

Novel Diagnostic Platforms for Pan-fungal Identification and Detection

Sean Zhang, MD, PhD, D(ABMM)

Associate Professor of Pathology

Johns Hopkins School of Medicine

Medical Director, Clinical Mycology Laboratory

Johns Hopkins Hospital

Co-founder and Chair, Fungal Diagnostic Laboratory Consortium (FDLC)

(<https://labs.pathology.jhu.edu/fdlc/>)



JOHNS HOPKINS
M E D I C I N E

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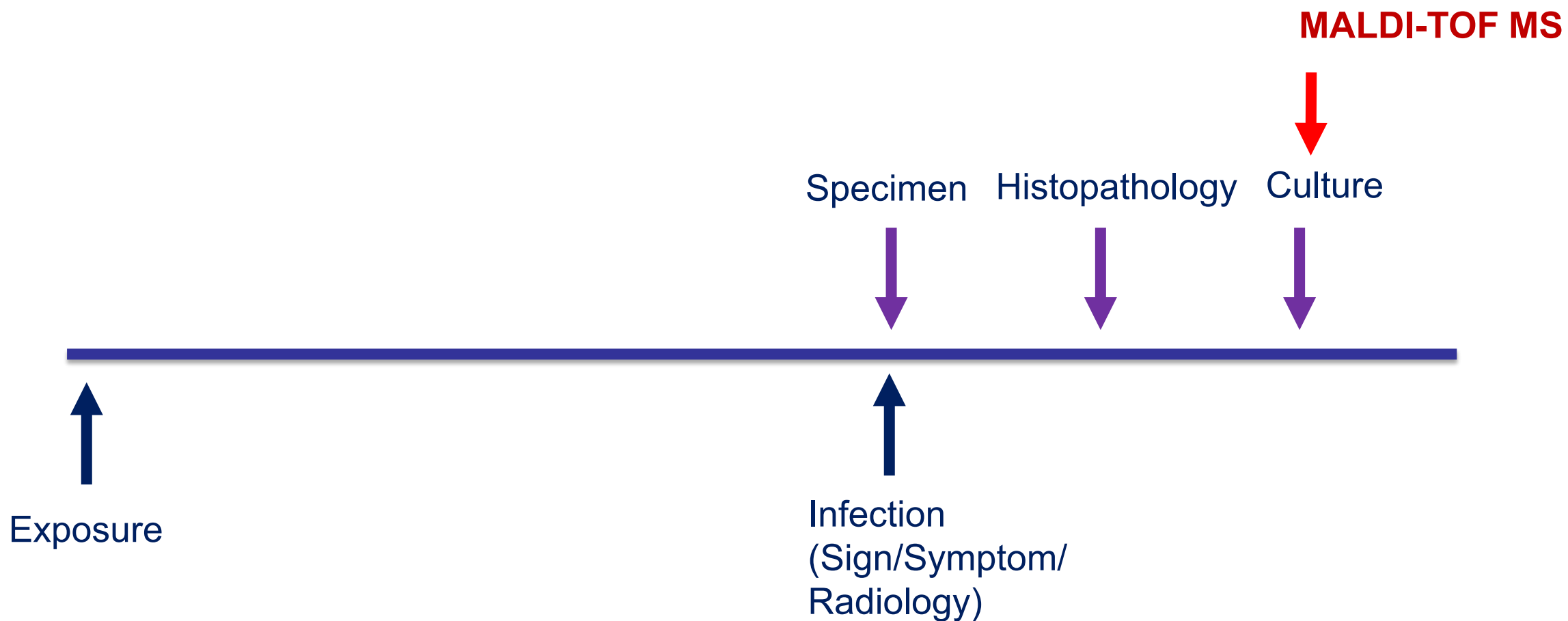
Disclosure

Received research grants and serve scientific board from the following companies:
Affinity Biosensors, Anvil Diagnostics, Applied BioCode, Bruker Daltonics, DiaSorin, ELITechGroup, Euroimmun, Fujifilm, Gotham Biotech, IMMY, Karius, Optimum Imaging Diagnostics, Pearl Diagnostics, Scanogen, T2 Biosystems, Vela Diagnostics, Zepto Life Technology.

Objective

- To provide an overview of current lab diagnostic methods for fungal organism identification and fungal disease diagnosis.
- To describe advances and progress to potentially enhance the detection and diagnosis of fungal diseases.

“Standard” of Care Lab Tests



MALDI-TOF MS for mold ID



	Bruker MALDI Biotyper® sirius RUO System	bioMerieux Vitek MS (new version vitek MS prime)
Extraction	-Extended Direct Transfer method (eDT) or Mycelium Transfer (MyT) method: pick up pea size colony from solid media culture -Full extraction: hyphae mass from liquid culture (database)	-Tube extraction: pick up 1-2cm circle of colony from solid media culture
Cutoff	≥1.7; <1,7 (NOI); No peak	≥99%
Database	Mold database: 226 species/groups (RUO) (MBT Filamentous Fungi Library + Ext, 2023) * Build up customer database	Mold database: 79 species (FDA approved) (Vitek MS v3.2)/347 taxa (SARAMIS V4 RUO)



Performance comparison of mold ID by Bruker vs bioMerieux Vitek MALDI-TOF MS



Methods

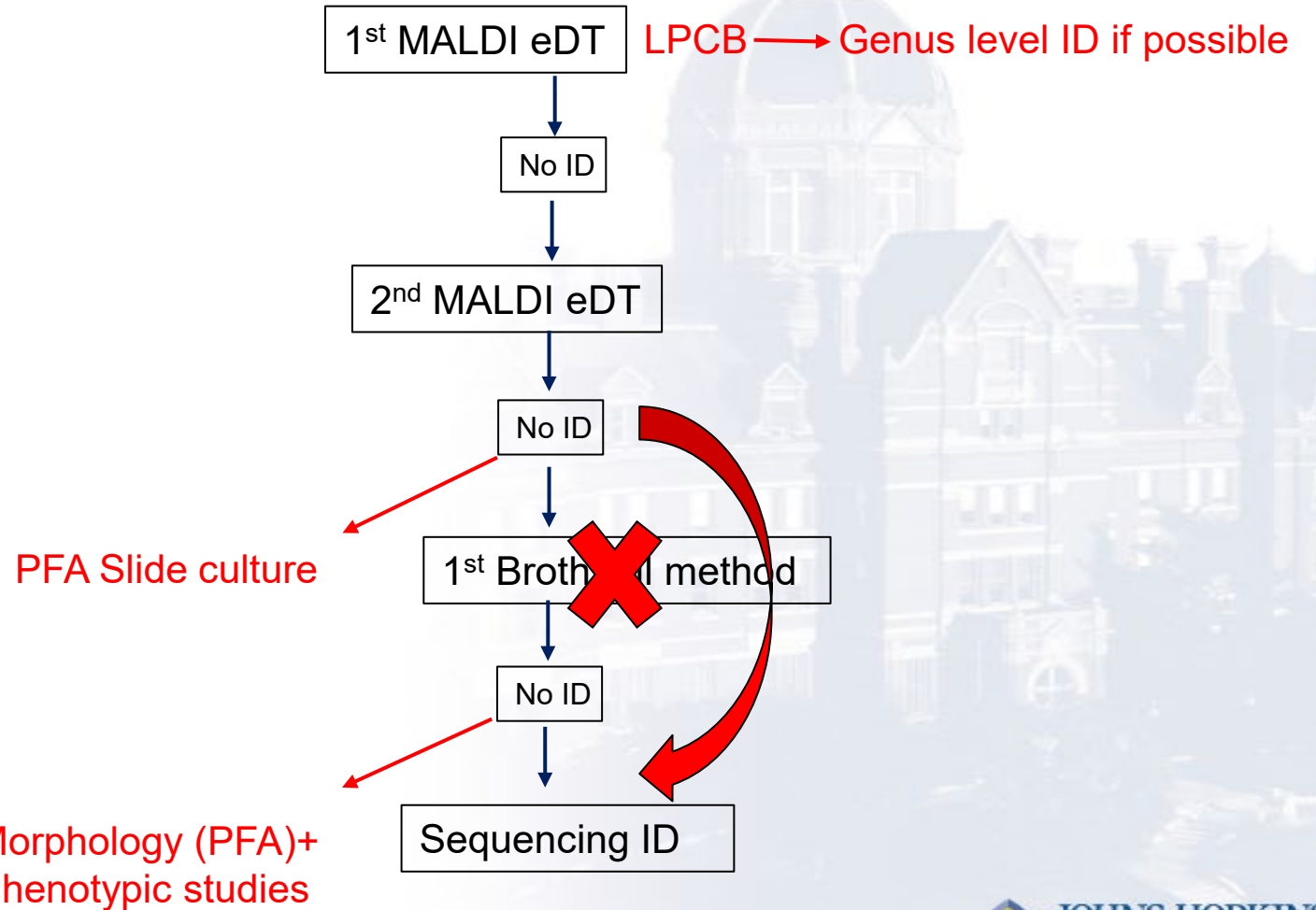
- St. Jude Children's Research Hospital (Vitek MS Knowledgebase Library 3.2)
- Johns Hopkins Hospital (Bruker MS Filamentous Fungi Library 4.0)
- 149 molds (listed in both Vitek or Bruker MS library) including 35 species/species complex
- Discrepant results was analyzed by sequencing and/or culture morphologies

Summary

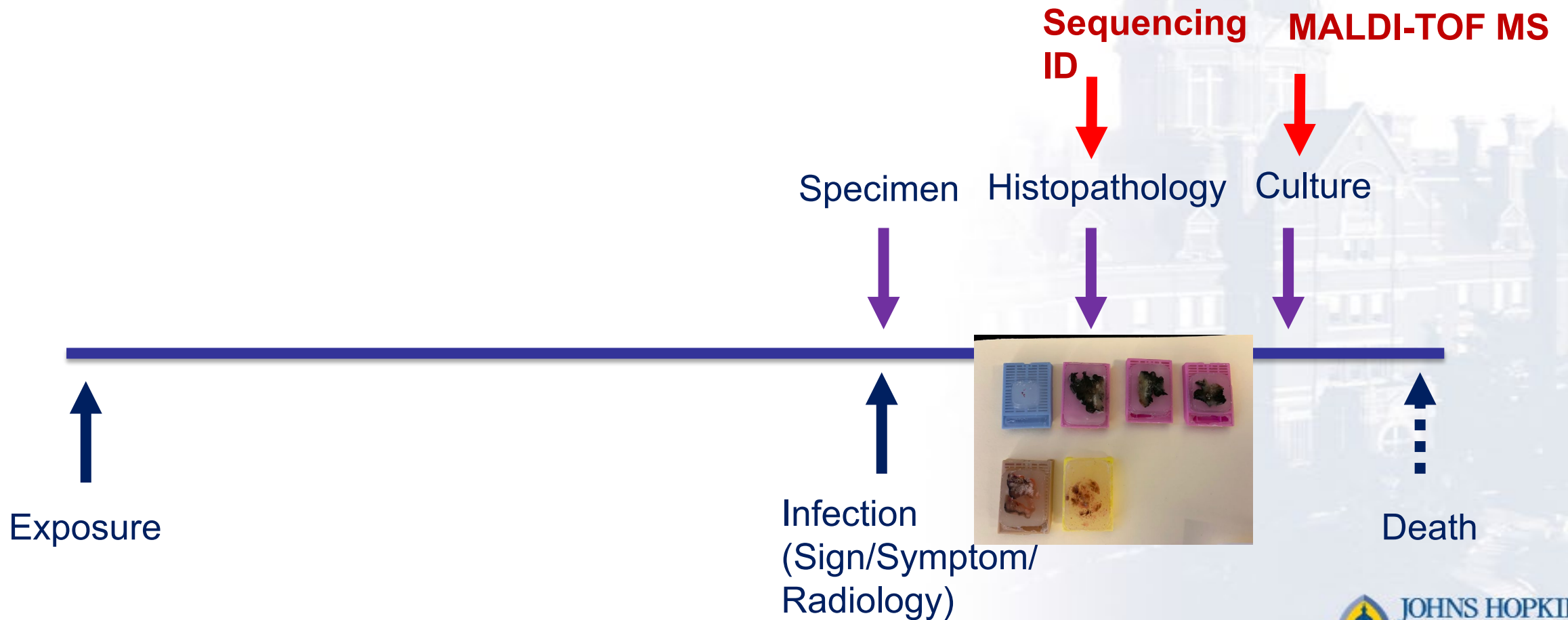
- 99% (148/149) concordant at genus level
- 81% (120/149) concordant at species level
- Few isolates (e.g. 5 *Fusarium*) concordant by both MS systems but identified different species/species complex by DNA sequencing.
- 3 isolates concordant by both MS systems but exhibited nomenclature inconsistencies
 - *Lecythophora* (VITEK)/*Coniochaeta hoffmannii* (Bruker)
 - *Microsporum gypseum* (VITEK)/*Nannizzia gypsea* (Bruker)
 - *Paecilomyces variotii* (VITEK)/*Byssochlamys spectabilis* (Bruker)

JHH Mold ID Algorithm

- MALDI is now our 1st line of method for mold ID using eDT
- Sent to MALDI as soon as a pea size mold colony appears in culture media
- >50% molds are identified to genus/species level in 24h (78% identified by MALDI <3d)

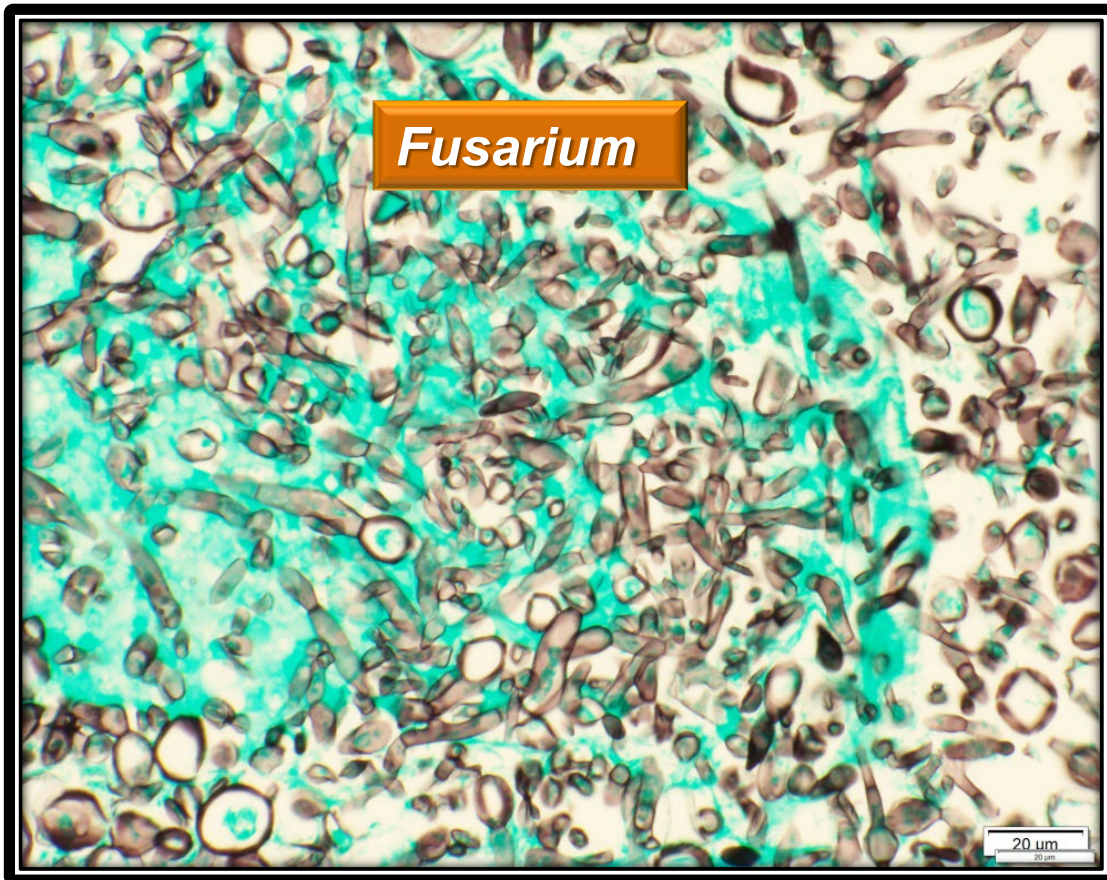


“Standard” of Care Lab Tests



Tissue – FFPE (Formalin-fixed Paraffin-embedded)

Lung biopsy GMS stain



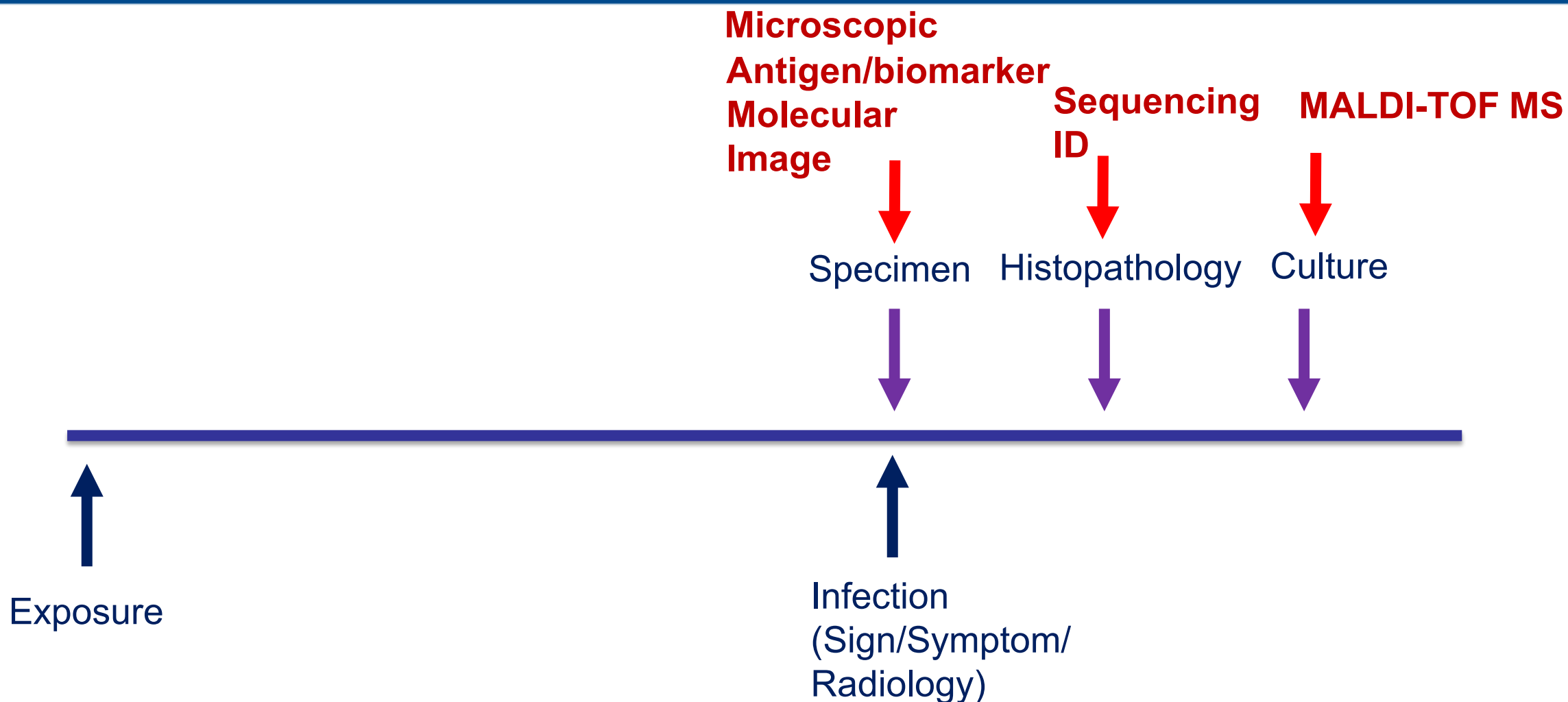
Molecular approaches (pan-fungal PCR/sequencing) to identify fungal pathogens from FFPE tissues

- Histopathological exam can be subjective, prone to diagnostic errors
 - 21% error rate
- Accurate species level identification, thus appropriate antifungal treatment can be initiated without delay
- No other choice
 - Tissue processed for histology but not for culture
 - Tissue culture fail to grow

Molecular Techniques for Genus and Species Determination of Fungi From FFPE Tissue in the Revised EORTC/MSGERC Definitions of Invasive Fungal Infection

- PCR/sequencing from tissue, but not immunohistochemistry or in situ hybridization, can be used for genus or species determination under the new EORTC/MSGERC guidelines, but **only when fungal elements are identified by histology.**
- PCR followed by sequencing should fulfill the definition of a proven fungal infection, identified to genus/species, even in the absence of culture

“Standard” of Care Lab Tests

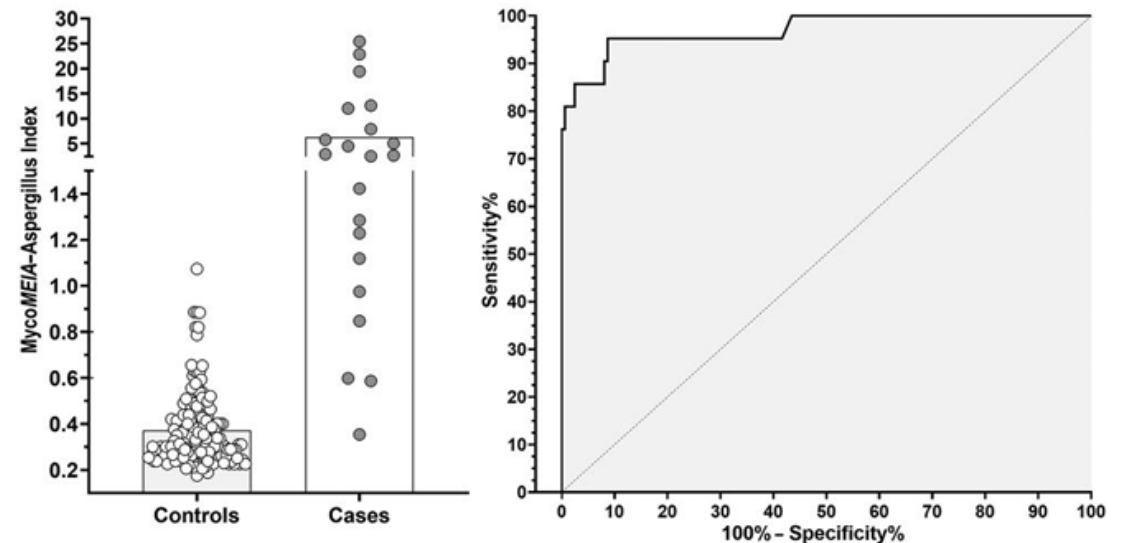


Fungal antigen assay

Name	Disease	Method	Sample type
Cryptococcus antigen (glucuronoxylomannan) (IMMY)	Cryptococcosis	LFA	Serum, CSF
<i>Candida</i> mannan (BioRad)	Candidiasis	EIA	Serum
Histoplasma/Blastomyces antigen (galactomannan) (MiraVista, IMMY, Gotham)	Histoplasmosis & Blastomycosis	EIA, LFA	Serum, urine
<i>Coccidioides</i> antigen (MiraVista)	Coccidioidomycosis	EIA	Serum, CSF
<i>Aspergillus</i> galactomannan (Platelia BioRad – FDA cleared)	Aspergillosis	EIA, LFA	Serum, BAL
(1,3)- β -D-glucan (BDG) (Fungitell Associated of Cape Cod – FDA cleared)	Invasive fungal disease	LAL	Serum, CSF

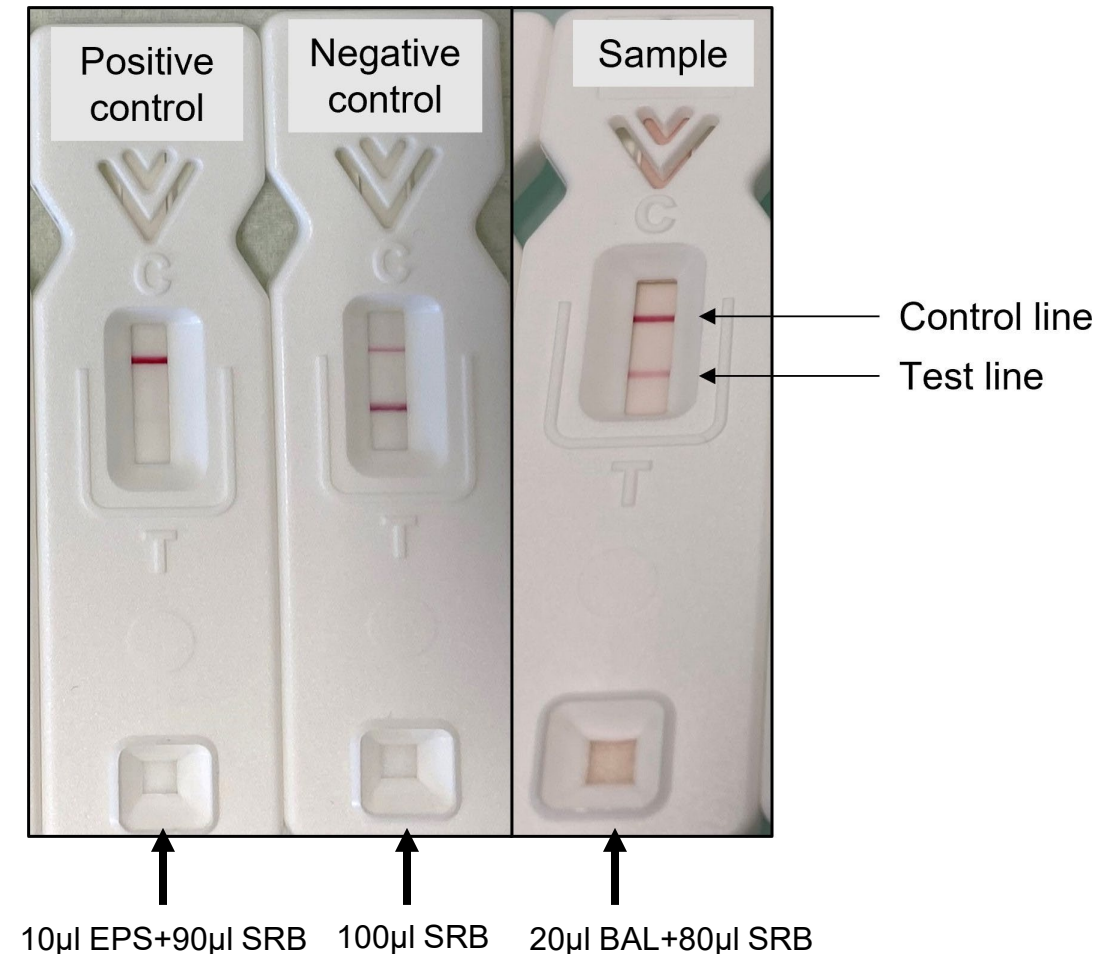
Novel urinary antigen assay (MycoMEIA) as an aid to diagnose invasive aspergillosis (IA)

- Detects *Aspergillus* galactofuranose in urine (recognized by mAb476)
- In testing 920 urine samples from 310 subjects, the assay showed ROC AUC of 0.97, per subject sensitivity of 91.2% and specificity of 89.2% for diagnosis of IA
- The assay has recently been approved by FDA



Detection of Mucorales antigen in BAL using newly developed LFD

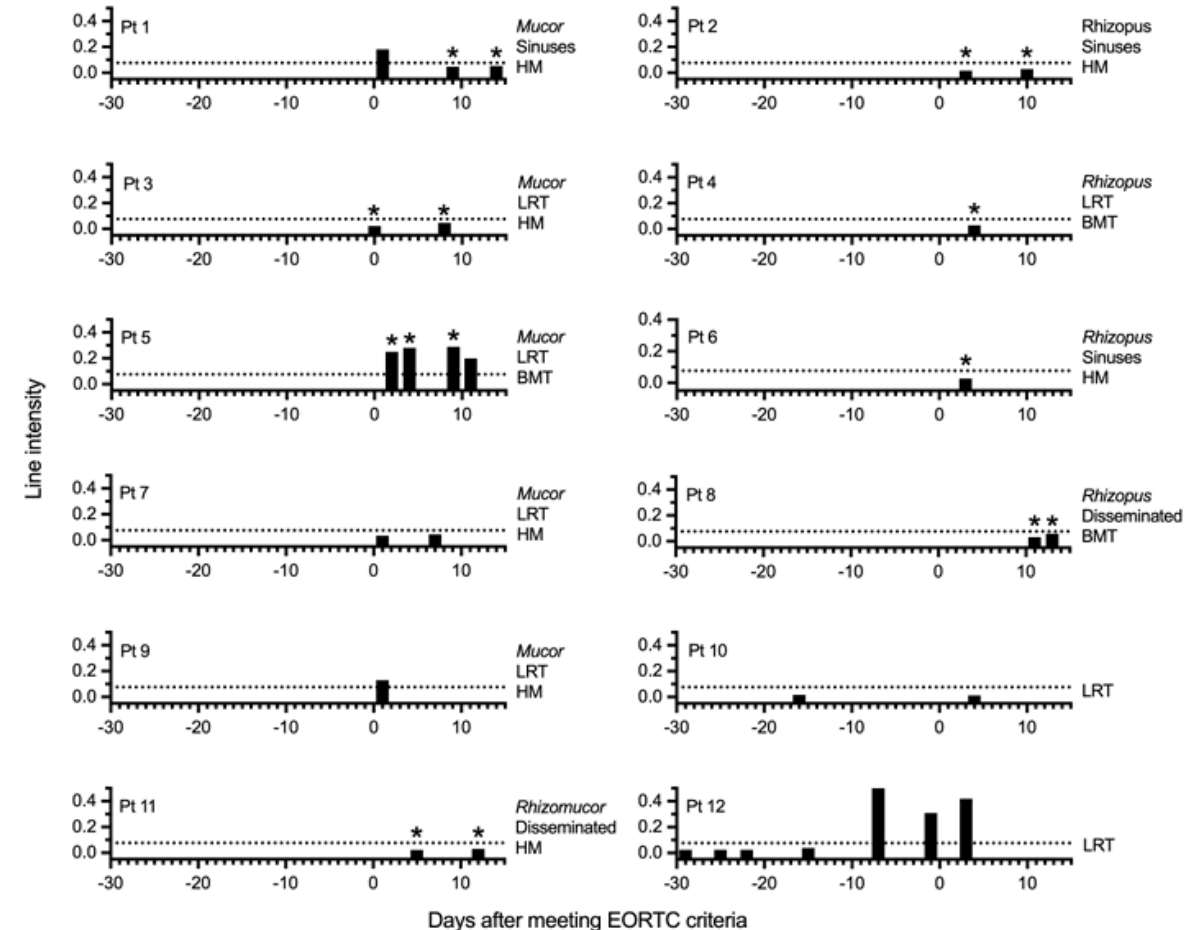
- TG11-LFD
 - A murine IgG2b mAb (TG11) binding to an **extracellular polysaccharide** (EPS) antigen secreted by all Mucorales
 - A competitive test: intensities of T line and C line after 30min as a.u. using a Cube reader; a pos results showing a reduced intensity of the T line (a.u. value ≤ 531 based on ROC curve at 0.739)
- Demonstrated sensitivity of 76.9% and specificity of 75.5% in 62 retrospective frozen BALs (pellets or supernatants)



Mucorales mannan

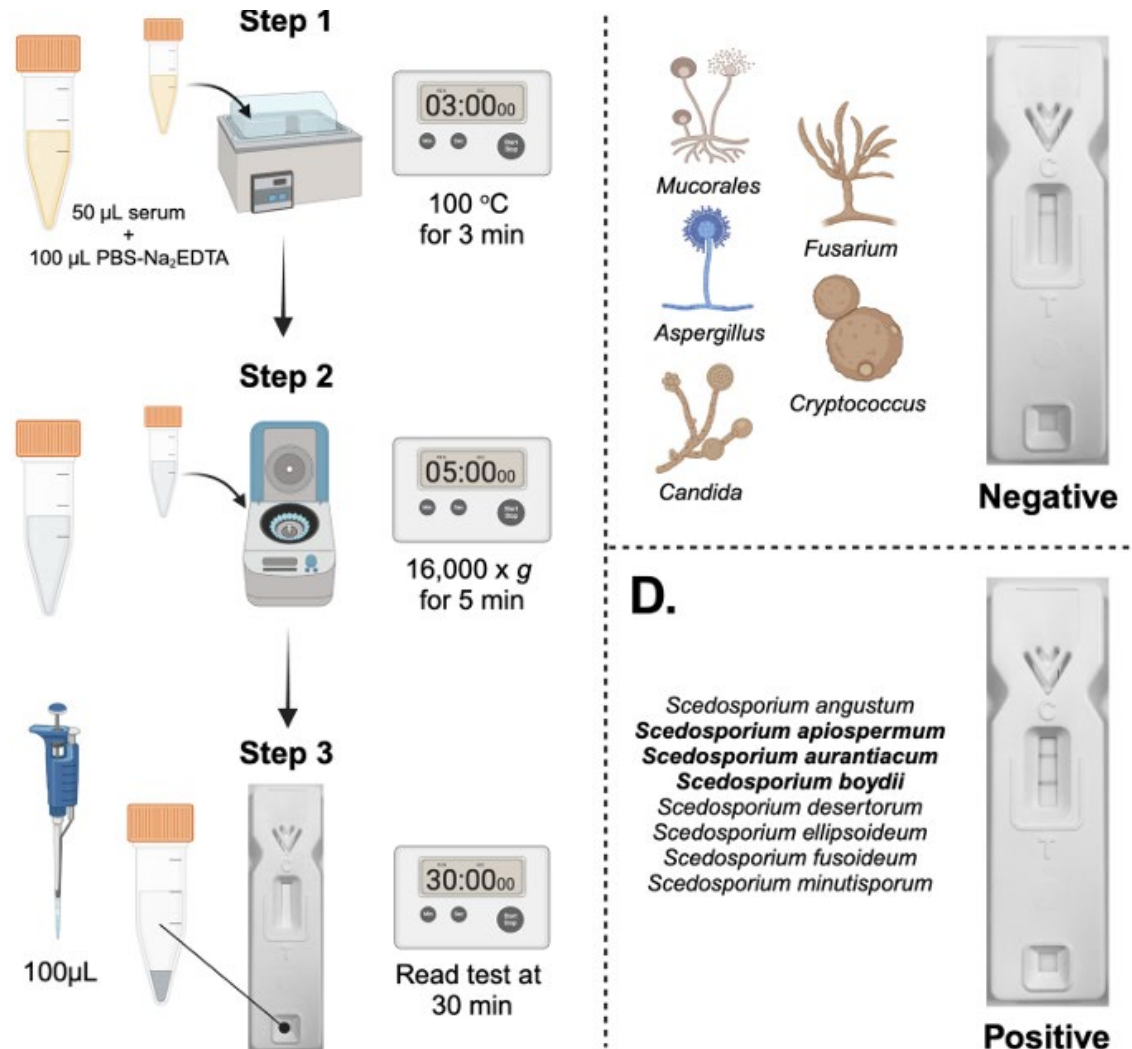
Lateral Flow Immunoassay (LFA)

- A mAb 7C11 in LFA (europium nanoparticles as the detector) in 15min (DxDiscovery, Inc.)
- LFA detected mannans from common Mucorales fungi (excl *Lichthiemia*) at LOD 0.12-1.2 ng/mL
- LFA detected Mucorales mannan in serum, BAL (100 µl) in a mouse model of pulmonary MCM
- Testing serum/plasma in 12 patients with probable/proven MCM at one or more timepoints
 - AMB treatment associate with neg LFA
 - 4/6 pts (without AMB) test positive
 - One pt showed high mannan 7 days before meeting EORTC criteria



Scedosporium-specific LFD (ScedLFD)

- A *Scedosporium*-specific IgG1 mAb HG12, recognizes extracellular polysaccharide (EPS) antigens between ~15 kDa – 250 kDa secreted during hyphal growth.
- 30-min LFD test performed in serum samples
- A proof-of-concept detection of *Scedosporium* EPS with LODs
 - ~0.81 ng/mL *S. apiospermum*
 - ~0.94 ng/mL *S. aurantiacum*
 - ~1.95 ng/mL *S. boydii*



Molecular testing: where we are?

- Few FDA cleared assays
- 2017 ESCMID-ECMM-ERS guideline for *Aspergillus* diseases:
 - “PCR should be considered in conjunction with other diagnostic tests”

2020 EORTC/MSG *Aspergillus* PCR

- At least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid
- Limited clinical outcome data

* European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) Consensus Group (Donnelly P et al CID 71:1367, 2020)
Ullmann et al. CMI 2018; 24:e1-e38

Positive blood cultures – Fungal detection by FDA approved commercial multiplex molecular assays

Assay Name	Vendor	Technology	Target	TAT	PPA/ NPA
LIAISON PLEX® Yeas BC kit	Luminex/	PCR coupled with	16 targets: <i>C.albicans</i> , <i>C.auris</i> , <i>C.dubliniensis</i> , <i>C.haemulonii/duobushaemulonii</i> , <i>Cryptococcus</i> <i>neoformans/gattii</i>	120min (fully automated)	100%/ 99.6%
FilmArray® Yeast BCID-2	BioFire/ BioMerieux	PCR coupled with microarray and melt-curve analysis	<u>6 targets</u> : <i>C.albicans</i> , <i>C.auris</i> , <i>C.glabrata</i> , <i>C.krusei</i> , <i>C.papapsilosis</i> , <i>C.tropicalis</i> , <i>Cryptococcus neoformans/gattii</i>	60min (fully automated)	98%/ 99.9% (antimicrobial stewardship)
ePlex® BCID- FP	GenMark/ Roche	PCR-based digital microfluidics coupled with eSensor® electrochemical detection system	<u>15 targets</u> : <i>C.albicans</i> , <i>C.auris</i> , <i>C.dubliniensis</i> , <i>C.famata</i> , <i>C.glabrata</i> , <i>C.guilliermondii</i> , <i>C.kefyr</i> , <i>C.krusei</i> , <i>C.lusitaniae</i> , <i>C.parapsilosis</i> , <i>C.tropicalis</i> , <i>Cryptococcus gattii</i> , <i>C.neoformans</i> , <i>Fusarium</i> sp, <i>Rhodotorula</i> sp.	100min (fully automated)	96.2%/ 99.8%

These molecular assays increase the speed for *Candida* species identification but not improve the sensitivity for diagnosing IC

Blood – Invasive Candidiasis (Candidemia)

(Direct detection of *Candida* in blood by commercial assays)



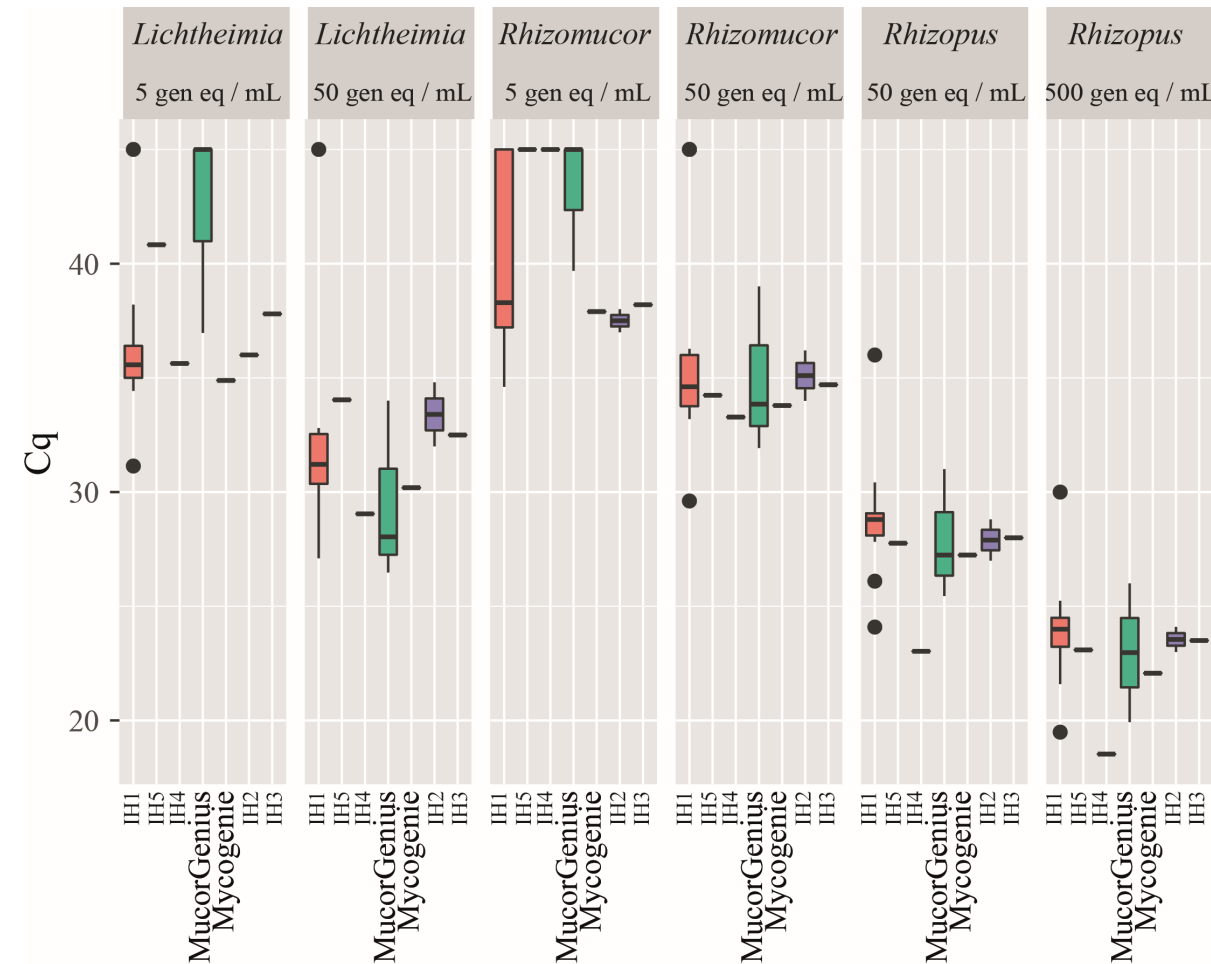
Assay	Vendor	Technology	Targets	Marked	TAT	Sensi/ Spec	Outcome (Clinical trial)
T2Candida	T2 Biosystems	Nanoparticles and T2 magnetic resonance detection platform	<i>C.albicans</i> / <i>C.tropicalis</i> , <i>C.parapsilosis</i> , <i>C.krusei</i> / <i>C.glabrata</i>	FDA (off-market)	~5h fully automated	89-91.1%/ 99.4% (27% in DS-IC)	Early detection; increase/retain sensitivity during antifungal therapy; antifungal stewardship
lightCycler® SeptiFast	Roche Mol Dx	Multiplex RT-PCR	<i>C.albicans</i> / <i>C.tropicalis</i> , <i>C.parapsilosis</i> , <i>C.krusei</i> / <i>C.glabrata</i>	CE-IVD	6h (3h hands on)	65%/ 86%*	No clinical trial data
Magicplex® Sepsis	Seegene Dx	PCR with electrophoresis separation/fluorescence detection	<i>C.albicans</i> / <i>C.tropicalis</i> , <i>C.parapsilosis</i> , <i>C.krusei</i> / <i>C.glabrata</i> ,	CE-IVD	6h	47%/ 66%*	No clinical trial data
RenDx Fungiplex® (<i>Candida</i>)	Renishaw D (Now under Bruker)	18S and 28S rDNA PCR with surface-enhanced Raman scattering detection (RT-PCR)	<i>C.albicans</i> , <i>C.dubiniensis</i> , <i>C.parapsilosis</i> , <i>C.tropicalis</i> , <i>C.glabrata</i> , <i>C.krusei</i> , <i>C.guilliermondii</i> , <i>C.lusitaniae</i>	CE-IVD	8h (2h hands on) (2h from extraction)	82.8%/ 87.5%*	No clinical trial data
SepsiTest®	Molzzy Mol Dx	16S/18S rRNA PCR +Sanger sequencing	Fungi in GenBank	CE-IVD	~11h (2.5h hands on)	48%/ 84%*	No clinical trial data

BAL – commercial NAAT assays performance (lack of clinical outcome data)

Disease	Assay	Vendor	Technology	Target	Performance
Aspergillosis	AsperGenius® Assay AsperGenius Resistance multiplex®	PathoNostics	Multiplex RT-PCR	28S rDNA <i>A.fumigatus</i> complex, <i>A.terreus</i> , <i>A.flavus</i> , <i>A.niger</i> <i>Cyp51A</i> of <i>A.fumigatus</i> (L98H, TR34, T289A, Y121F)	84% sensi, 80% spec
Aspergillosis	MycoGENIE® assay	AdamTech	RT-PCR	28S rDNA, <i>Aspergillus</i> sp/ <i>A.fumigatus</i> with <i>Cyp51A</i> (TR34/L98H)	71.1% sensi, 92% spec
Aspergillosis	Fungiplex® <i>Aspergillus</i> Fungiplex® Azole-R (RenDx Fungiplex®)	Bruker (Renishaw Dx)	RT-PCR	18S and 28S rDNA <i>A.fumigatus</i> , <i>A.flavus</i> , <i>A.niger</i> , <i>A.terreus</i> <i>Cyp51A</i> (TR34/TR46)	
Mucormycosis	MucorGenius®	PathoNostics	Multiplex RT-PCR	<i>Rhizopus</i> , <i>Mucor</i> , <i>Lichtheimia</i> , <i>Cunninghamella</i> , <i>Rhizomucor</i>	
<i>Pneumocystis</i>	Several commercial assays (Curetis Unyvero LRT FDA-cleared)	Several vendors	RT-PCR	mtLSU, mtSSU, MSP, CDC2	Variable

Interlaboratory assays to assess qPCR detection of Mucorales DNA in serum

- Two panels to 26 labs in Europe
 - Panel A (6 sera spiked Mucorales DNA + 1 neg control)
 - Panel B (6 Mucorales DNA extracts)
- For extraction: 6 automated platforms and 5 manual extraction methods
- For qPCR: 5 in-house assay; 2 commercial (MucorGenius [PathoNostics], MycoGENIE [Ademtech])
- Volume of serum for DNA extraction and DNA template volume for PCR amplification are the main parameters to improve Mucorales qPCR (irrespective of extraction platforms and qPCR assay/platform)



Detection of fungal cell-free DNA (cfDNA) in plasma is a novel testing modality for the non-invasive diagnosis of IFI



- Fungal cell-free DNA in serum or plasma could be high due to the extensive angioinvasion and tissue damage by fungal organisms, along with a high copy number of rRNA.
 - Plasma > serum
 - PCR vs mNGS
- Over 15,000 patient (>18,000 plasma samples) tested from 2018 to 2021, opportunistic fungi were detected (4%) by KARIUS test (mcf-DNA mNGS)
 - *Aspergillus* sp (374)
 - *Pneumocystis jirovecii* (258)
 - Mucorales (196)
 - Dematiaceous fungi (33)

Aspergillus plasma cell-free DNA PCR is superior to serum galactomannan for the diagnosis of invasive aspergillosis

- Tested in 238 immunocompromised patients with suspected IFD from 2020-2022 in a single medical center (43 cases of proven/probable IA based on 2020 EORTC/MSGERC definitions) *[not for screening]
 - cfDNA PCR showed better sensitivity than serum GM in HM/HSCT patients (92% vs 67.9%, $p < 0.05$)
 - Comparable specificity between the two methods (91.8% vs 89.8%)
- *Aspergillus* **plasma** cfDNA PCR (not **serum** cfDNA PCR) is more sensitive than serum GM for non-invasive diagnosis of IA.
- Combination of plasma cfDNA PCR with serum GM reached sensitivity of 100% in patients with new diagnoses of IA

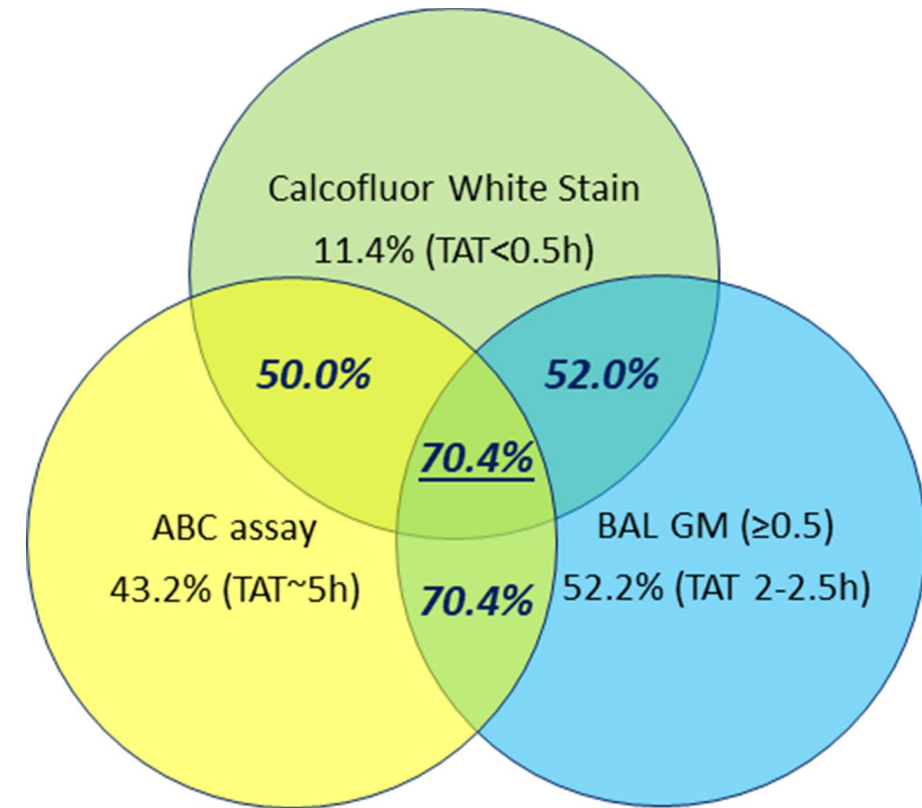
Combination of molecular tests and biomarkers to increase fungal diagnostic sensitivity and specificity



- Combination of *Aspergillus* PCR with serum GM improves the diagnostic accuracy, early detection and intervention of invasive aspergillosis
- Combining *Candida* PCR with serum Beta-D-glucan increases sensitivity and specificity to detect invasive candidiasis
- T2Candida in combination with Platelia *Candida* Ag may be beneficial for rapid detection of *Candida* species in children with concern for invasive candidiasis.

A multiplex PCR assay using barcoded magnetic bead technology for detecting fungal pathogens in BAL

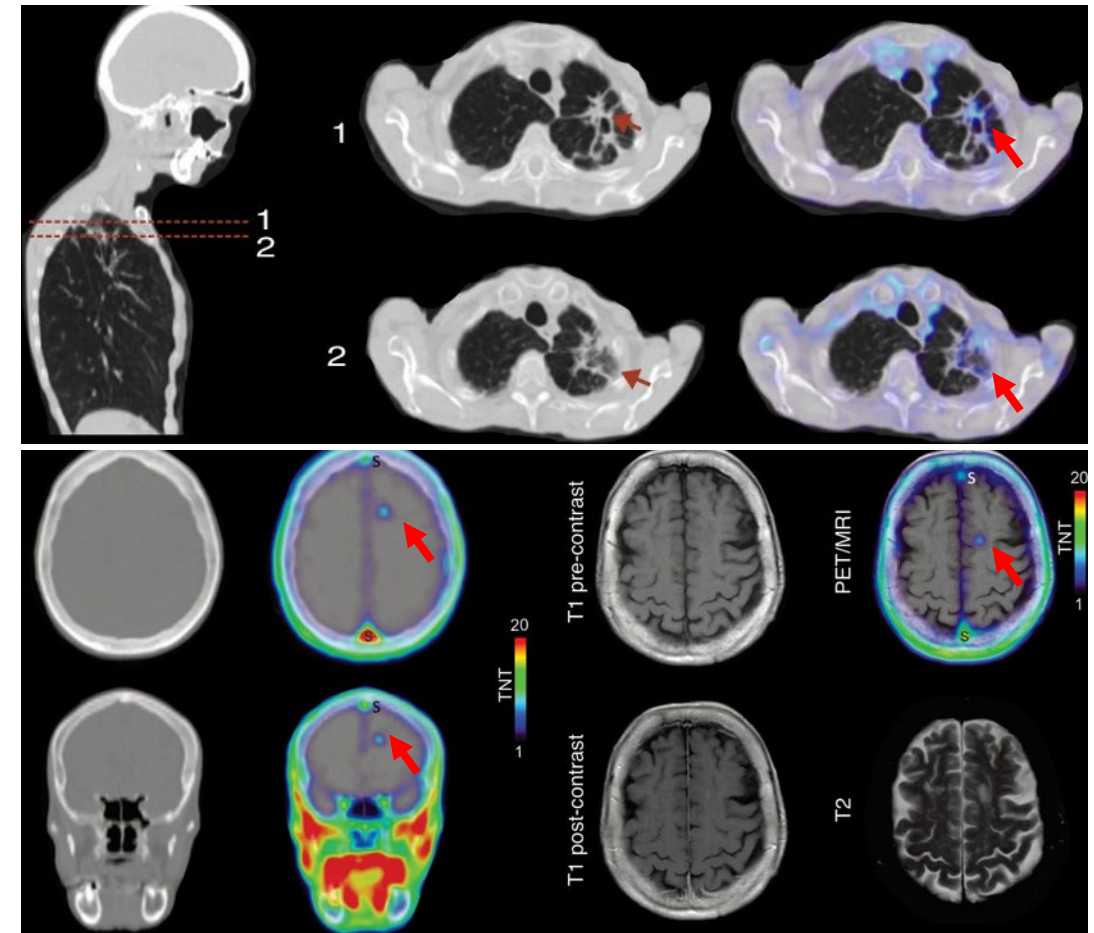
- Applied BioCode (ABC) assay detects 22 fungal pathogen in BAL in <5 h (4 *Aspergillus* species, 8 Mucorales fungi, 2 *Fusarium* species, *Scedosporium apiospermum*, *Lomentospora prolificans*)
- In 323 patients (44 proven/probable IFI, 279 non-IFI), when the ABC assay and BAL GM (≥ 0.5) were combined, sensitivity reached 70.4% in 5h.
- The ABC assay sensitivity was not affected by antifungal therapy



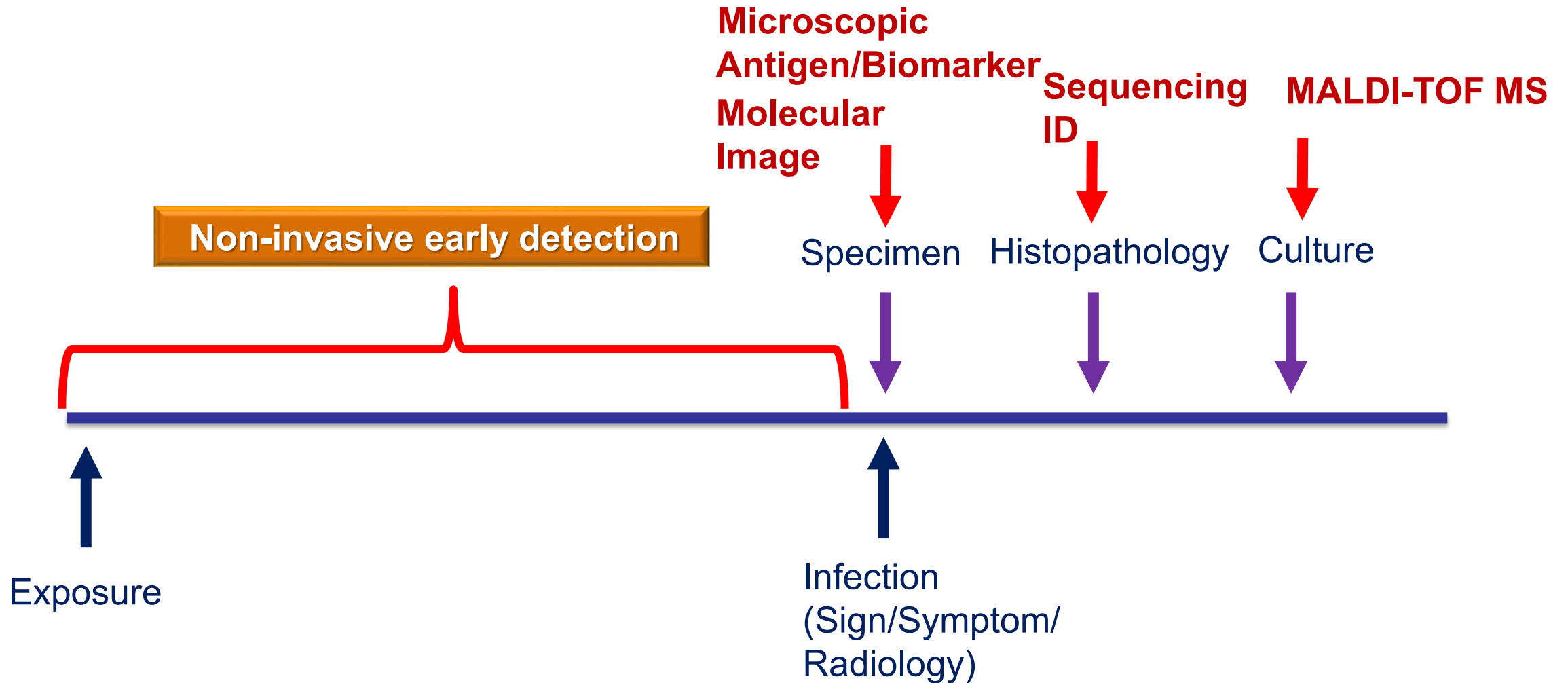
TAT: Test turnaround time

^{18}F -FDS (Fluorodeoxysorbitol) PET (Positron Emission Tomography) for IMI detection

- ^{18}F -FDS (a radioanalog of sorbitol) can be easily synthesized from commercially available ^{18}F -Fluorodeoxyglucose (^{18}F -FDG)
- ^{18}F -FDS selectively and rapidly accumulates intracellularly in clinically relevant viable molds (not mammalian cells).
- In mice, ^{18}F -FDS detected and localized IMI at different body sites.
- First-in-human study enrolled 9 pts: whole-body ^{18}F -FDS PET/CT scan
 - 4 pts with proven IMI by EORTC/MSGERC
 - 5 pts (4 ILDs and 1 breast ca)

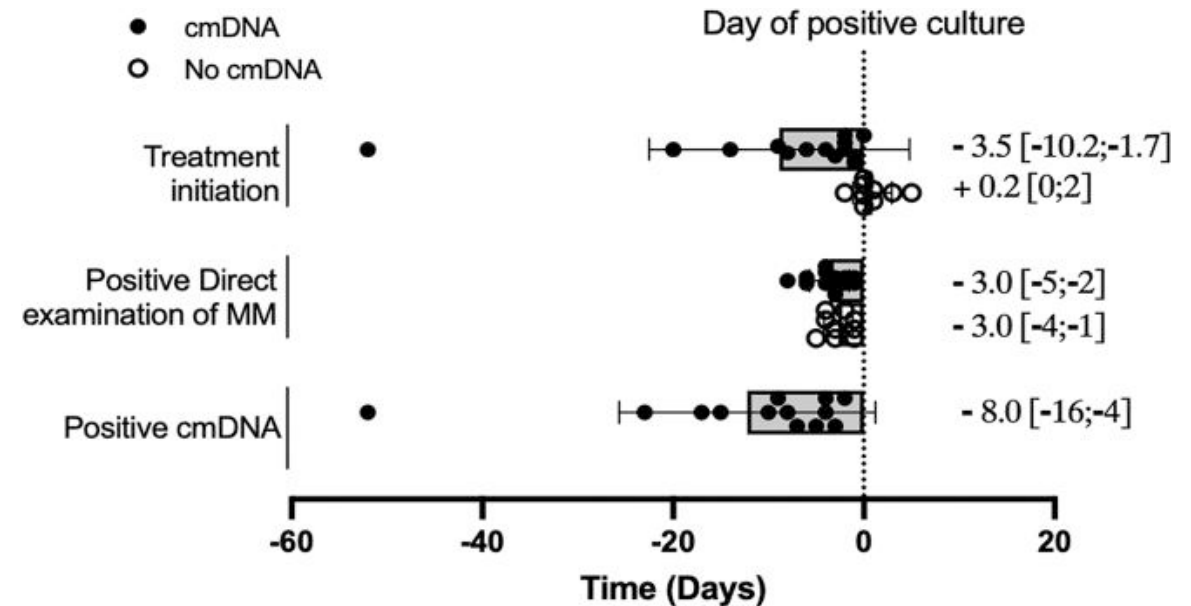


Beyond “Standard” of Care Lab Tests – can we improve early detection of fungal diseases?



Weekly screening of circulating Mucorales DNA (cmDNA) improves survival in invasive mould mucormycosis (IWM)

- A total of 37 IWM pts included
- LDT in serum or plasma (*Rhizopus/Mucor*, *Rhizomucor*, *Lichtheimia*)
- cmDNA detection allowed earlier diagnosis of IWM with a median of -8 days than mycological culture
- This early detection was associated with early initiation of AMB combined with surgery
- 100-day mortality rate was reduced from 63.6% to 30.8% (more in pts with TBSA $\geq 30\%$, $p < 0.05$) in cmDNA group.



Take-home messages

- While recent progress has been made, there remains a need for a proactive pre-emptive non-invasive diagnostic workup to enhance sensitivity and early detection of invasive fungal infections (IFIs) in high-risk patient population.
- Combining multiple test modalities (not rely on a single test) may improve the overall diagnosis of IFI.
- More prospective **multicenter** clinical trials are needed to better assess the clinical performance of these promising new assays for early screening, disease diagnosis, and outcome improvement, as well as to support FDA clearance.
- Accumulating data on improved diagnosis are needed to further support the inclusion of these new diagnostic assays into diagnostic guidelines as well as the EORTC/MSG criteria.



Fungal Diagnostic Laboratory Consortium (FDLC) <https://labs.pathology.jhu.edu/fdlc/>



- A mission of bring joint forces to improve and enhance laboratory capacities for diagnosis of fungal diseases
- The FDLC brings together 33 clinical laboratories and medical centers in the US and Canada to form a laboratory network for fungal diagnostics.
- The network covers a diverse of geographic and climate regions, including 29 centers across 17 States in the US and 5 centers across 3 Provinces in Canada.
- The network includes CLIA-certified and CAP-credited tertiary hospital laboratories (80% $\geq 1,000$ beds) that provide >30,000 fungal diagnostic tests each month to a variety of patient populations including pediatric and adult cancer, stem cell transplant, solid organ transplant and other immunosuppressed patients.
- The network provides a platform for collaboration, facilitating R&D in fungal diagnostics, and supporting multicenter clinical trials to evaluate novel diagnostic assays.

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