk factors for invasive mould infections


***Risk factors for invasive aspergillosis***

Haematological malignancy
Neutropenia
Allogeneic haematopoietic stem-cell transplantation
Solid organ (lung) transplantation

**Invasive aspergillosis in the ICU**:
Prolonged treatment with corticosteroids before admission to the ICU
Chronic obstructive pulmonary disease
Liver cirrhosis

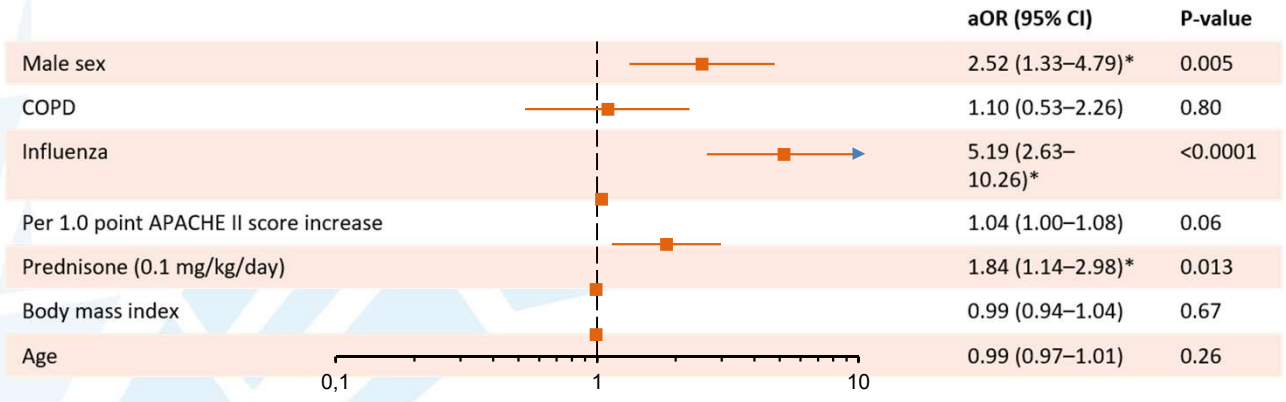
**Critically ill with viral pneumonitis**
Influenza
COVID-19

Meersseman W, *et al. Clin Infect Dis.* 2007;45(2):205–16;
Wauters J, *et al. Intensive Care Med.* 2012;38(11):1761–8;
Dewi IMW, *et al. Curr Opin Microbiol.* 2021;62:21–7.

# Growing evidence for influenza as an independent risk factor for invasive aspergillosis

## Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study

*Alexander F A D Schauwvlieghe*, Bart J A Rijnders*, Nele Philips, Rosanne Verwijs, Lore Vanderbeke, Carla Van Tienen, Katrien Lagrou, Paul E Verweij, Frank L Van de Veerdonk, Diederik Gommers, Peter Spronk, Dennis C J J Bergmans, Astrid Hoedemaekers, Eleni-Rosalina Andrinopoulou, Charlotte H S B van den Berg, Nicole P Juffermans, Casper J Hodiamont, Alieke G Vonk, Pieter Depuydt, Jerina Boelens, Joost Wauters, on behalf of the Dutch-Belgian Mycosis study group*




- IPA diagnosed in 19% of 342 patients admitted to the ICU with influenza
- Median of 3 days after admission to the ICU
- Incidence similar for influenza A and B
- 90-day mortality significantly higher in cases of IPA in influenza cohort (51% vs 28%)
- Influenza is an independent risk factor for IPA

Schauwvlieghe AFAD, *et al. Lancet Resp Med.* 2018;6(10):782–92.

# Study of invasive pulmonary aspergillosis in critically ill patients

## Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study

Alexander F A D Schauwvlieghe*, Bart J A Rijnders*, Nele Philips, Rosanne Verwijs, Lore Vanderbeke, Carla Van Tienen, Katrien Lagrou, Paul E Verweij, Frank L Van de Veerdonk, Diederik Gommers, Peter Spronk, Dennis C J J Bergmans, Astrid Hoedemaekers, Eleni-Rosalina Andrinopoulou, Charlotte H S B van den Berg, Nicole P Juffermans, Casper J Hodiamont, Alieke G Vonk, Pieter Depuydt, Jerina Boelens, Joost Wauters, on behalf of the Dutch-Belgian Mycosis study group

ORIGINAL

2

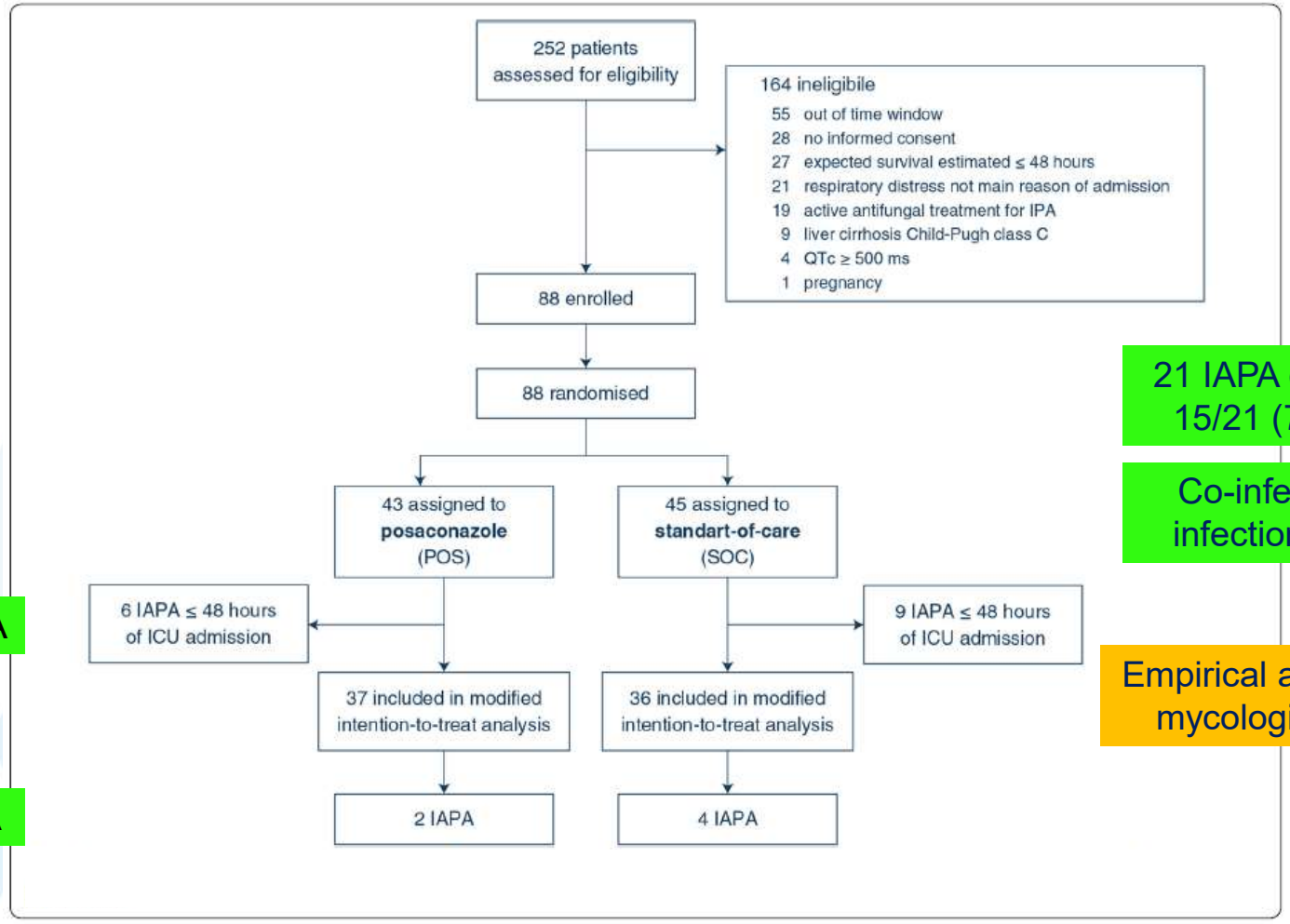
## Posaconazole for prevention of invasive pulmonary aspergillosis in critically ill influenza patients (POSA-FLU): a randomised, open-label, proof-of-concept trial

Lore Vanderbeke[1,2], Nico A. F. Janssen[3,4], Dennis C. J. J. Bergmans[5], Marc Bourgeois[6], Jochem B. Buil[4,7], Yves Debaveye[8,9], Pieter Depuydt[10], Simon Feys[1,2], Greet Hermans[2,8], Oscar Hoiting[11], Ben van der Hoven[12], Cato Jacobs[2], Katrien Lagrou[1,13], Virginie Lemiale[14], Piet Lormans[15], Johan Maertens[1,16], Philippe Meersseman[1,2], Bruno Mégarbane[17], Saad Nseir[18], Jos A. H. van Oers[19], Marijke Reynders[20], Bart J. A. Rijnders[21], Jeroen A. Schouten[22], Isabel Spriet[23,24], Karin Thevissen[25], Arnaud W. Thille[26], Ruth Van Daele[23,24], Frank L. van de Veerdonk[3,4], Paul E. Verweij[4,7], Alexander Wilmer[1,2], Roger J. M. Brüggemann[4,27] and Joost Wauters[1,2*] on behalf of the Dutch-Belgian Mycosis Study Group

- 12 ICUs in Belgium, the Netherlands and France
- 2017–2020
- Adult patients with positive influenza PCR were admitted to the ICU due to respiratory distress
- At study inclusion, patients were evaluated for the presence of an invasive fungal infection consisting of bronchoscopy with BAL within 48h of ICU admission (if safe)

Schauwvlieghe AFAD, *et al. Lancet Resp Med.* 2018;6(10):782–92;
Vanderbeke L, *et al. Intensive Care Med.* 2021;47(6):674–86.

# The majority of invasive aspergillosis is diagnosed within 48h of ICU admission in influenza patients




Early IAPA

Late IAPA

21 IAPA cases (21/88 patients, 24%)
15/21 (71%) diagnosed within 48h

Co-infection instead of secondary infection in majority of the patients

Empirical antifungal therapy + diagnostic mycological work-up within 24–48h?

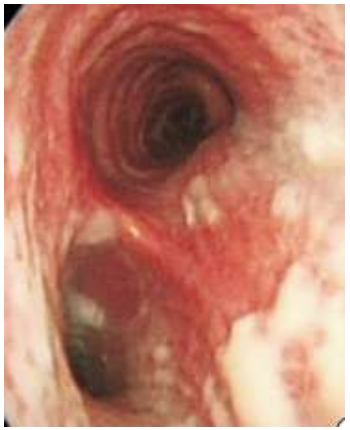
Vanderbeke L, *et al. Intensive Care Med.* 2021;47(6):674–86.

# *Aspergillus* tracheobronchitis

## Invasive pulmonary aspergillosis

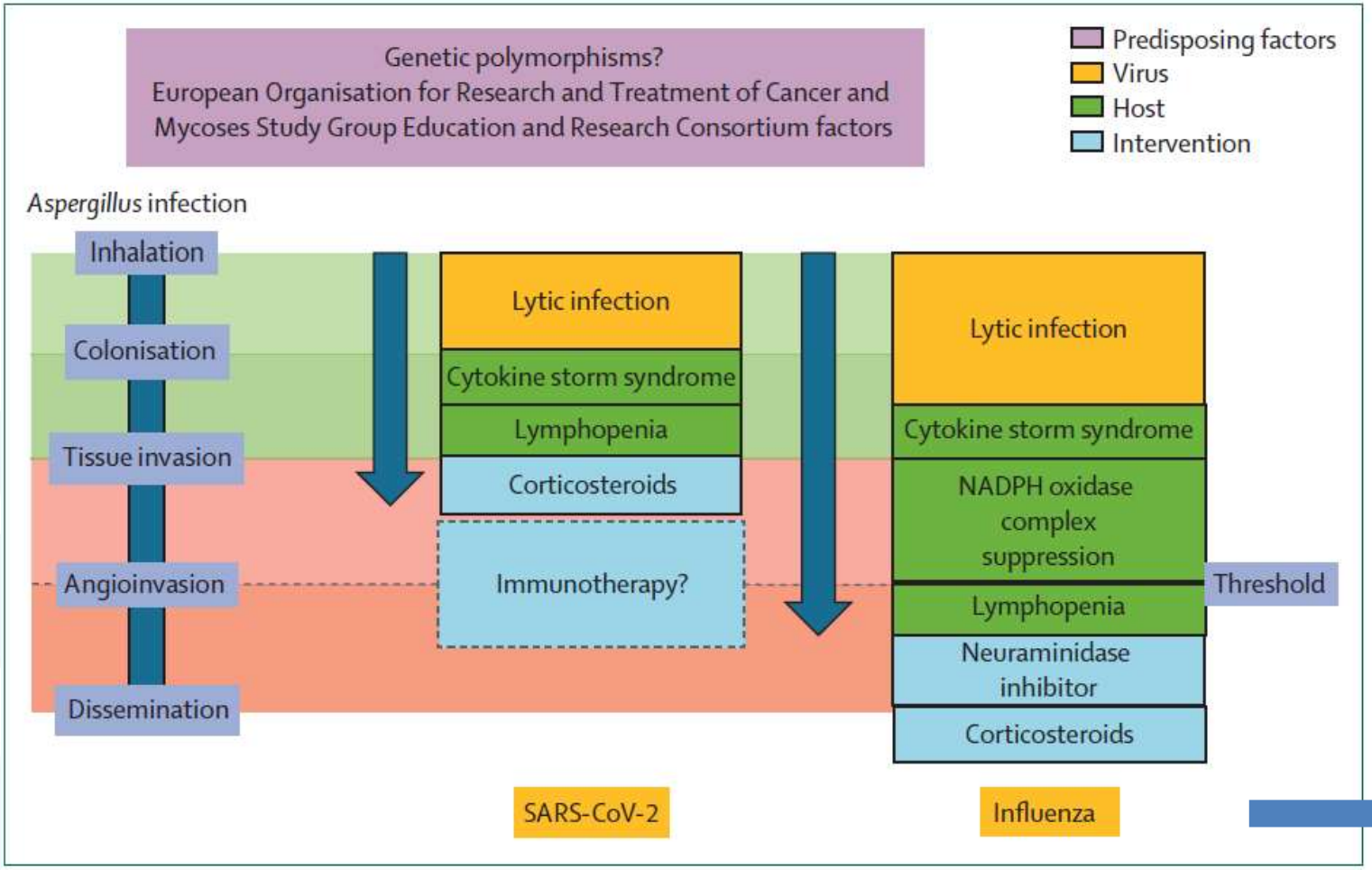
Alveolar **AND/OR** Airway disease (= tracheobronchitis)

- Up to **56**% if severe **influenza**, **early** after ICU admission, high mortality
- Up to **20**% if severe **COVID-19**, **later** after ICU admission
- Also in lung transplant recipients



Van de Veerdonk FL, *et al. Lancet Respir Med.* 2021;9(7):795–802;
Cho BH, *et al. Tuberc Respir Dis (Seoul)*. 2014;77(5):223–6.

# Factors that contribute to invasive *Aspergillus* spp. tracheobronchitis disease progression ultimately leading to angioinvasion




- High fungal burden
- Deep tissue invasion
- Angioinvasion

Van de Veerdonk FL, *et al. Lancet Respir Med.* 2021;9(7):795–802.

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Accepted Manuscript Posted Online

JCM Accepted Manuscript Posted Online 8 September 2021
J Clin Microbiol doi:10.1128/JCM.01229-21


1 ***Aspergillus*** **test profiles and mortality in critically-ill COVID-19 patients**

2

3 Mehmet Ergün[1,2], Roger J.M. Brüggemann[1,3], Alexandre Alanio[4,5], Sarah Dellière[4,5],

4 Andreas van Arkel[6], Robbert G. Bentvelsen[6,7], Tom Rijpstra[8], Simone van der Sar-van

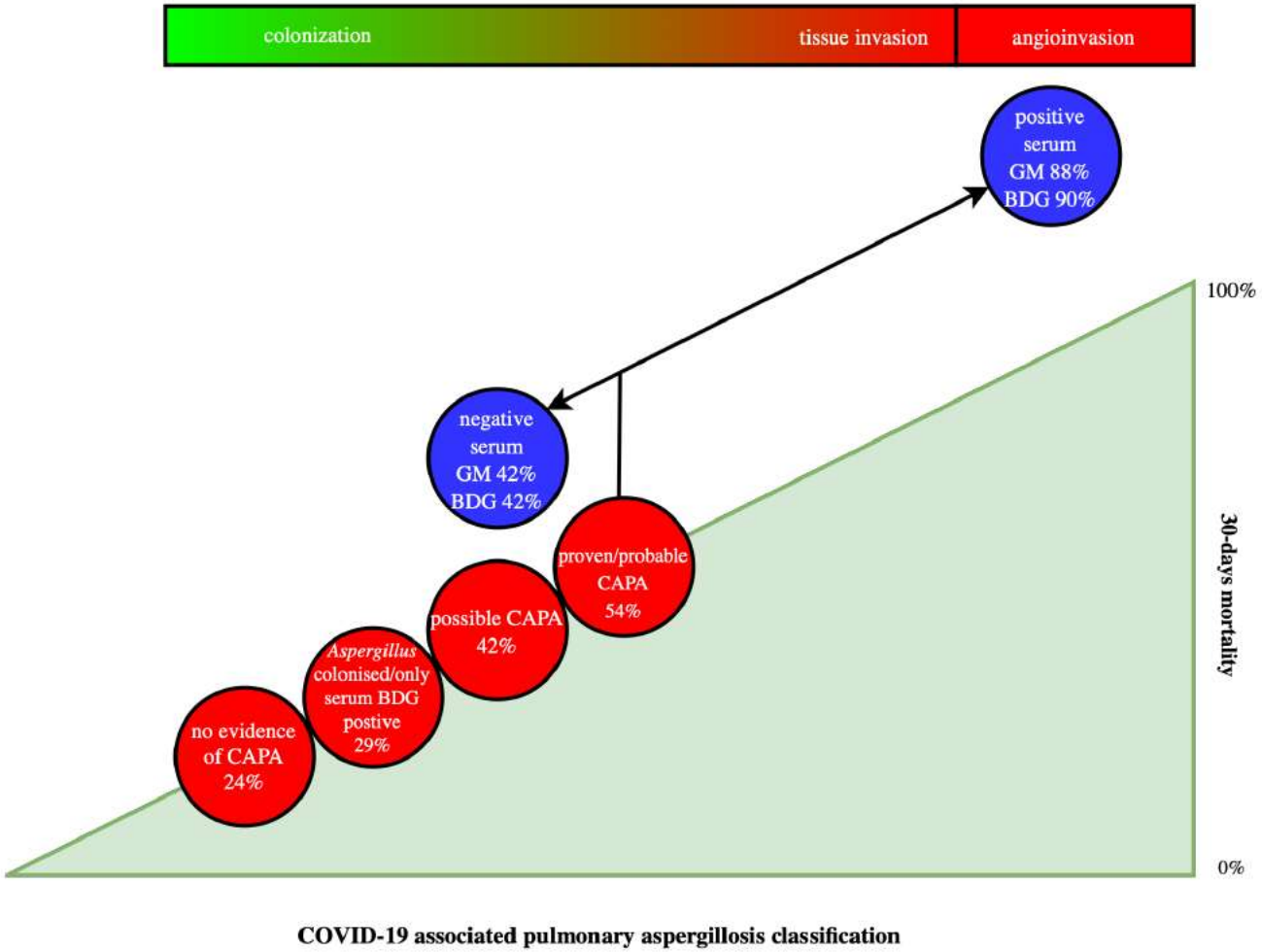
5 der Brugge[9], Katrien Lagrou[10,11], Nico A.F. Janssen[1,12], Jochem B. Buil[1,2], Karin van

6 Dijk[13], Willem J.G. Melchers[1,2], Monique H.E. Reijers[1,14], Jeroen A. Schouten[15,16], Joost

7 Wauters[17], Alan Cordey[18], Shuchita Soni[18], P. Lewis White[18], Frank L. van de

8 Veerdonk[1,12], and Paul E. Verweij[1,2]

published online September 2021.




**COVID-19 associated pulmonary aspergillosis classification**

Multinational case-control study:
219 critically ill COVID-19 cases

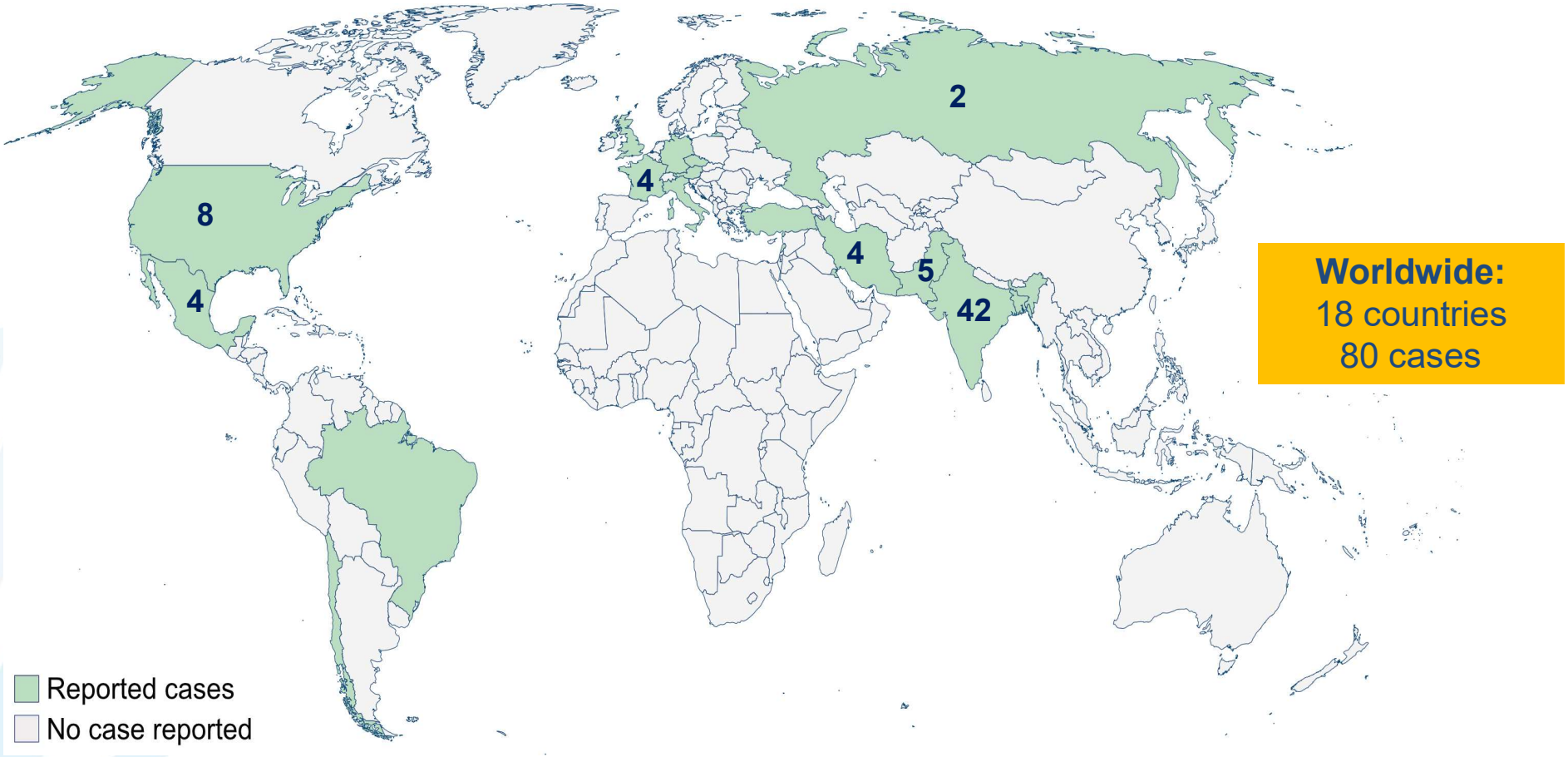
Classified using the 2020 ECMM/ISHAM consensus case definition

CAPA is a complex disease probably involving a continuum

Need for biomarker that distinguishes respiratory tract colonization and tissue invasive CAPA disease

M. Ergün et al. JCM 2021

# Emergence of Covid-associated mucormycosis (CAM)




**Worldwide:**
18 countries
80 cases

CAM, COVID-19-associated mucormycosis

Hoenigl M, *et al*. *The Lancet Microbe*. 2021. [Epub ahead of print].

# Emergence of Covid-associated mucormycosis (CAM)

- 77.5% of patients were male
- 95% had additional risk factors:
  - Diabetes: 82% (40/42 Indian cases), 55/66 uncontrolled or poorly controlled
  - Hypertension: 19%
  - Chronic kidney diseases: 6%
  - Haematological malignancies: 6%
- 79% were treated with systemic corticosteroids
- Clinical presentation:
  - Rhino-orbital cerebral infection: 74%
  - Pulmonary disease: 25%

Diabetic COVID-19 patients receiving corticosteroids may be particularly vulnerable to the development of CAM

Hoenigl M, *et al*. *The Lancet Microbe*. 2021. [Epub ahead of print].

# The importance of diagnostic tests for invasive fungal disease

There is a need for **rapid** diagnostic tests with a good performance in patients with **various underlying diseases**

**Galactomannan/other *Aspergillus* antigen**:

- ELISA
- Lateral flow assay
- Blood/BAL

**BDG**

- Batch format
- Single-test format
- Blood (and CSF), not BAL

**PCR**

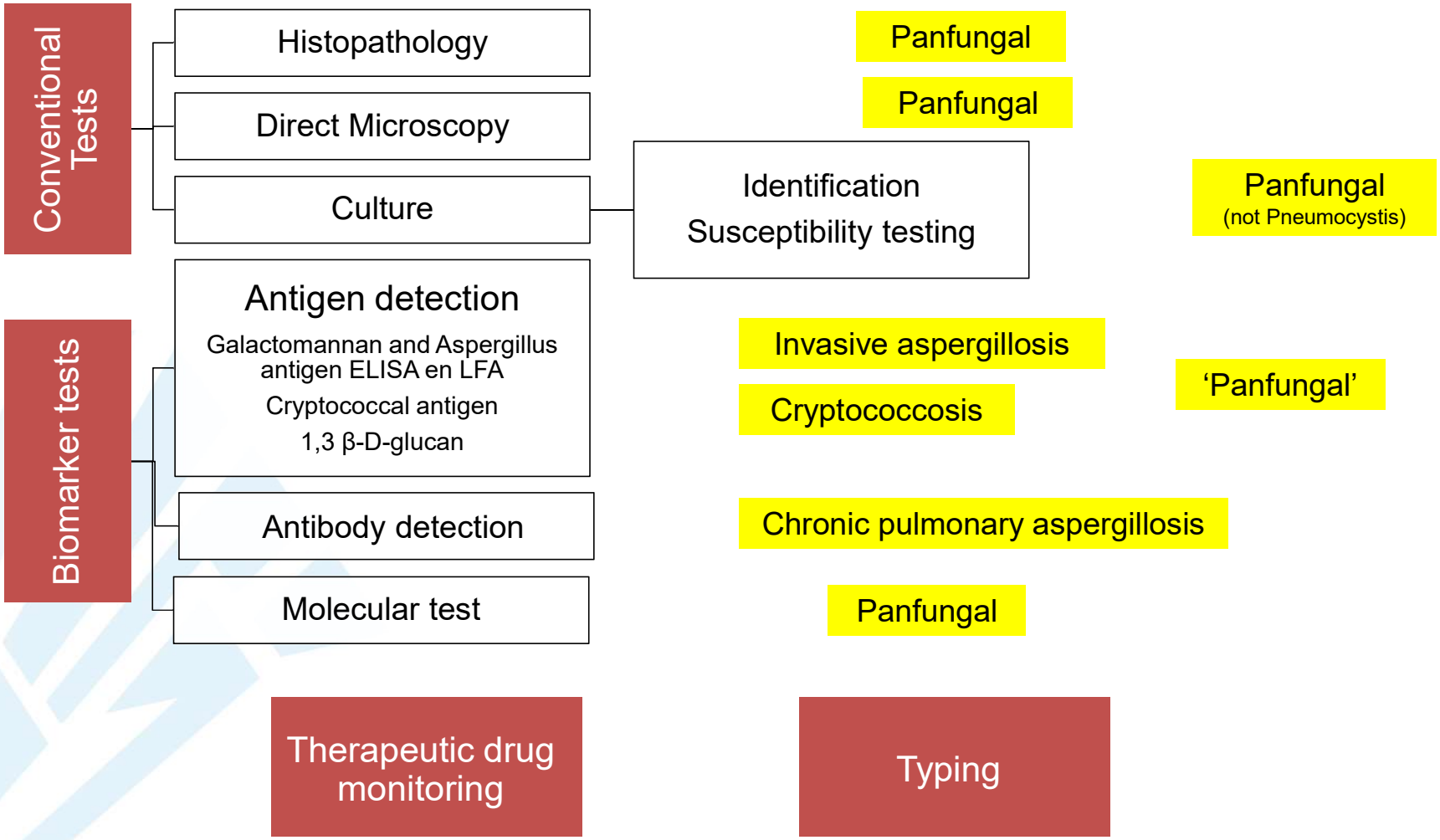
- *Aspergillus*, including resistance markers
- Mucorales

More commercial assays are becoming available

Histopathology and culture remain essential investigations

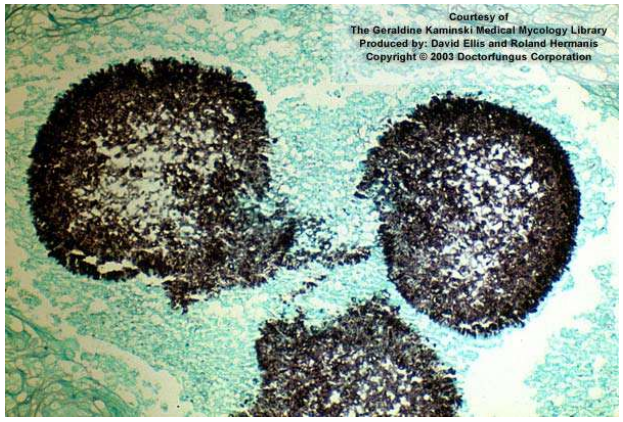
Palacios CF, and Moffarah AS. *Diagnostics (Basel)*. 2021;11(7):1226.

# Tests for the diagnosis and treatment of fungal infections

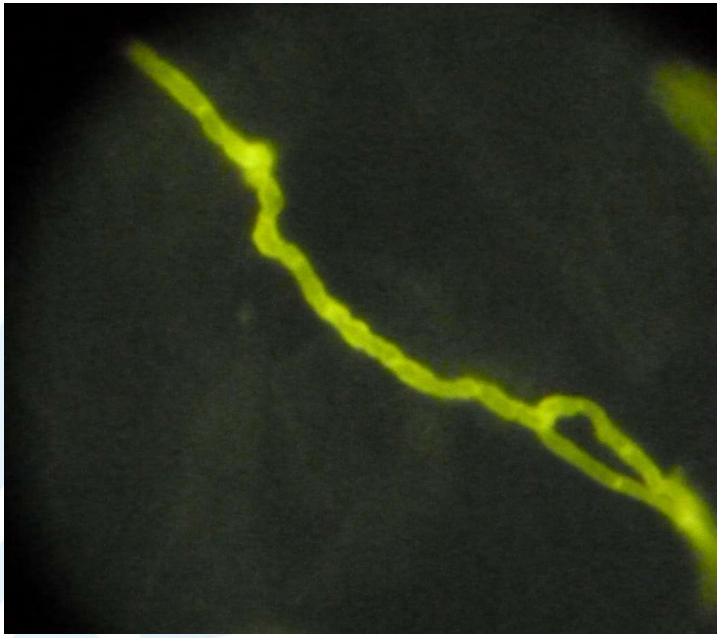


# Histopathology

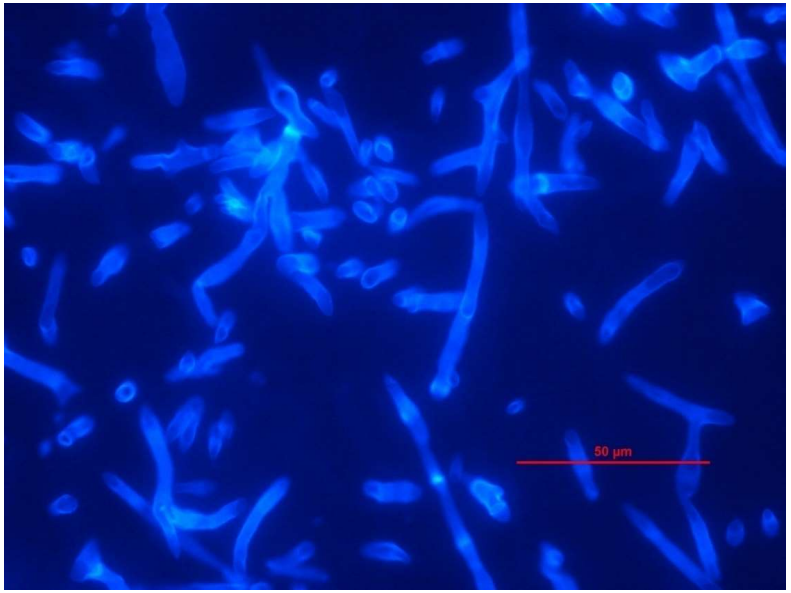
- Demonstrates true tissue invasion: defines proven fungal infection
- Reveals the relation of fungal elements to tissue structures
- Sometimes the only means to establish a diagnosis
- Rapid
- Biopsy should be taken whenever possible



# Rapid direct microscopy: optical brighteners recommended



Dermatophyte




Mucorales

# Culture- based methods

- Important for species identification and susceptibility testing
- Blood culture: *Candida spp*, *Fusarium spp*, *Scedosporium spp*
- CSF
  - Highly sensitive for cryptococcal meningitis (98%), marker of response to treatment (↔ Ag test)
  - Sensitivity lower for CNS aspergillosis or candidiasis (parenchyma rather than meningeal involvement)
- Biopsies: homogenization reduces the culture yield of Mucorales
- Sensitivity BAL culture for invasive aspergillosis is no more than 50%
- Sensitivity of blood cultures for invasive candidiasis is about 50%

# Identification of fungi

- Macroscopic examination
- Microscopic examination
  - use media that promote conidiation such as potato flake agar, potato dextrose agar, diluted Sabouraud agar (Takashio medium)
  - examen cultures regularly (manner of conidiation or sporulation is obscured in old cultures)
- MALDI-TOF MS
- Sequencing rDNA Internal Transcribed Spacer (ITS) region:
  - suffices for species recognition in many groups
  - in well-studied genera such as *Aspergillus* or highly speciose groups such as *Fusarium*, ribosomal genes provide insufficient resolution to recognize all siblings within species complexes
  - short segment of DNA that is unique to particular species and able to differentiate it from all others = **barcode**

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# Validation of a New Web Application for Identification of Fungi by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

A. C. Normand,[a] P. Becker,[b] F. Gabriel,[c] C. Cassagne,[a] I. Accoceberry,[c] M. Gari-Toussaint,[d] L. Hasseine,[d] D. De Geyter,[e] D. Pierard,[e] I. Surmont,[f] F. Djenad,[a] J. L. Donnadieu,[g] M. Piarroux,[h] S. Ranque,[a] M. Hendrickx,[b] R. Piarroux[a]

**Free online available:**

https://biological-mass-spectrometry-identification.com/msi/

Access limited to users

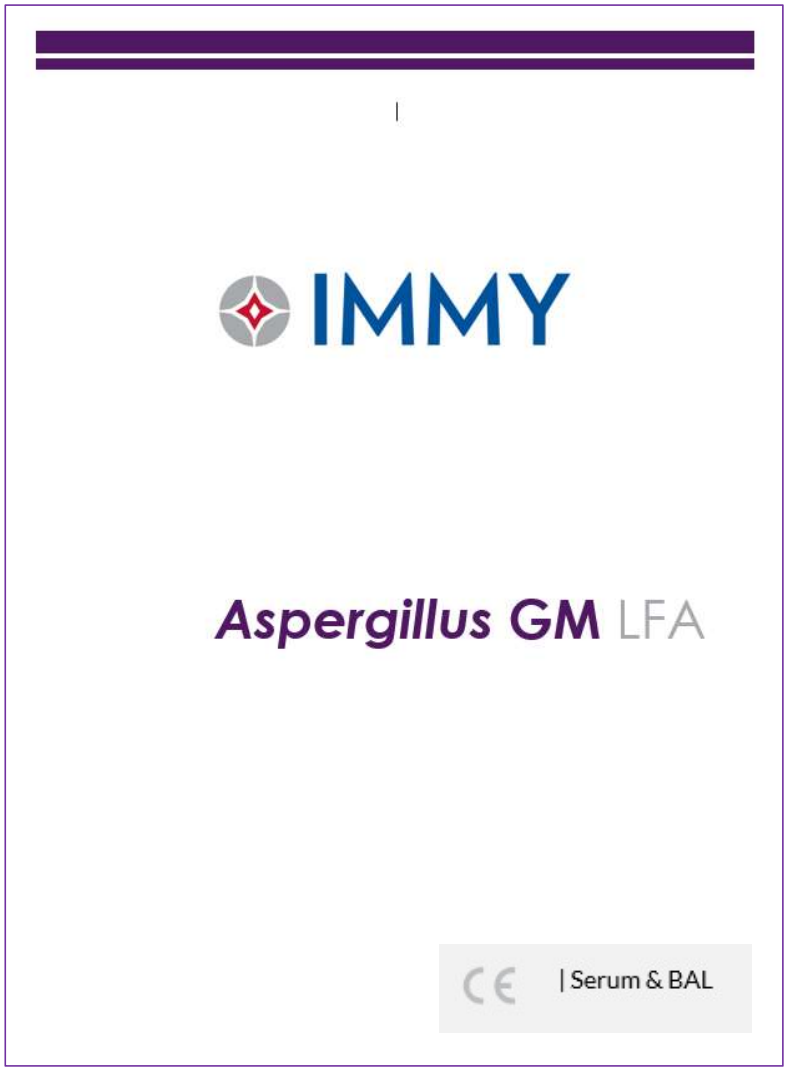
2017, 55: 2661-2670.

# ANTIGEN DETECTION ASSAYS

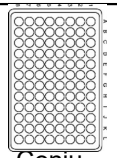
# Galactomannan detection: characteristics

- **Detection in serum**:
  - Good sensitivity in neutropenic patients
  - Sensitivity is much lower in non-neutropenic patients
  - Sensitivity is lower in patients on Aspergillus-active antifungal treatment
  - Early marker: is used for screening of patients with a high risk of developing invasive aspergillosis
- **Detection in BAL:**
  - Good sensitivity in both neutropenic and non-neutropenic patients
  - Requested upon suspicion of an invasive fungal infection (screening not possible)
  - Optimal cutoff value for BAL (index= 0.8-1) somewhat higher than for serum (index = 0.5)

# CE-IVD Aspergillus lateral flow tests finally available

# Procedure GM ELISA versus lateral flow assays

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GM** |  Sample |  Buffer |  |  |  Conju-gate |  Super-natans |  Incubate |  Wash 5x |  Chromo-gen |  Incubate |  Stopping solution |  Readout |

*Courtesy Toine Mercier*

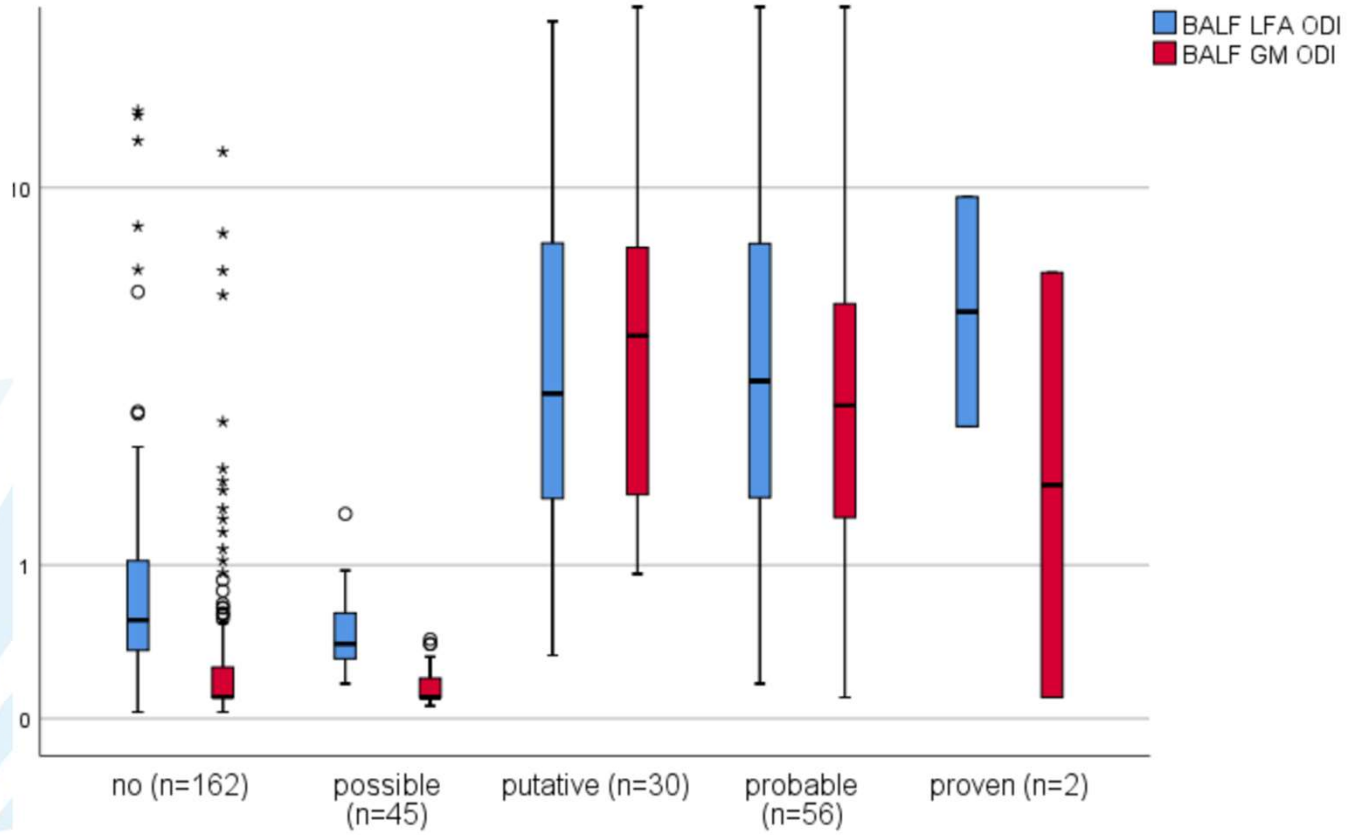
# *Aspergillus* galactomannan lateral flow assay demonstrates good diagnostic performance

- Retrospective, multicentre study on BAL fluid samples
  - University of California (San Diego), the Medical University of Graz (Austria) and the Mannheim University Hospital (Germany)
- 296 patients with various underlying diseases (65% without underlying haematological malignancy)
- Two proven IA, 56 probable IA, 30 putative IA, 45 possible IA, 162 no IA
- IMMY *Aspergillus* galactomannan LFA with cube reader

BAL, bronchoalveolar lavage; IA, invasive aspergillosis; LFA, lateral flow assay

Jenks JD, *et al. Clin Infect Dis*. 2020. [Epub ahead of print].

# *Aspergillus* galactomannan lateral flow assay demonstrates good diagnostic performance




**Invasive pulmonary aspergillosis classification**

BALF, bronchoalveolar lavage fluid; GM, galactomannan; LFA, lateral flow assay; ODI, optical density index

Jenks JD, *et al. Clin Infect Dis*. 2020. [Epub ahead of print].

# Combination of blood antigen tests for diagnosis of invasive aspergillosis in haematology patients

- Prospectively collected serum samples from 239 haematology patients, 2017–2019
- Five proven IA, 36 probable IA, 188 controls
- **No mould-active prophylaxis**
- **Antigen detection assays**
  - Wako BDG test
  - GM ELISA (bioRAD)
  - *Aspergillus* lateral flow tests: IMMY (LFA) and OLM (LFD)

Mercier T, *et al. Clin Infect Dis.* 2021;72(9):1577–84.

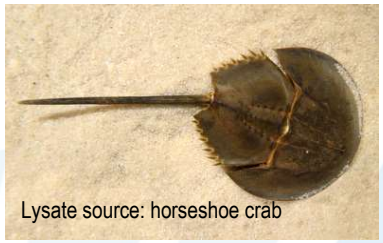
# Combination of blood antigen tests for diagnosis of invasive aspergillosis in haematology patients

| | SENS (%) | SPEC (%) | NPV (%) | PPV (%) |
|---|---|---|---|---|
| GM ELISA | 44 | 99 | 89 | 93 |
| LFA | 49 | 95 | 90 | 69 |
| BDG ≥11 | 27 | 98 | 86 | 79 |
| BDG ≥2.359 | 46 | 90 | 89 | 51 |
| LFA **OR** GM ELISA | 54 | 94 | 90 | 66 |
| LFA **OR** BDG ≥2.359 | 63 | 86 | 92 | 50 |
| GM ELISA **OR** BDG ≥2.359 | 60 | 88 | 91 | 53 |
| LFA AND BDG ≥2.359 | 32 | 99 | 87 | 93 |

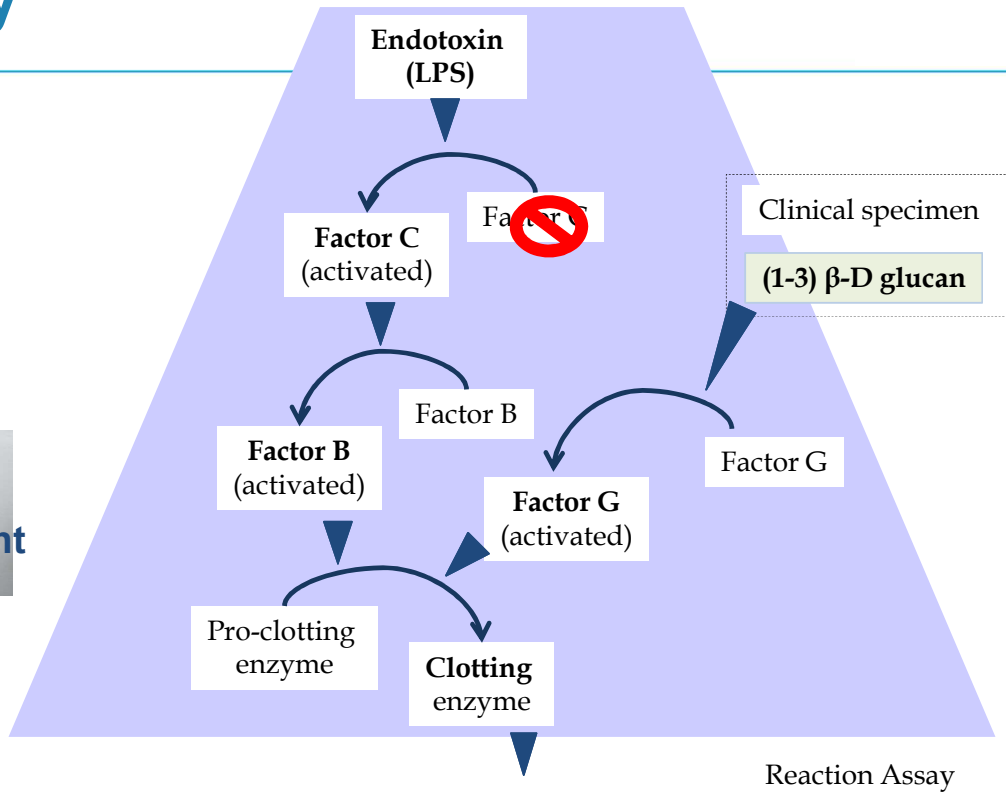
- Optimal combination: BDG + GM ELISA or LFA
- LFA can replace GM ELISA

Mercier T, *et al. Clin Infect Dis.* 2021;72(9):1577–84.

# 1, 3 β-D-Glucan assay








**Fungitell**

**Wako**

# 1, 3 β-D-Glucan detection

- **'Panfungal'**:
  - *Aspergillus, Candida,* many other fungi
  - **NO detection of Mucorales and *Cryptococcus***

- Several commercial kits available (different cut-off values): Fungitell™ in US and Europe, WAKO recently also in Europe
- Approved for serum testing only. Lower specificity than GM detection in BAL (frequent Candida colonization of respiratory tract)
- Fungitell test not really user-friendly
- Several causes for false positivity were reported but recent research suggests elevated β-D-glucan might be due to microbial (*Candida*) translocation from the gut

**(1→3)-β-D-Glucan: A Biomarker for Microbial Translocation in Individuals with Acute or Early HIV Infection?**

Martin Hoenigl[1,2,3*†], Josué Pérez-Santiago[1†], Masato Nakazawa[4], Michelli Faria de Oliveira[1], Yonglong Zhang[5], Malcolm A. Finkelman[5], Scott Letendre[1,6], Davey Smith[1] and Sara Gianella[1*]

frontiers in Immunology

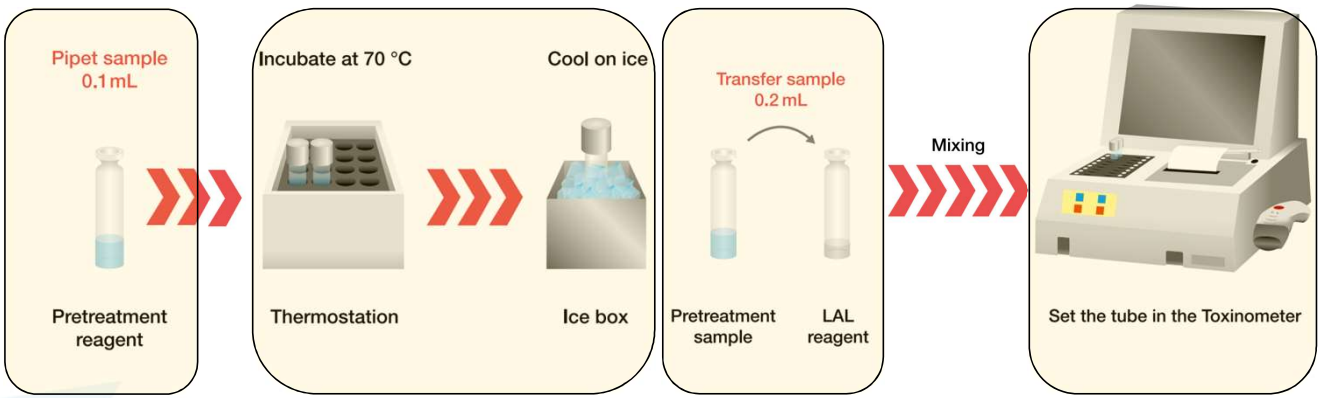
ORIGINAL RESEARCH
published: 03 October 2016
doi: 10.3389/fimmu.2016.00404

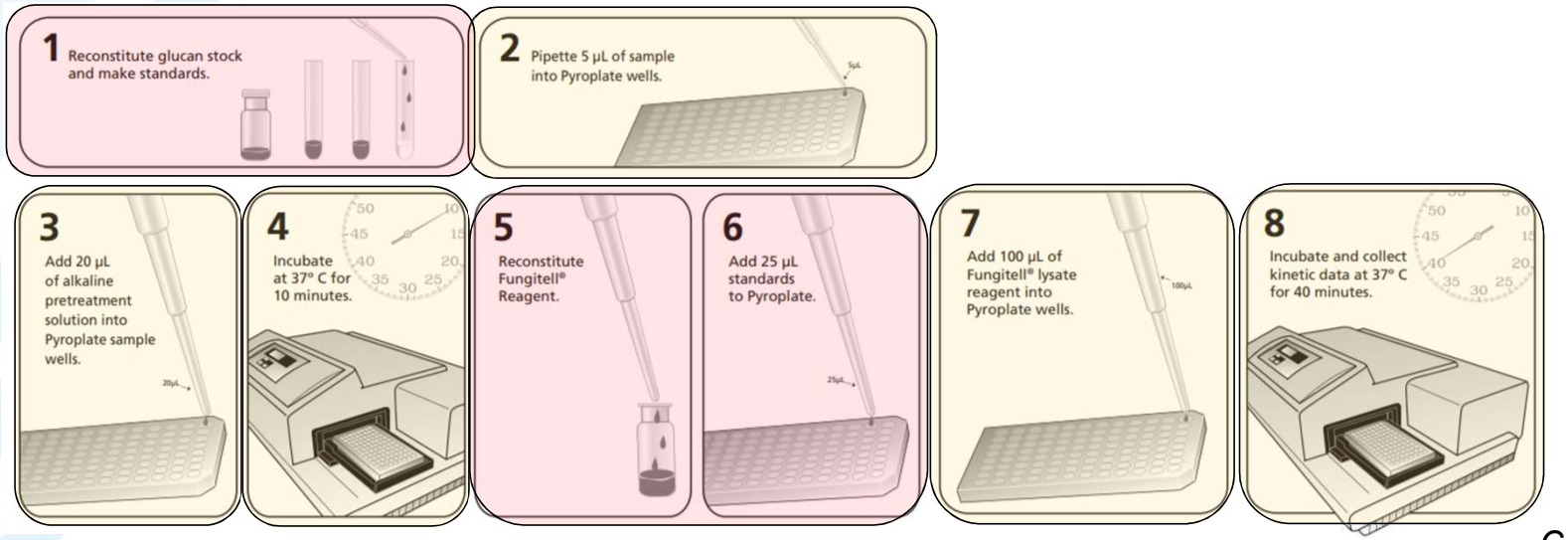
# Main indications/use 1,3- β-D-glucan detection

- Screening of patients at high risk of candidemia in ICU
  - to stop empiric antifungal therapy
  - to withhold antifungal therapy
- Diagnostic tool for *Pneumocystis jirovecii* infection when no BAL is possible
  - infection highly unlikely if negative
  - contributes to the diagnosis of PCP
- Diagnostic test for invasive fungal infection in combination with other biomarkers in at risk patients

**Detection on blood samples**

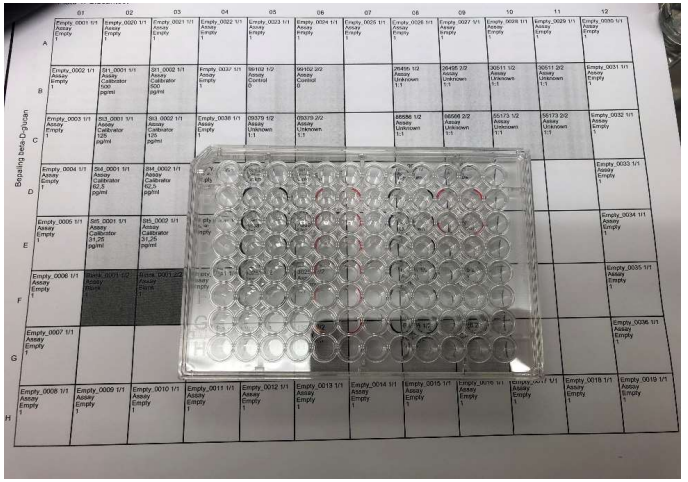
# (1,3)-β-D-glucan detection: Wako versus Fungitell



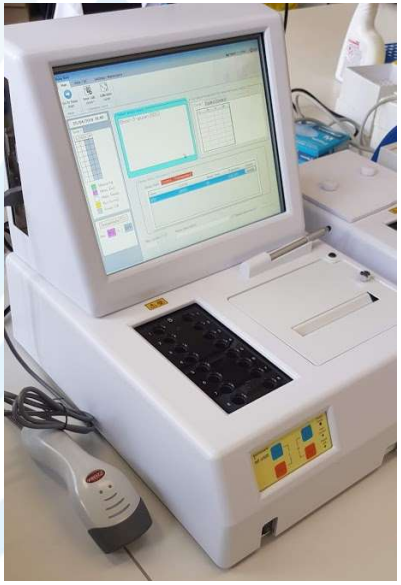




*Courtesy Toine Mercier*

# Fungitell assay



# Wako assay




# MOLECULAR TESTS

# PCR-based diagnostics

- Studies focus mainly on *Aspergillus*, *Candida, Pneumocystis jirovecii,* mucorales or ‘panfungal’
- Very useful on microscopy positive, culture negative sterile site samples
- Best diagnostic yield in combination with other biomarkers for other sample types

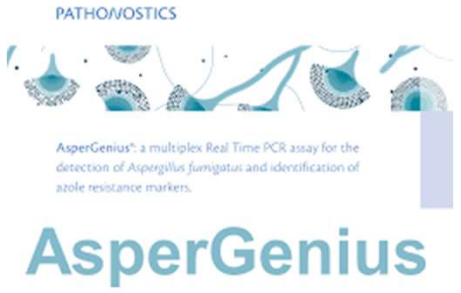
# Commercial assay for molecular diagnosis of azole resistance: AsperGenius® assay

**Species multiplex PCR detects and differentiates**:

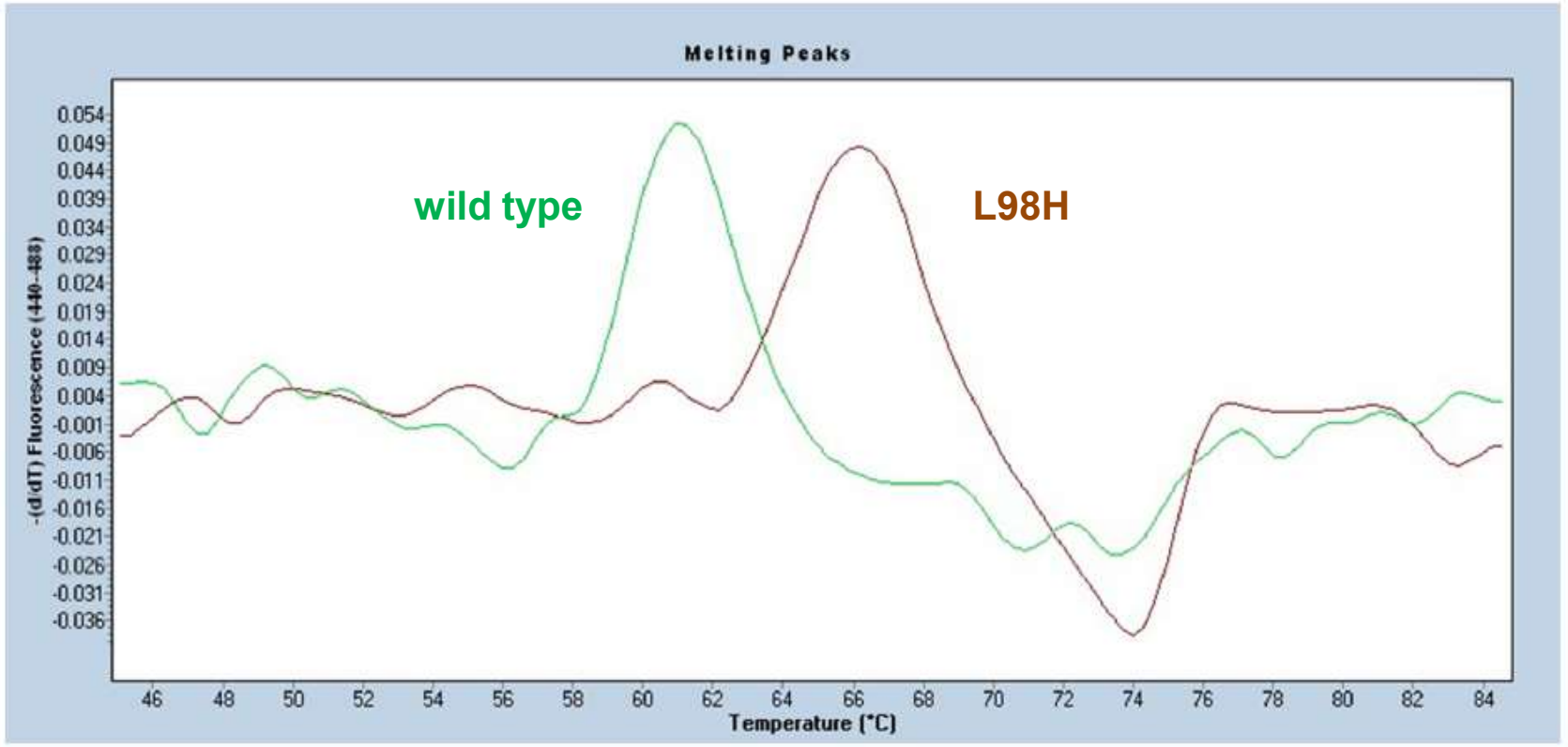
- *Aspergillus fumigatus* complex
- *Aspergillus terreus*
- *Aspergillus species*

**Resistance multiplex PCR detects:**

- L98H
- TR34
- T289A
- Y121F



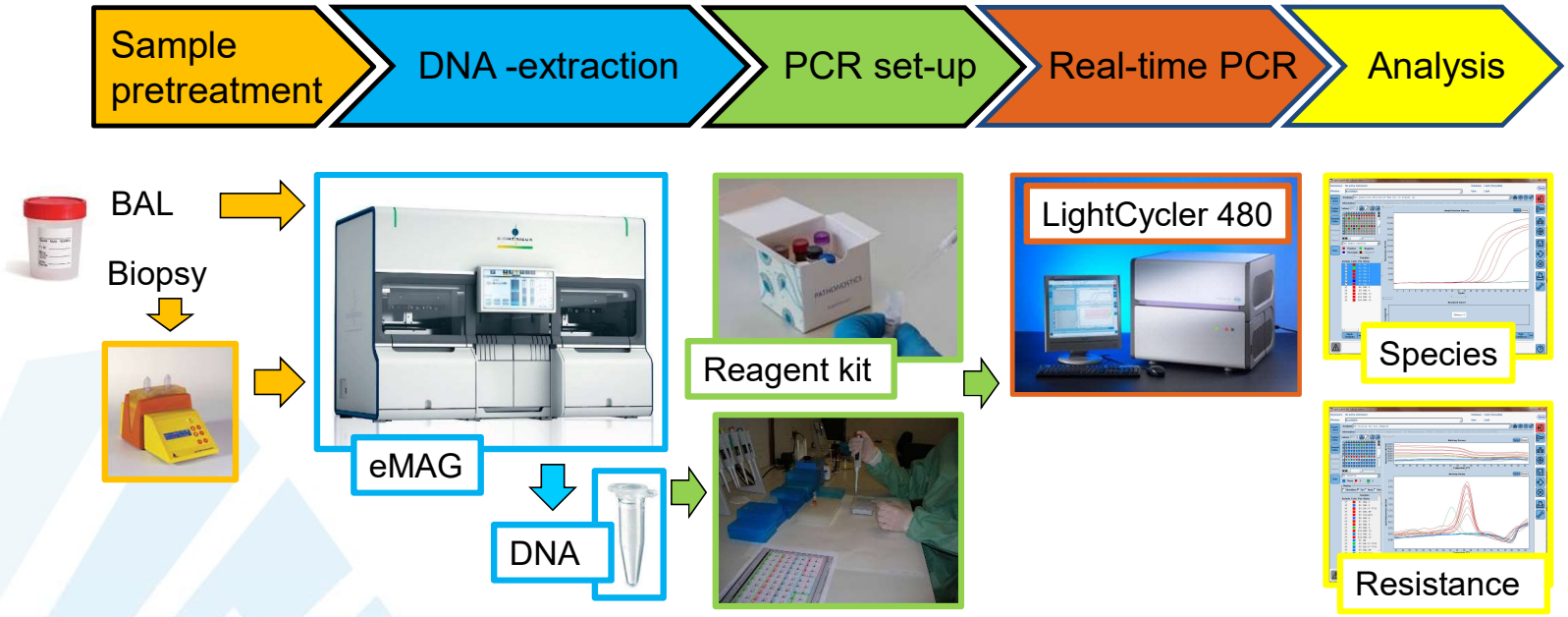

Validated for BAL samples




AsperGenius® based melting curve analysis of a potentially present L98H mutation in a BAL sample containing *A. fumigatus* DNA. The sample contained *A. fumigatus* wild type DNA (green curve in the range 61.0–64.0°C) compared to the L98H positive control DNA (brown curve in the range 65.5–68.5°C).

P. Postina et al. Frontiers in Microbiology 2018 Mar 27;9:555.

# Procedure AsperGenius test (Pathonostics)




| **Assay-time** (minutes) | | | | | | **Total** |
|---|---|---|---|---|---|---|
| ✋ | (15) | 25 | 30 | 10 | 15 | 95 |
| 🕒 | (20) | 145 | 30 | 120 | 15 | 330 |

✋ Hands-on time  🕒 Total time

**Detection of resistance is possible with molecular methods, exclusion of resistance not!**

Clinical Infectious Diseases
MAJOR ARTICLE

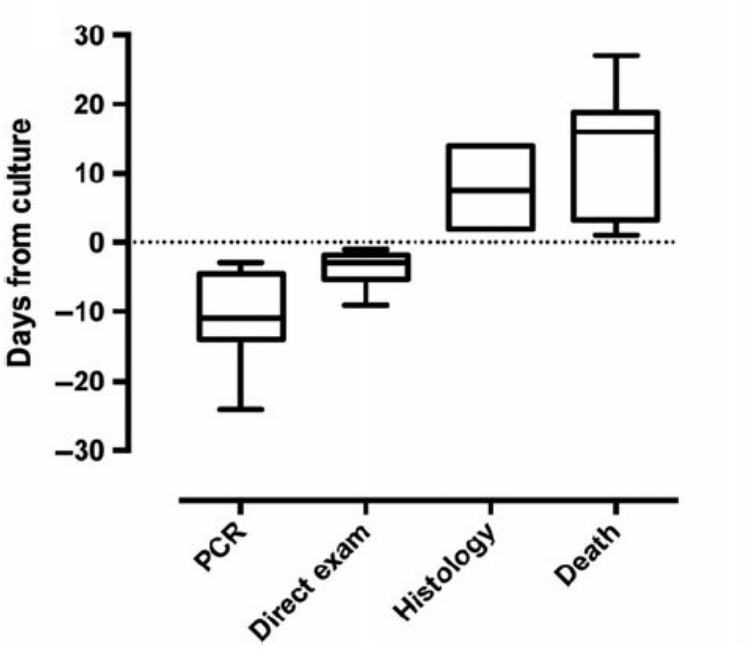
# Detection of Circulating Mucorales DNA in Critically Ill Burn Patients: Preliminary Report of a Screening Strategy for Early Diagnosis and Treatment

Matthieu Legrand,[1,2,3] Maud Gits-Muselli,[4] Louis Boutin,[1] Dea Garcia-Hermoso,[5,6] Véronique Maurel,[1] Sabri Soussi,[1] Mourad Benyamina,[1] Axelle Ferry,[1] Maïté Chaussard,[1] Samia Hamane,[4] Blandine Denis,[7] Sophie Touratier,[8] Nicolas Guigue,[4] Emilie Fréalle,[9] Mathieu Jeanne,[10] Jean-Vivien Shaal,[11] Charles Soler,[12] Maurice Mimoun,[2,13] Marc Chaouat,[2,13] Matthieu Lafaurie,[7] Alexandre Mebazaa,[1,2,3] Stéphane Bretagne,[2,4,5,6] and Alexandre Alanio[2,4,5,6]

- Patients and samples: 77 severely ill burn patients (418 samples), admitted between Oct 2013 - Feb 2016
- Assay: three real-time PCR assays for circulating mucorales DNA in plasma, identification at the genus level
- Retrospective part: Oct 2013 – Jan 2015, plasma collected at 3, 7, 14 and 28 days after admission, n =31, 5 developed invasive wound mucormycosis
- Prospective part: after Feb 2015, patients were screened twice weekly, liposomal amphotericin-B imitated based on a positive qPCR, n = 44, 3 developed invasive wound mucormycosis
- Primary endpoint: time between cmDNA detection and standard diagnosis
- Secondary endpoints: time from cmDNA detection and treatment initiation and mortality

# Time between PCR positivity and standard diagnosis

DNA detected 11 days before culture based diagnosis

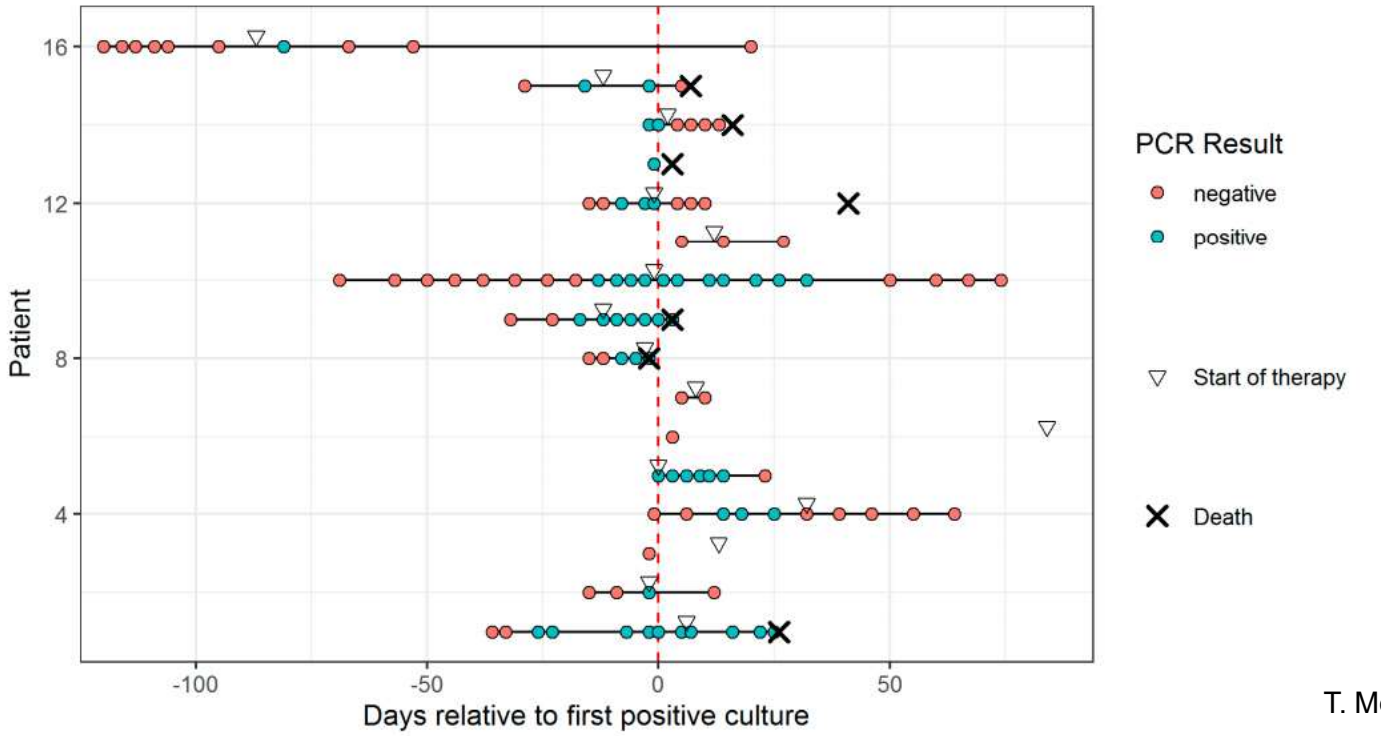



Time from admission to positive plasma qPCR was 7 days and to positive culture 16 days

M. Legrand et al. CID 2016, 63: 1312-1317

# Commercial assay for molecular detection of Mucorales species : MucorGenius® assay

- Serial blood samples from patients with culture-positive invasive mucormycosis
- Overall sensitivity of 75%.
- A positive test preceded a positive culture result by up to 81 days (median eight days).
- After initiation of appropriate therapy, the average levels of circulating DNA decreased after one week and stabilized after two weeks.




T. Mercier et al. Journal of Fungi 2019

Clinical Infectious Diseases
SUPPLEMENT ARTICLE

# Molecular Techniques for Genus and Species Determination of Fungi From Fresh and Paraffin-Embedded Formalin-Fixed Tissue in the Revised EORTC/MSGERC Definitions of Invasive Fungal Infection

Shawn R. Lockhart,[1] Ralf Bialek,[2] Christopher C. Kibbler,[3] Manuel Cuenca-Estrella,[4] Henrik E. Jensen,[5] and Dimitrios P. Kontoyiannis[6]

**Fungal elements seen in tissue samples by histopathology and identified by PCR followed by sequencing should fulfill the definition of a proven fungal infection, identified to genus/species, even in the absence of culture.**

Lockart SR et al. CID 2021, Suppl 2

Journal of *Fungi*

MDPI

*Article*

# Introduction of a Comprehensive Diagnostic and Interdisciplinary Management Approach in Haematological Patients with Mucormycosis: A Pre and Post-Intervention Analysis

Malene Risum [1], Jannik Helweg-Larsen [2], Søren Lykke Petersen [3], Peter Kampmann [3], Ulrik Malthe Overgaard [3], Daniel El Fassi [4,5], Ove Juul Nielsen [3], Mette Brabrand [6], Niclas Rubek [7], Lars Munksgaard [8], Marianne Tang Severinsen [9,10], Bendt Nielsen [11], Jan Berg Gertsen [12], Åsa Gylfe [13], Ulla Hjort [14], Angeliki Vourtsi [15], Rasmus Krøger Hare [1] and Maiken Cavling Arendrup [1,5,16,*]

8 November 2020

# Management of mucormycosis in haematological patients

Case-series based review 2012 – 2019: 13 cases

2016 – 2017: implementation of aggressive, multidisciplinary management approach

**Multidisciplinary**

Treating physician, surgeon and microbiologist

**Management approach**

- Early combination therapy with liposomal amphotericin B 5 mg/kg + isavuconazole
- Imaging supported evaluation of extension
- Early diagnostic biopsies or immediate resection with consideration of risk of surgical complications
- Real time Blankophor microscopy of removed tissue and biopsies from resection margins – guiding subsequent surgical revisions
- Repeated surgical resections performed until radical resection is achieved
- Topical amphotericin B treatment when possible
- Dose adjustment according to species, susceptibility pattern, anatomic localisation and TDM results

M. Risum et al. JOF 2020

## Data suggest improved treatment outcomes for mucormycosis

| | 2012-2015 | 2016-2019 |
|---|---|---|
| Median N° samples/patient for culture/PCR | 3/3 | 10.5/10 |
| Six months mortality | 3/5 | 0/7 |

Only 2 pulmonary infections

M. Risum et al. JOF 2020

# ANTIFUNGAL SUSCEPTIBILITY TESTING

# When to perform antifungal susceptibility testing?

- All isolates from patients invasive infections
  - *Candida*:
    - Isolates from normal sterile sites
    - Higher change of resistance in patients exposed to antifungal agents before
  - *Aspergillus*:
    - All invasive infections
    - Resistance mainly develops in the environment and thus also occurs in azole naïve patients
  - Rare moulds, rare yeasts
- Difficult to treat mucosal infections, e.g. recurrent vulvovaginal candidiasis

# Reference methods for antifungal susceptibility testing

## *Candida* spp.

| Parameter | CLSI (M27-4th edition) | EUCAST (E.Def 7.3.1) |
|---|---|---|
| Format | Microdilution | Microdilution |
| Culture medium | RPMI + MOPS, pH 7.0 | RPMI + MOPS + 2% glucose, pH 7.0 |
| Final inoculum | 0.5 - 2.5 x$10^3$ CFU /ml | 0.5 - 2.5x$10^5$ CFU/ml |
| Incubation time | 24h | 24h |
| Wells | Round bottom | Flat bottom |
| Reading | Visual | Spectrophotometric |
| MIC endpoint | 50% inhibition (100% for AMB) | 50% inhibition (90% for AMB) |

## *Aspergillus* spp.

| Parameter | CLSI (M38-3th edition) | EUCAST (E.Def 9.3.1) |
|---|---|---|
| Format | Microdilution | Microdilution |
| Culture medium | RPMI + MOPS, pH 7.0 | RPMI + MOPS + 2% glucose, pH 7.0 |
| Final inoculum | 0.5 - 4 x$10^4$ CFU /ml | 1 - 2.5 x$10^5$ CFU/ml |
| Incubation time | 48h | 48h |
| Wells | Round bottom | Flat bottom |
| Reading | Visual | Visual |
| MIC endpoint | 100% inhibition | 100% inhibition |

# Commercial methods

- Etest, Vitek, Sensititre yeast one,…
- Standardised against CLSI and not to EUCAST
  - EUCAST breakpoints can not be applied as such
    (mainly risk for misclassification of echinocandin results)
- Validation mainly for *Candida* spp (check literature for other fungi)

EUCAST Version 10.0, valid from 2020-02-04/ CLSI M27, 4th edition, nov 2017, CLSI M59, 3th edition June 2020

| Antifungal | susceptible | | resistant | |
|---|---|---|---|---|
| | CLSI S ≤ | EUCAST S ≤ | CLSI R> | EUCAST R> |
| **Fluconazole** | | | | |
| C. albicans, C. parapsilosis, C. tropicalis | 2 | 2 | 4 | 4 |
| C. glabrata | - | 0.001 | 32 | 16 |
| **Anidulafungin** | | | | |
| C. albicans | 0.25 | 0.03 | 0.5 | 0.03 |
| C. tropicalis | 0.25 | 0.06 | 0.5 | 0.06 |
| C. krusei | 0.25 | 0.06 | 0.5 | 0.06 |
| C. parapsilosis | 2 | 4 | 4 | 4 |
| C. glabrata | 0.12 | 0.06 | 0.25 | 0.06 |
| **Amphotericin B** | | | | |
| C. albicans, C. glabrata, C. krusei, C. tropicalis | | 1 | 2 (ECV) | 1 |
| C. parapsilosis | | 1 | 1 (ECV) | 1 |

## SENSITITRE™ YEASTONE™ PLATE FORMAT

Plate Code: **YO10**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | POS | AND 0.015 | AND 0.03 | AND 0.06 | AND 0.12 | AND 0.25 | AND 0.5 | AND 1 | AND 2 | AND 4 | AND 8 | AB 0.12 |
| B | MF 0.008 | MF 0.015 | MF 0.03 | MF 0.06 | MF 0.12 | MF 0.25 | MF 0.5 | MF 1 | MF 2 | MF 4 | MF 8 | AB 0.25 |
| C | CAS 0.008 | CAS 0.015 | CAS 0.03 | CAS 0.06 | CAS 0.12 | CAS 0.25 | CAS 0.5 | CAS 1 | CAS 2 | CAS 4 | CAS 8 | AB 0.5 |
| D | FC 0.06 | FC 0.12 | FC 0.25 | FC 0.5 | FC 1 | FC 2 | FC 4 | FC 8 | FC 16 | FC 32 | FC 64 | AB 1 |
| E | PZ 0.008 | PZ 0.015 | PZ 0.03 | PZ 0.06 | PZ 0.12 | PZ 0.25 | PZ 0.5 | PZ 1 | PZ 2 | PZ 4 | PZ 8 | AB 2 |
| F | VOR 0.008 | VOR 0.015 | VOR 0.03 | VOR 0.06 | VOR 0.12 | VOR 0.25 | VOR 0.5 | VOR 1 | VOR 2 | VOR 4 | VOR 8 | AB 4 |
| G | IZ 0.015 | IZ 0.03 | IZ 0.06 | IZ 0.12 | IZ 0.25 | IZ 0.5 | IZ 1 | IZ 2 | IZ 4 | IZ 8 | IZ 16 | AB 8 |
| H | FZ 0.12 | FZ 0.25 | FZ 0.5 | FZ 1 | FZ 2 | FZ 4 | FZ 8 | FZ 16 | FZ 32 | FZ 64 | FZ 128 | FZ 256 |

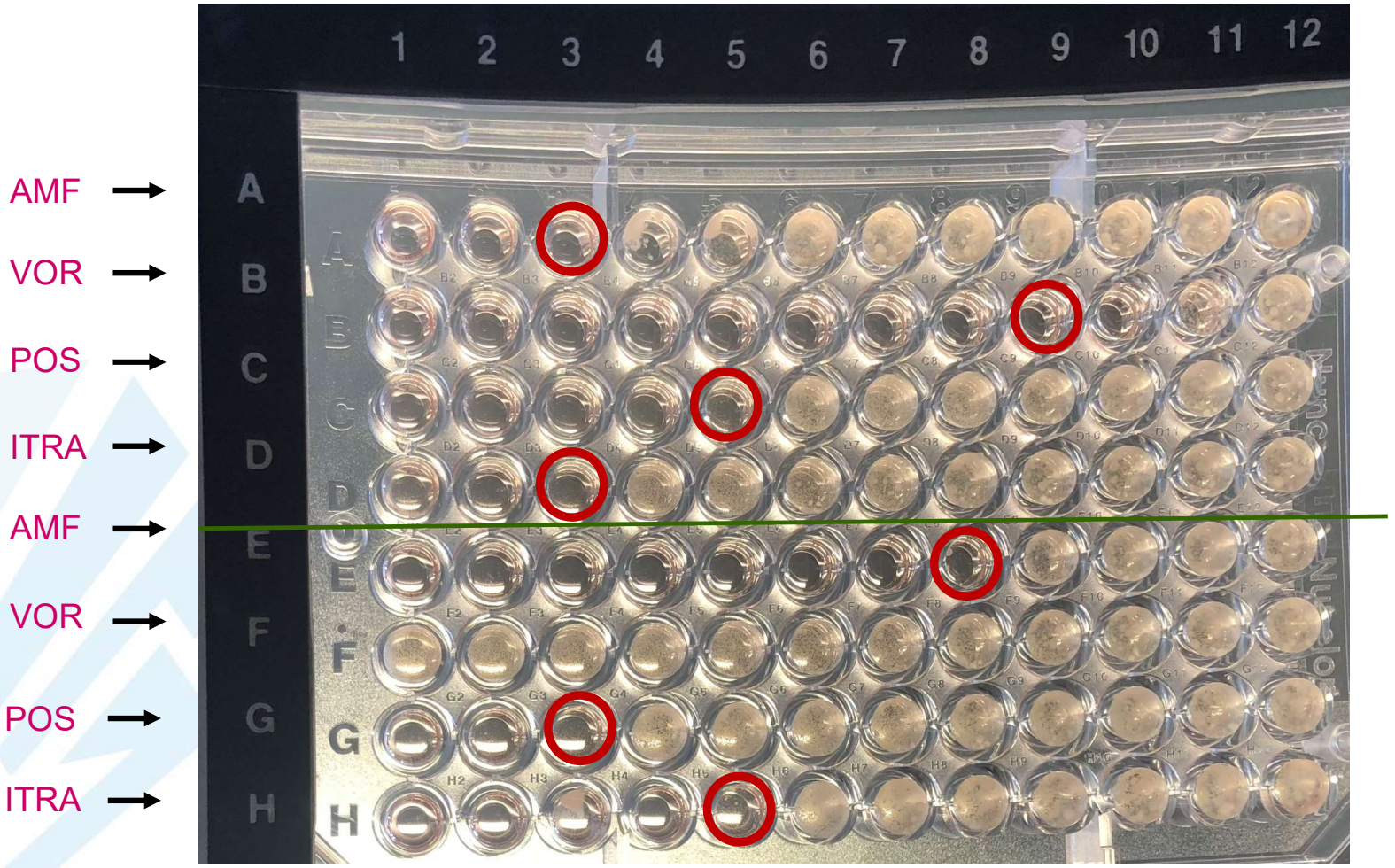
**ANTIMICROBICS**

| | |
|---|---|
| POS | Positive Control |
| AND | Anidulafungin |
| AB | Amphotericin B |
| MF | Micafungin |
| CAS | Caspofungin |
| FC | 5-Flucytosine |
| PZ | Posaconazole |
| VOR | Voriconazole |
| IZ | Itraconazole |
| FZ | Fluconazole |

The YeastOne system is available in a dry-form 96-well panel with the colorimetric growth indicator Alamar Blue

# Sensititre YeastOne antifungal panel

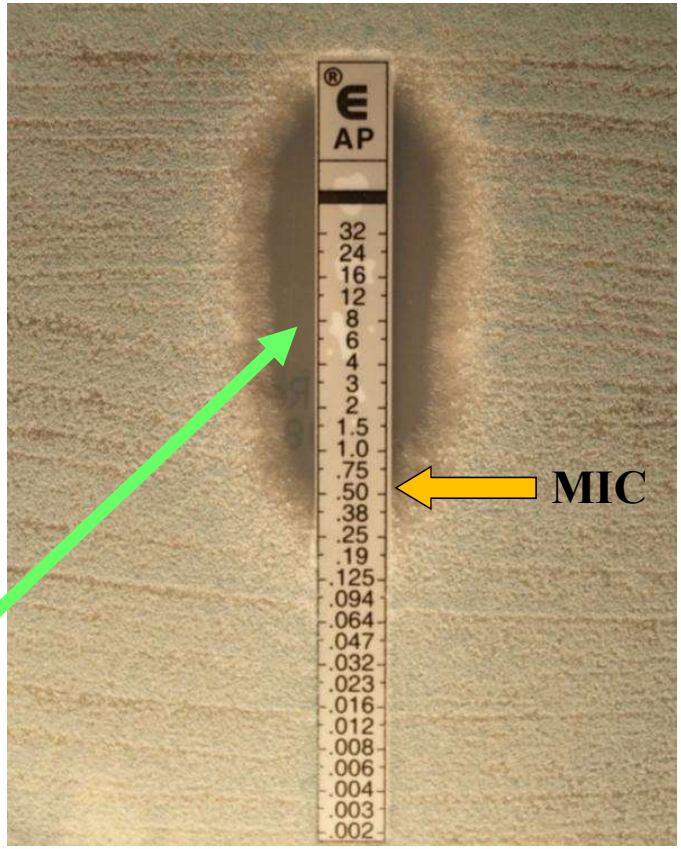
# EUCAST susceptibility testing



# Amphotericin B: fungicidal

Easy to read

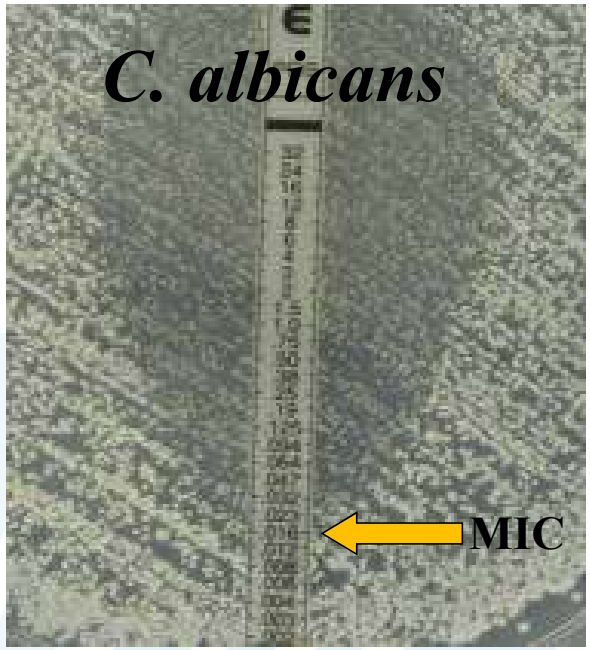
The first well showing a distinct color change as compared to the positive growth well is the MIC.




**Complete inhibition**

# Azoles

## Yeasts

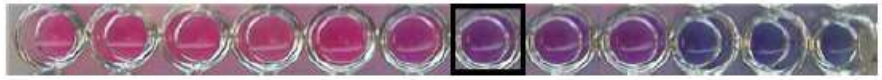



Can be difficult to read, trailing endpoint

Reading guide: when trailing endpoints occur, read the MIC at the first point of significant inhibition of growth i.e. the so-called 80% inhibition as judged by the naked eye.

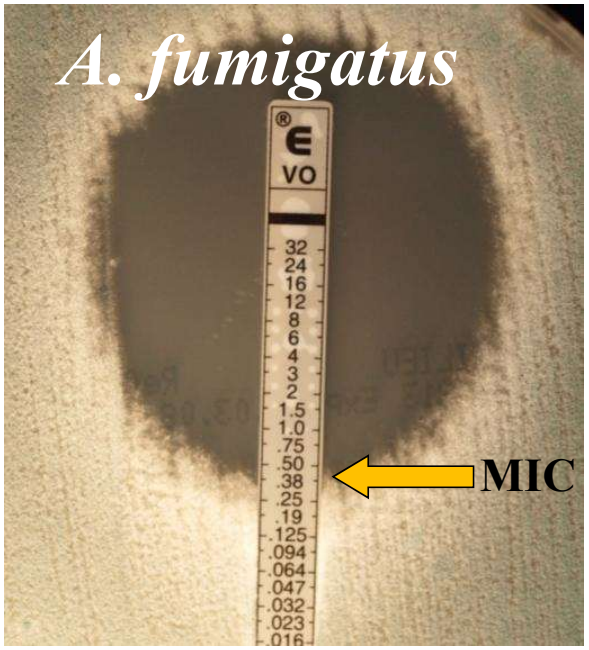
Reading course (AB BIODISK, Solna Sweden): MIC = concentration at which normal colony morphology changes to a different colony morphology

Trailing endpoint: This occurs when a slight color change persists and is often identical in several concentrations. The MIC should be read as the first well showing a less intense color change compared to the positive growth control well.



## Filamentous fungi

Easy to read

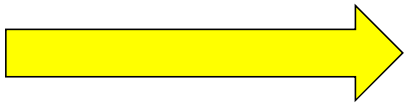


# National Reference Center of Leuven: what do we do?

***Candida* species**

Sensititre Yeast One

+ CLSI breakpoints



unexpected results/difficult to read

**All other fungi**

***Candida auris***

EUCAST reference method

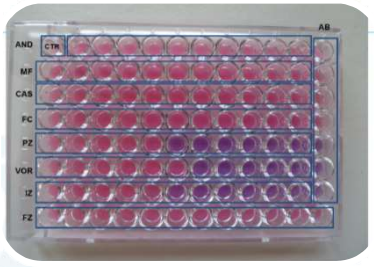
+ EUCAST breakpoints

# Triazole resistance detection in Aspergillus

ISOLATE

ISOLATE

SAMPLE



**MIC determination**

- CLSI/EUCAST
- (Commercial systems)

**Triazole resistance screening agar**

**Molecular methods**

- Always perform susceptibility testing if antifungal therapy is intended – contact the lab!
- Both azole-susceptible and azole-resistant phenotypes can be simultaneously present in culture, test multiple colonies!

# Therapeutic drug monitoring

- Importance is increasingly recognized
- Highest recommendation for voriconazole, posaconazole, also recommended for itraconazole, isavuconazole and flucytosine at least for specific circumstances
- Clinical input (access to medical dossier?) and judgement is essential
- Turn-around time is important
  - Performing on site versus shipment to reference laboratory
  - Quality control is essential
  - Commercial assays are now available

HR Ashbee et al. JAC 2014, 69: 1162-1176.

THE LANCET
Infectious Diseases

# Global guideline for the diagnosis and management of rare yeast infections: an initiative of the ECMM in cooperation with ISHAM and ASM

*Sharon C-A Chen*, John Perfect*, Arnaldo L Colombo*, Oliver A Cornely*, Andreas H Groll, Danila Seidel, Kerstin Albus, Joao N de Almedia Jr, Guillermo Garcia-Effron, Nicole Gilroy, Cornelia Lass-Flörl, Luis Ostrosky-Zeichner, Livio Pagano, Tamas Papp, Riina Rautemaa-Richardson, Jon Salmanton-García, Andrej Spec, Joerg Steinmann, Sevtap Arikan-Akdagli, Dorothee E Arenz, Rosanne Sprute, Luisa Duran-Graeff, Tomas Freiberger, Corrado Girmenia, Michelle Harris, Souha S Kanj, Maryam Roudbary, Olivier Lortholary, Joseph Meletiadis, Esther Segal, Felipe Francisco Tuon, Nathan Wiederhold, Tihana Bicanic, Jagdish Chander, Yee-Chun Chen, Po-Ren Hsueh, Margaret Ip, Patricia Munoz, Isabel Spriet, Elvis Temfack, Luis Thompson, Anna Maria Tortorano, Aristea Velegraki, Nelesh P Govender**

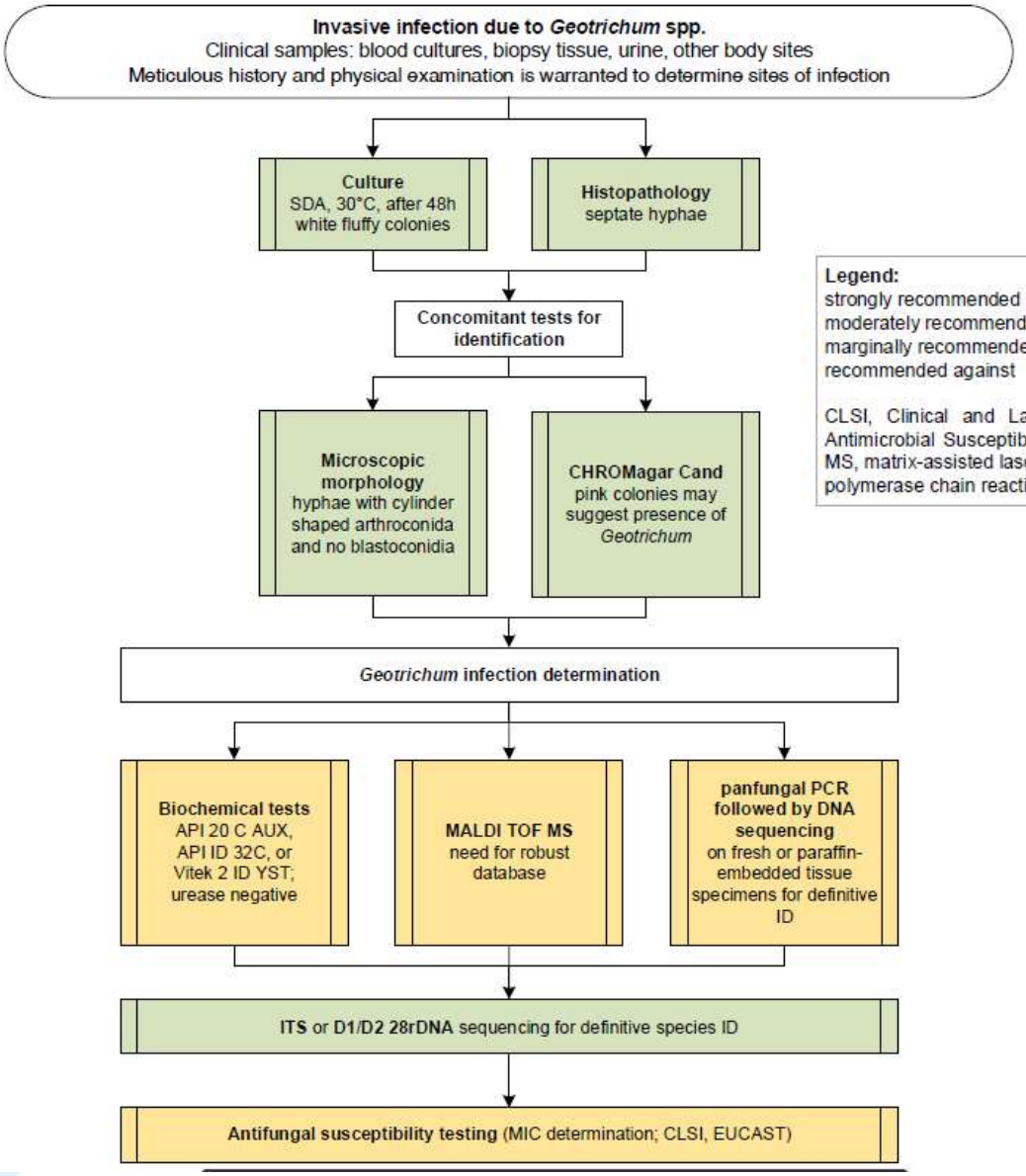
## Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: C-A Chen S, Perfect J, Colombo AL, et al. Global guideline for the diagnosis and management of rare yeast infections: an initiative of the ECMM in cooperation with ISHAM and ASM. *Lancet Infect Dis* 2021; published online August 19. https://doi.org/10.1016/S1473-3099(21)00203-6.

237 pages!

Published Online August 2021




**Legend:**

strongly recommended

moderately recommended

marginally recommended

recommended against

CLSI, Clinical and Laboratory Standards Institute; DNA, deoxyribonucleic acid; EUCAST, European Committee on Antimicrobial Susceptibility Testing; rDNA, ribosomal DNA; ID, identification; ITS, internal transcribed spacer; MALDI TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction; SDA, Sabouraud dextrose agar

THE LANCET
Infectious Diseases

# Global guideline for the diagnosis and management of rare mould infections: an initiative of the European Confederation of Medical Mycology in cooperation with the International Society for Human and Animal Mycology and the American Society for Microbiology

*Martin Hoenigl, Jon Salmanton-García, Thomas J Walsh, Marcio Nucci, Chin Fen Neoh, Jeffrey D Jenks, Michaela Lackner, Rosanne Sprute, Abdullah M S Al-Hatmi, Matteo Bassetti, Fabianne Carlesse, Tomas Freiberger, Philipp Koehler, Thomas Lehrnbecher, Anil Kumar, Juergen Prattes, Malcolm Richardson, Sanjay Revankar, Monica A Slavin, Jannik Stemler, Birgit Spiess, Saad J Taj-Aldeen, Adilia Warris, Patrick C Y Woo, Jo-Anne H Young, Kerstin Albus, Dorothee Arenz, Valentina Arsic-Arsenijevic, Jean-Philippe Bouchara, Terrence Rohan Chinniah, Anuradha Chowdhary, G Sybren de Hoog, George Dimopoulos, Rafael F Duarte, Petr Hamal, Jacques F Meis, Sayoki Mfinanga, Flavio Queiroz-Telles, Thomas F Patterson, Galia Rahav, Thomas R Rogers, Coleman Rotstein, Retno Wahyuningsih, Danila Seidel, Oliver A Cornely*

## Supplementary appendix

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Supplement to: Hoenigl M, Salmanton-García J, Walsh TJ, et al. Global guideline for the diagnosis and management of rare mould infections: an initiative of the European Confederation of Medical Mycology in cooperation with the International Society for Human and Animal Mycology and the American Society for Microbiology. *Lancet Infect Dis* 2021; published online Feb 16. https://doi.org/10.1016/S1473-3099(20)30784-2.

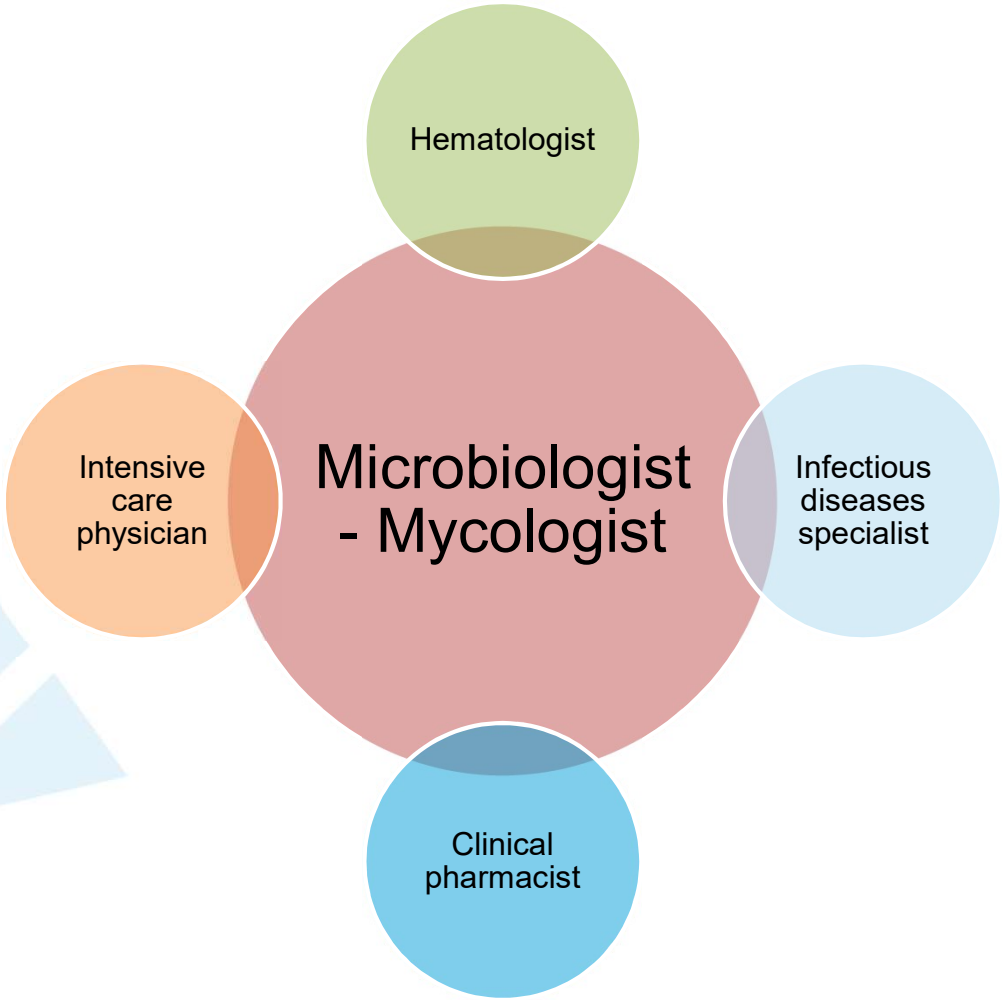
263 pages!

# Some reflections…

- Every single test has obvious limitations. Combination testing enhances the opportunity to detect biomarkers that may vary differentially at various stages of infection
- Optimal strategy depends on clinical setting (screening, diagnosis, mould-active prophylaxis, epidemiology including triazole resistance) and practical considerations
- GM LFA (IMMY) has a similar performance to GM ELISA
- Diagnosis of IA in the ICU setting is based largely on GM detection in BAL fluid but this test does not allow discrimination between colonisation and infection. There is an urgent need for biomarkers for tissue invasion and airway involvement
  - GM detection in blood of influenza patients admitted to the ICU is a useful test:
    - Negative result does not exclude the diagnosis of IA but...
    - Positive result increases the diagnostic certainty compared with a GM BAL-only positive patient, is a marker of angioinvasion and is useful for treatment follow-up

# Some reflections…

- Detection of circulating Mucorales DNA in blood appears to be a good, fast diagnostic test that often precedes diagnosis by culture/histopathology
- Discordant results will occur when combining different biomarkers!
  - High certainty about lack of invasive mould disease if different biomarkers are negative, and therefore withhold antifungal therapy
  - High certainty of presence of invasive mould disease if different biomarkers are positive


Hematologist
Intensive care physician
Microbiologist - Mycologist
Infectious diseases specialist
Clinical pharmacist