ASIA FUNGAL WORKING GROUP
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INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

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Diagnosis of IFIs and tests available

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Disclosure

- No conflict in interest

Diagnostic approach to IFI

The diagnosis of IFI is a combination of :

- host factors (neutropenia or critically ill fever refractory to broad-spectrum anti-bacterials)

  PLUS

- clinical, radiological and microbiological criteria

Hsu LY, Lee DG, Yeh SP, et al. Epidemiology of invasive fungal diseases among patients with haematological disorders in the Asia-Pacific: a prospective observational study. Clin Microbiol Infect 2015;21:594, e7-11.

Invasive fungal infections (IFI)

- In critically ill non-haematological patients, invasive candidiasis is the commonest fungal infection
- In haematological neutropenic patients, invasive aspergillosis (IA) is the commonest mould infections; others are mucormycosis and fusariosis
- HIV-related opportunistic fungal infections
- Endemic mycoses in immunocompromised hosts
- Others : immunosuppressive agents, structural organ abnormalities

Hsu LY, Lee DG, Yeh SP, et al. Epidemiology of invasive fungal diseases among patients with haematological disorders in the Asia-Pacific: a prospective observational study. Clin Microbiol Infect 2015;21:594, e7-11.

Invasive candidiasis in the critically ill

Critically ill: Invasive candidiasis (IC)

- The overall incidence of invasive fungal infection of the ICU is low (<1%)
- Moderate-risk patients are ICU residents for ≥4 days or in septic shock
- **High-risk patients** with severe acute or necrotizing pancreatitis, or recurrent leaks of the GI tract

Hall AM et al . Prediction of invasive candidal infection in critically ill patients with severe acute pancreatitis. Crit Care. 2013;17(2):R49.
Ostrosky-Zeichner L et al. Multicenter retrospective development and validation of a clinical prediction rule for nosocomial invasive candidiasis in the intensive care setting. Eur J Clin Microbiol Infect Dis. 2007;26(4):271-6.
Hadley S et al Candidemia as a cause of septic shock and multiple organ failure in nonimmunocompromised patients. Crit Care Med. 2002;30(8):1808-14.
Harrison D et al. Development and validation of a risk model for identification of non-neutropenic, critically ill adult patients at high risk of invasive Candida infection: the Fungal Infection Risk Evaluation (FIRE) Study. Health Technol Assess. 2013;17(3):1-156H

Independent Risk factors for IC

Multi-variate analysis

- Recurrent gastrointestinal perforation
- Anastomotic leakage
- Abdominal drain (GI surgery)
- TPN via central vascular access

TPN : nutrient-rich components supporting bacterial and fungal growth, and bowel rest enable gut translocation; indwelling parenteral access devices encourage candida colonization and biofilm formation.

Bassett et al , Infect Dis Ther (2022) 11:827–840
Yeşilbağ et al , J Surg Med. 2021;5(1):97-102
Chulakadabba et al , RTA Med J 2022;75(3):149-57
Swindell K et al, he Journal of infectious diseases, 2009

Invasive candidiasis :

- **Culture Is the gold standard for diagnosis**
- The median *Candida* concentration within a first positive blood culture is 1 colony-forming unit (CFU)/mL
- The median time to positivity is 2–3 days, and can take as long as 8 days
- *Candida glabrata* : the longest time to positivity
- It take an additional 24–48 h for species identification
- Based on autopsy-proven invasive candidiasis and antemortem blood culture results, the sensitivity of blood culture ranged from 63%–83%
- In patients with hematologic malignancies, daily blood cultures and additional sets during febrile episodes, sensitive was 71%

Ibáñez-Martínez et al, Rev Esp Quimioter 2017;30 (Suppl. 1): 16-21
Clancy CJ et al , Diagnosing invasive candidiasis. J Clin Microbiol 2018; 56: e01909-17.
Pfeiffer CD et al. Quantitation of Candida CFU in initial positive blood cultures. J ClinMicrobiol 2011; 49: 2879–83.
Clancy et al. Clinical Infectious Diseases 2013;56(9):1284–92

Identification of *Candida* spp

- From culture positive to yeast identification using conventional methods, requires 24-48H

New techniques shorten the time to identification :

1) Peptid Nucleic Acid Fluorescence In-Situ Hybridization system (PAN-FISH)

- It is performed from positive blood cultures in 90 minutes
- Allows the rapid identification of
- *C. albicans/C. parapsilosis* (green fluorescence),
- *C. glabrata/C. krusei* (red fluorescence) *and*
- *C. tropicalis* (yellow fluorescence).

2) T2 Candida Panel

- To lyses the *Candida* cells, amplifies the DNA with pan-*Candida* PCR primers
- To differentiate 5 *Candida* species (*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis* and *C. krusei)*
- The limit of detection ranging from 1 CFU/mL
- Sensitivity and specificity of 91.1% and 99.4% respectively
- The mean time to *Candida* detection and species identification was < 5 hours

Monday et al. J Fungi (Basel). 2021 Mar 3;7(3):178. doi: 10.3390/jof7030178

3) MALDI-ToF: Matrix-Assisted Laser Desorption–Ionization Time-Of-Flight mass spectrometry

- Highly accurate, comparable with sequencing
- The database can be expanded in house
- Is able to identify *Candida* isolates to species level in less than 15min

A caveat (exception to MALDI-ToF)

Unable to identify emerging resistant-*Candida* spp :

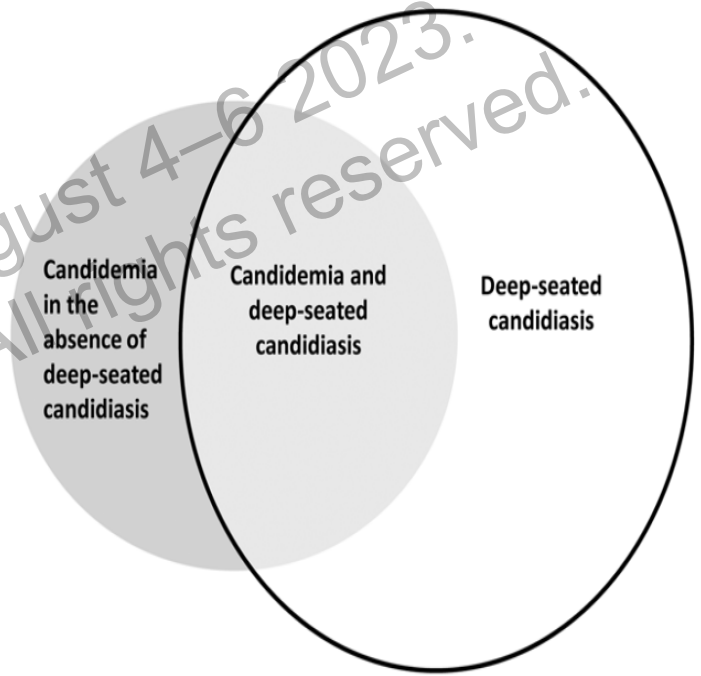
- *Candida auris*
- *Candida haemulonii* complex and its related species

Invasive candidiasis

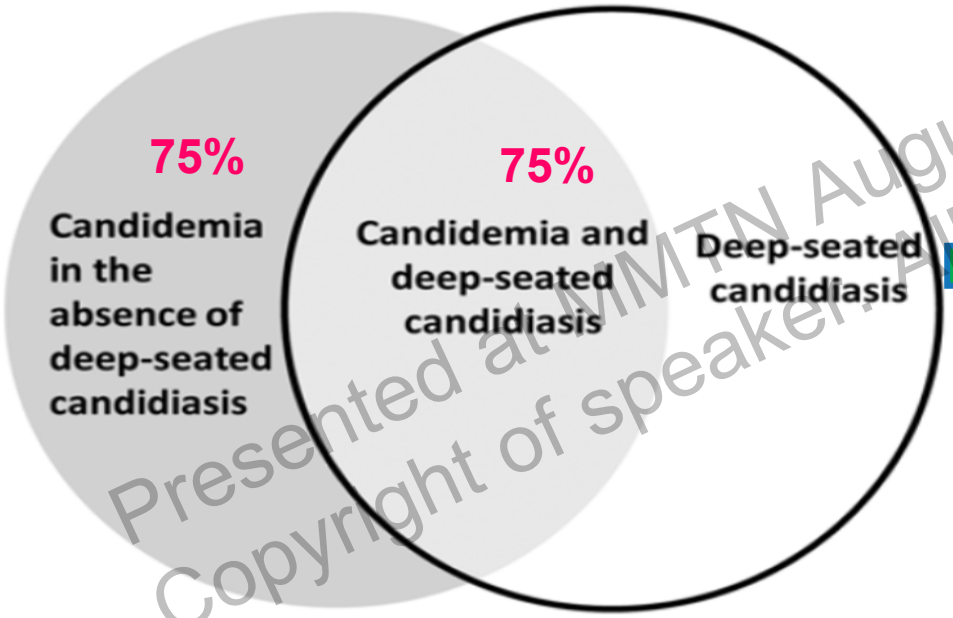
(i) Candidemia in the absence of deep-seated candidiasis

(ii) Candidemia associated with deep-seated candidiasis

(iii) Deep-seated candidiasis in the absence of candidemia.



Blood cultures identify the majority patients with candidaemia



In intra-abdominal candidiasis:
the sensitivity of intra-abdominal fluid or cultures from infected sites is < 50%, and only 4–20% of cases will be candidaemic

CID 2013;56(9):1284 – 92

Fungal biomarkers

Non-cultured based diagnostic methods for IC

False Negative blood culture may be seen in :

✓Deep-seated candidiasis due to early or transient or intermittent candidaemia in low concentration.

✓Absence of viable *Candida* within the circulation

Fungal biomarkers

A) Mannan/ anti-mannan

	Sensitivity	Specificity
Mannan	58%	93%
Ant-mannan	59%	86%
Mannan+ Anti-mannan	83%	86%

- Best performance in patients with *C.albicans*, *C. glabrata*, or *C. tropicalis* infections, less sensitive in *C parapsilosis* and *C krusei* .
- Time to positivity is 6-7 days before culture results; 16 days before radiological evidence of IC .
- Immunosuppressed hosts may not mount adequate IgG response and positive results may not distinguish acute from past infections.
- False positive Mn and anti-Mn in patient with candida colonization.

Mikulska et al. Crit Care 2010. 14:R222
Prella et al. . Diagn Microbiol Infect Dis 2005,51:95-101

B) C. albicans germ tube antibody assay (CAGTA)

- Indirect immunofluorescence test for the detection of anti-mycelium Ig G hyphal protein (Hwp1) of *Candida* spp expressed **during tissue invasion and biofilm formation.**
- The sensitivity of 42-96% and specificity of 54-100%, respectively
- Sensitivity may be lower for infections caused by *C.tropicalis*
- Study showed better sensitivity in candidaemic patients with deep seated candidiasis (69%), BUT very low sensitivity in patient without deep seated infection (5%)

When combined with 1,3-ßDG, or anti-Mn and/or Mannan antigens: increased the sensitivity, with NPP 99.6-99.8%

A positive CAGTA test in a patient with candidemia suggests deep-seated candidiasis.

Martinez-Jimenez et al. Med Mycol 2014, 52:270 –275
Martínez-Jiménez a et al. *J Antimicrobial Chemotherapy*, 2015, 70(8), 2354–2361

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c) Beta D-glucan (BDG)

- Pan-fungal biomarker, present in cell wall of most pathogenic fungi, excluding *Cryptococcus* species, *Blastomyces* species (yeast phase), and *Mucorales*
- In meta-analyses:

	Sensitivity	Specificity
candidaemia	75-80%	60-80%
Intra-abdominal candidiasis	60-77%	64-75%

- Performance improved if two consecutive results are positive
- Lower sensitivity for *C krusei* and *C parapsilosis*
- 1-3-ßDG may be positive in average of 10 days before clinical manifestations of IC, and in patients with intra-abdominal candidiasis without candidemia, an average of 5 days before confirmation of the diagnosis.

Onishi A et al. J Clin Microbiol 50:7–15
Hansen et al . PLoS One 7:e42282.

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Commercial *Beta-D glucan* kits :

	Cut off (pg/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPP (%)
Fungitec G test ES	20	90.9 (10/11)	91.5 (140/153)	43.5 (10/23)	99.3 (140/141)
Fungitell β-D-glucan assay kit	80	63.6 (7/11)	91.5 (140/153)	35.0 (7/20)	97.2 (140/144)
Fungitec G test MKII	20	100 (11/11)	90.8 (139/153)	44.0 (11/25)	100 (139/139)
β-Glucan test Wako	11	100 (11/11)	96.1 (147/153)	64.7 (11/17)	100 (147/147)
European version of β-Glucan test Wako	11	100 (11/11)	96.1 (147/153)	64.7 (11/17)	100 (147/147)

Fungus (1-3)-β-D-Glucan Test (Chemiluminescence immunoassay -CLIA Method) , Cut off : 100pg/ml

Turn around time : < 120min

- False-positive results may occur due to hemodialysis with cellulose membranes, wound packing with gauze, albumin, or intravenous immunoglobulin.
  - Positive results can also result from other fungal infections : *Pneumocystis Jiroveci*, Histoplasmosis, Fusariosis, *Candida* colonization or invasive infection
  - Limited data in pediatric population and cutoff is not well-defined

Martinez-Jimenez et al. Med Mycol 2014, 52:270 –275
Martínez-Jiménez a et al. *J Antimicrobial Chemotherapy*, 2015, 70(8), 2354–2361

BDGlucan in haematological patients

- BDG is non-specific
- Not able to differentiate *Aspergillus, Candida, Pneumocystis jirovecii, or Fusarium*
- A negative result does not exclude IFD
- While two consecutive positive BDG are associated with very high probability of true positivity
- May combined with GM to increase specificity

Interpretation of fungal biomarkers

To consider :

- Risk factors (High vs Low risk)
- Clinical characteristics
- Exposure to antifungal agent (as prophylaxis)
- Adults vs children

How to interpret the fungal biomarker ?

- Non-culture biomarkers at best is to assign a probability of infection, but IT IS **NOT** a confirmative diagnostic test.

- The positive and negative predictive values (PPV and NPV, respectively) will depend on **the prevalence** of the disease in a defined population and/or the **pre-test probability** of the disease in an individual

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Clancy CJ, Nguyen MH. J Clin Microbiol 2018. 56:e01909-17

1

TABLE 2 Prevalence of candidemia in different populations and anticipated PPVs and NPVs of nonculture tests[a]

		Mannan/antimannan and BDG[b]		PCR[c]		T2Candida[d]	
Prevalence (%)	Representative patient	PPV (%)	NPV (%)	PPV	NPV	PPV	NPV
0.4	Any hospitalized patient from whom a blood culture is collected	1	99.9	3	>99.9	15	>99.9
1	Patient admitted to ICU	4	99.7	8	99.9	**31**	**99.9**
2	Patient with febrile neutropenia and baseline rate of candidemia prior to empirical antifungal treatment	7	99.5	16	99.8	**47**	**99.8**
3	Patient with sepsis, shock, or >3–7-day stay in ICU	11	99.2	**22**	**99.6**	**67**	**99.7**
10	Patient at increased risk for candidemia based on clinical prediction models	**31**	**97**	**50**	**98.8**	**82**	**99**

In short

- Treatment decisions based on non-culture results will depend upon clinical judgment and must be individualized
- In a patient is likely to have invasive candidiasis, and a positive result significantly increases that possibility

or

- in a patient has some risk factors for candidemia, but a negative result makes the disease extremely unlikely

Invasive Aspergillosis

Risk factors for Invasive aspergillosis

- Prolonged neutropenia (< 0.5 x 10^9) > 10 days.

The duration and severity of neutropenia are associated with increased risk of IPA

- Allogenic stem cell transplant
- Immunosuppressants :

✓ T-lymphocyte immunosuppressants such as calcineurin inhibitors and TNFα inhibitors,
✓ B-cell immunosuppressants eg ibrutinib;
✓ Chimeric antigen receptor T-cell (CAR-T) therapy, venetoclax

- Graft vs host disease
- Steroid: A dose >0.3 mg /kg prednisone > 3 weeks

Nucci et al. Clin. Chest Med. 2009, 30, 295–306
Meersseman et al. Clin. Infect. Dis. 2007, 45, 205–216.
J Peter Donnelly et al . Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the EORTC and the Mycoses Study Group Education and Research Consortium, *CID*, 2020,71(6) : 1367–1376

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Non-conventional risk factors

Any risk factors leading to injury of alveolar epithelial cells

1. Critically ill in ICU : IA prevalence of 0.017%
2. Post viral pneumonia :

i. Influenza-associated pulmonary aspergillosis (IAPA)

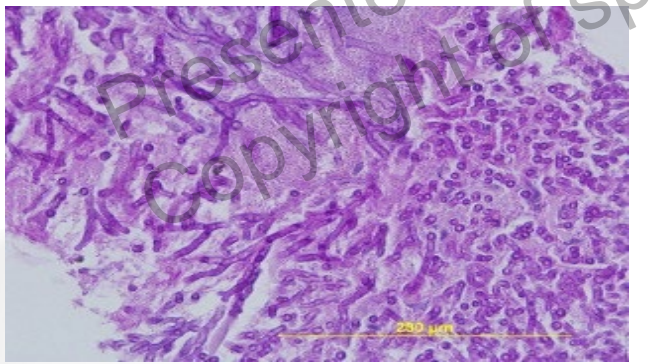
ii. COVID-associated pulmonary aspergillosis (CAPA)

Invasive aspergillosis in non-neutropenic patients is associated with bad prognosis, with mortality rates exceeding 80%, mainly due to delayed diagnosis

Baddley et al , *BMC Infect Dis* **13**, 29 (2013)
Cornillet A et al, *Clin Infect Dis.* 2006;43:577–584.

Diagnosis of invasive aspergillosis (IA)

- Histopathological examination and culture remain the gold standard for the diagnosis of IA
- Conventional blood culture has low sensitivity
- *Aspergillus* is grown from sputum in only 8% to 34%, and from broncho-alveolar lavage (BAL) in 45% to 62% of patients with invasive aspergillosis
- Culture from respiratory samples; difficult to differentiate infection from colonization
- Often unable to obtain biopsied tissue for HPE



HPE : septated hyphae with acute angle branching

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Paterson et al . Medicine (1999);78: pp. 123–138.

Galactomannan (GM)

- Serum GM can be positive before the clinical diagnosis of IA
- With GM :diagnosis could be made a mean of 6–13 days before patient becoming symptomatic or evidence of CT changes in neutropenic HM patient
- Critically ill in ICU, serum GM positivity precedes Aspergillus culture detection of IA by a mean of 4.3 days
- A meta-analysis : sensitivity of serum galactomannan assay of 71% and specificity of 89% for haematological patients .
- However, in non-neutropenic patients the sensitivity and specificity of the test dropped to 22%-37% and 84%-87.1%, respectively

The galactomannan assay is more useful in patients who have a haematological malignancy or who have undergone allogeneic HSCT than in solid-organ transplant recipients or non-neutropenic patients

Maertens et al, J Clin Microbiol 1999
Zhou W et al, *J Clin Microbiol.* 2017;55:2153–2161

GM interpretation

- Interpret with consideration of clinical characteristic, CT scan of lungs and sinuses and histopathology and culture results

False positive results :

✓ Galactomannan contamination (piperacillin-tazobactam, amoxicillin-clavulanate)

✓ Cross-reactive mycosis :
Penicillium spp., *Paecilomyces* spp., *Fusarium* spp. and *Histoplasma capslatum*

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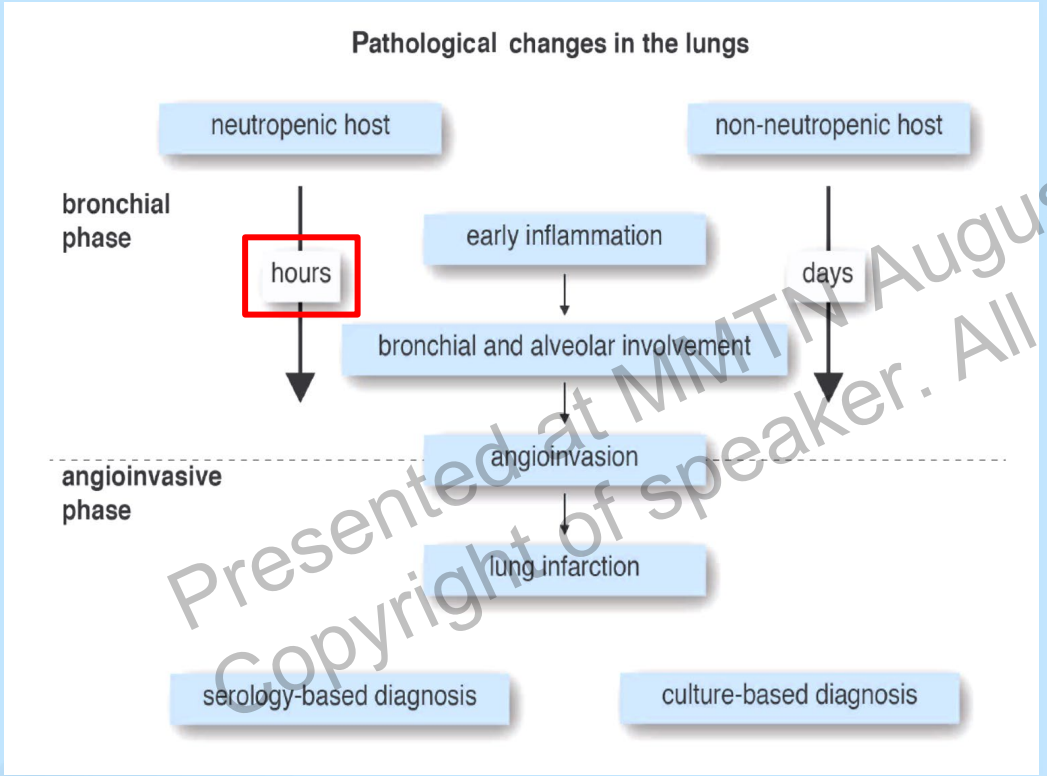
Haese et al. J. Clin. Microbiol. **2012**, 50, 1258–1263

Aspergillosis



Positive cultures from respiratory secretions occurred in:

- 17% of patients with the angioinvasive form of IPA
- 83% of patients with radiological signs of bronchoalveolar IPA

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Galactomannan in BAL

- GM levels appeared earlier in BAL fluid as compared to serum.
- The sensitivity and specificity of the test in BAL are higher than in serum
- The Serum GM index is higher in severely neutropenic patients (<100 PMN) than in other (≥100 PMN) patients.
- In non-neutropenic patients, IA is not always associated with angioinvasion
- False-positive results may be due to colonization

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Cordonnier et al, Clinical microbiology Infectionis. 2009. 15(1)

European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSG) 2020

GM Antigen detected in plasma, serum, BAL, or CSF

Any 1 of the following:

- Single serum or plasma: ≥1.0
- BAL fluid: ≥1.0
- Single serum or plasma: ≥0.7 and BAL fluid ≥0.8
- CSF: ≥1.0

Aspergillus PCR

Any 1 of the following:

- Plasma, serum, or whole blood 2 or more consecutive PCR tests positive
- BAL fluid 2 or more duplicate PCR tests positive
- At least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid

Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus

Maertens et al. Clin. Infect. Dis. **2007**, 44, 1329–1336
Lamoth et al. Clin. Infect. Dis. **2012**, 54, 633–643.

Lancet Infect Dis 2021; 21: e149–62

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Galactomannan

- Galactomannan enzyme immunoassay (GM-EIA)

Point –of –care :

- Aspergillus GM lateral flow assay (IMMY)
- Aspergillus-specific lateral flow device test (OML)

Good agreement LFA/LFD and GM-EIA test in

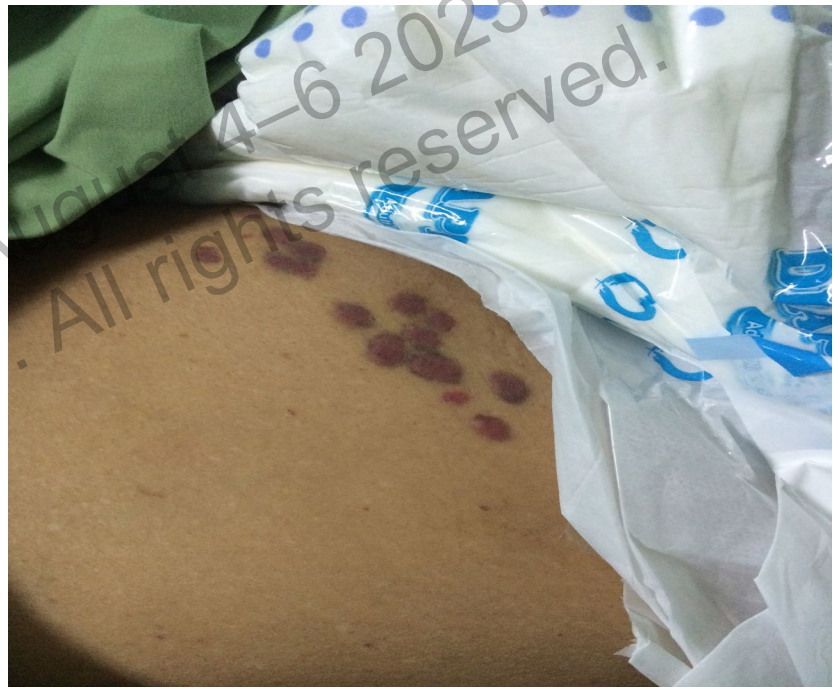
- haematology patients (serum and BAL)
- Covid-19 patients (CAPA) in ICU (Serum)
- In CAPA : A retrospective study showed, GM LFA in BAL > sensitive than serum, due to less invasive disease

Giusiano et al. Med Mycol. 2022 May 18;60(5):myac026. doi: 10.1093/mmy/myac026
Autier et al,. J Clinc Microbilogy. 2022. 60(1)
Adhan et al. Mycopathologia. 2023. doi: 10.1007/s11046-023-00749-7

Skin manifestation: Cutaneous findings can be the first indicator of IFIs in susceptible patients

Disseminated candidiasis :

- asymptomatic, erythematous to violaceous papules, vesicular centers that can progress to necrotic, purpuric plaques and tense hemorrhagic bullae.



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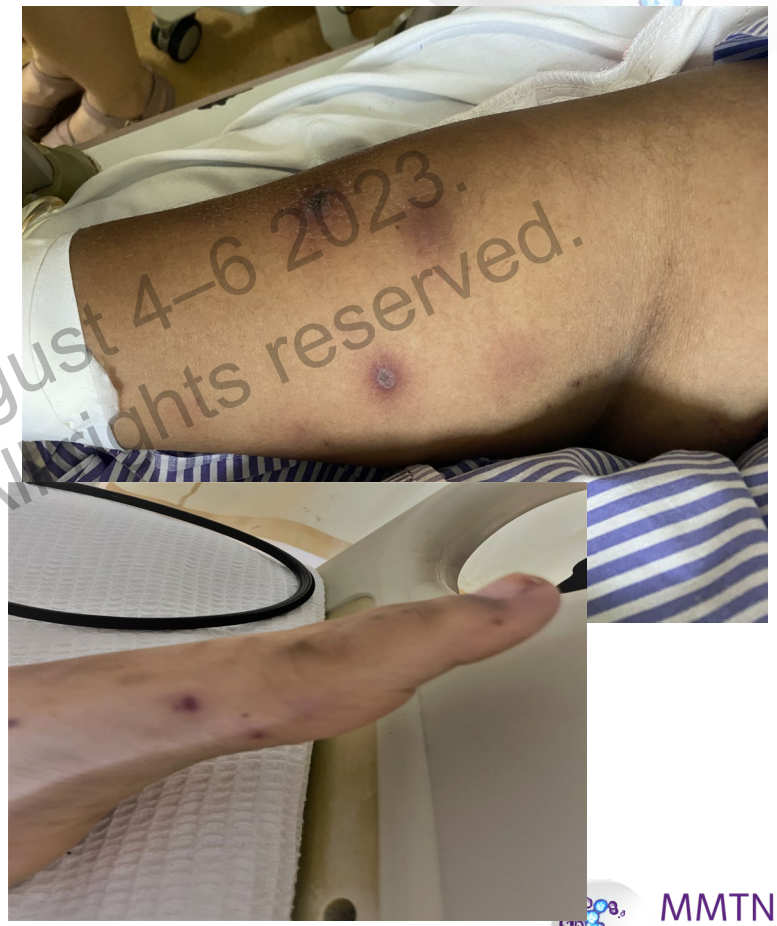
Schimmelpfennig et al, Bone Marrow Transplant. 2001;27:753-755.
Sheilds et al. J Am Aca Derm. 2019 . https://doi.org/10.1016/j.jaad.2018.04.059

- IFI : Ecthyma gangrenosume-like lesions, necrotic papulonodules, deep subcutaneous nodules with central eschar

- Cutaneous involvement can be seen in approximately 75% of cases of disseminated *Fusarium* infection.
- Lesions are often tender



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Role of imaging in IFIs

Invasive candidiasis

In neutropenic host:

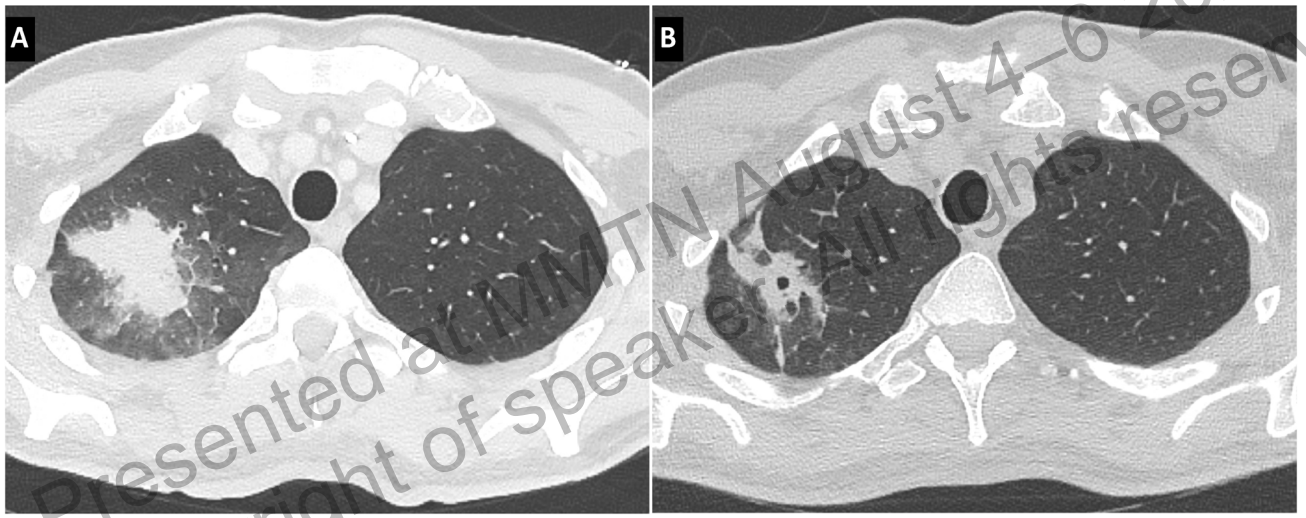
The clinical signs of hepato-splenic candidiasis (e.g. persistent fever, hepatosplenomegaly, increased alkaline phosphatase) as well as fungal endophthalmitis typically develop after neutrophil recovery.

Invasive aspergillosis

- Lungs are affected in 30 % of febrile neutropenic patients and allogeneic hematopoietic stem cell transplant (aSCT) recipients
- In approximately 60 % of the patients with a normal CXR, HRCT showed pulmonary infiltrates.
- In only 10 % of patients with a normal chest x-ray and a normal HRCT, pneumonia occurred during follow-up.
- CT thorax a reduction in the time to diagnosis, from 7 to 1.9 days

Caillot D et al. J Clin Oncol. 1997;15(1):139-47

Early CT scan is crucial in neutropenic hosts



Air-crescent sign
Cavitation – neutrophil granulocytes reconstitution

Ill-defined nodules - coagulation necrosis
Halo sign (non-specific) - edema and hemorrhage that surrounds the zone of infarction

Ledoux et al. *J. Fungi* **2023**, *9*(2), 131; https://doi.org/10.3390/jof9020131

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CT imaging in neutropenic patients

1) **Halo signs**

- This sign is transient and is seen only in the first 10 days of angioinvasion after which it disappears
- The sensitivity 13-92% depending on the timing of CT (higher when it is systematically performed)

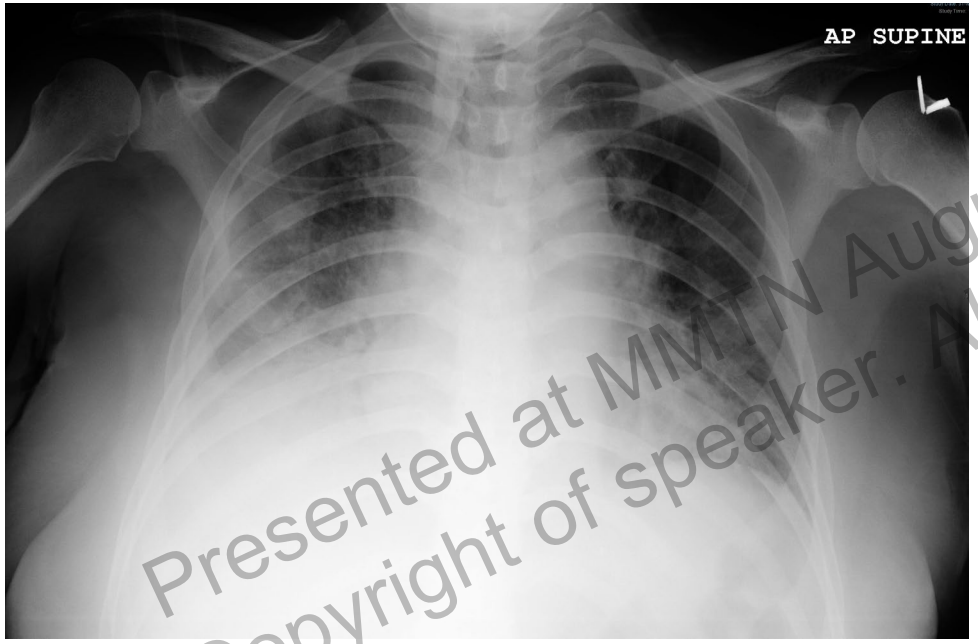
2) **Air crescent sign** It is seen in 50% cases usually after two weeks of disease onset when the patient's immune response recovers.

It is asso with high sensitivity (80%) and specificity (60–98%) in neutropenic patients.

Nevertheless,in non-neutropenic patients, both signs are uncommon, have a lower sensitivity (5–24%). **Atypical radiographic findings may be present in these patients.**

Greene RE et al, Clin Infect Dis. 2007;44(3):373-79.
Gotway MB et al, JCAT. 2002;26(2):159-173.
Dai Z et al, *Respirology.* 2013;18:323–331.

Other IFIs



RVD, CD4: 34 cells/UL

Pleural fluid exam :

Yeast cells +

Culture: *Cryptococcus neoformans*

Serum cryp Ag 1: 64

Blood culture : NG

Cryptococossis

- Standard diagnostics are India ink microscopy, cryptococcal antigen (CrAg), and culture.
- Culture is gold standard, takes 72 hours to 2 weeks
- Culture can detect ~10^2 CFU yeast/mL

- India ink microscopic testing is able to detect >10^3 CFU yeasts/mL in CSF, sensitivity of ~60%–80% in HIV-infected and ~50% in non-HIV cryptococcal meningitis.
- Centrifugation of the sample enhanced the sensitivity of the culture by 100-fold.

Severance et al. Mykosen 1982. 26 (1) 29-33

Cryptococcal Ag

- Detection of Cryptococcal capsular galactoxylomannan antigen in serum and CSF have been available commercially for over 2 decades
- Able to detect both *C. neoformans* and *C. gattii species complex*

The methods:

- latex agglutination-based antigen system (LA),
- enzyme immunoassay-based assay (EIA),
- lateral flow assay (LFA).

- SE CrAg is highly sensitive for detecting meningitis in HIV-infected persons.
- Individuals with antigenemia can remain asymptomatic for weeks to months before clinical meningitis occurs
- As serum or plasma CrAg titers rise from 1:160 to 1:320 to 1:640, the probability of CSF involvement increases .
- CrAg titers of ≥1:1,280 have near-universal central nervous system (CNS) involvement
- However in symptomatic patients, despite a negative Cryp Ag, assessment of the CSF and culture should be performed in case of false negative result

Rachel M Wake and others, Cryptococcal Antigenemia in Advanced Human Immunodeficiency Virus Disease: Pathophysiology, Epidemiology, and Clinical Implications, *Clinical Infectious Diseases*,2023.76-4, 764–770
Ford N, Shubber Z, Jarvis JN, Chiller T, Greene G, Migone C, Vitoria M, Doherty M, Meintjes G. 2018. CD4 cell count threshold for cryptococcal antigen screening of HIV-infected individuals: a systematic review and meta-analysis. *Clin Infect Dis* 66:S152–s159.
Rajasingam etal. J Clin Microb. 2019, 57 (1)

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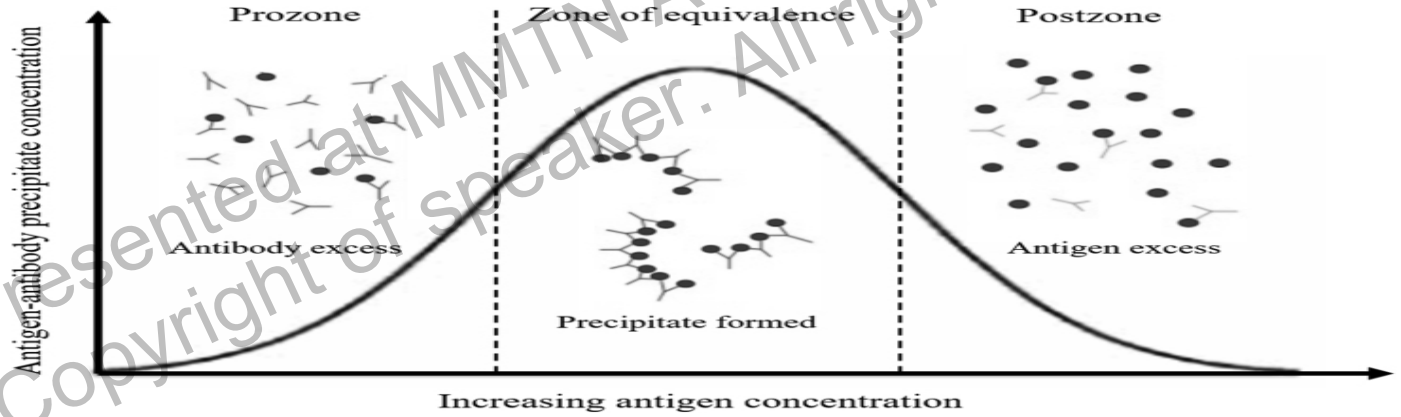
HIV positive

False negative :

- ✓ “postzone” effect : very high CrAg concentrations > greater than 0.140 mg/ml
- ✓ A capsular variant



Hevey MA, George IA, Rauseo AM, et al. Performance of the lateral flow assay and the latex agglutination serum cryptococcal antigen test in cryptococcal disease in patients with and without HIV. J Clin Microbiol **2020**
Tadeo KK, Nimwesiga A, Kwizera R, et al. Evaluation of the diagnostic performance of a semiquantitative cryptococcal antigen point-of-care assay among HIV-infected persons with cryptococcal meningitis. J Clin Microbiol **2021**; 59:e0086021.

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Non-HIV

- Cryp Ag test is less sensitive in non-HIV patients than in PLWH.
- LA is less sensitive in Localised pulmonary (23.5%) than disseminated cryp (78.1%) in NON-HIV

False-negative tests are more common In Non-HIV

TABLE 3 Cryptococcal antigen test sensitivity by disease presentation and HIV status[a]

Infection type	Population	% LA sensitivity (95% CI)	% LFA sensitivity (95% CI)	*P* value
Localized pulmonary	PLWH	100 (0–100)	—	—
	HIV-negative	23.5 (10.8–41.2)	90.9 (58.7–99.8)	<0.001
Disseminated	PLWH	94.7 (86.9–98.5)	100 (65.1–100)	0.517
	HIV-negative	78.1 (66.9–86.9)	82.6 (61.2–95.1)	0.641

[a]PLWH, people living with HIV; LA, latex agglutination; LFA, lateral flow assay; CI, confidence interval; —, not applicable.

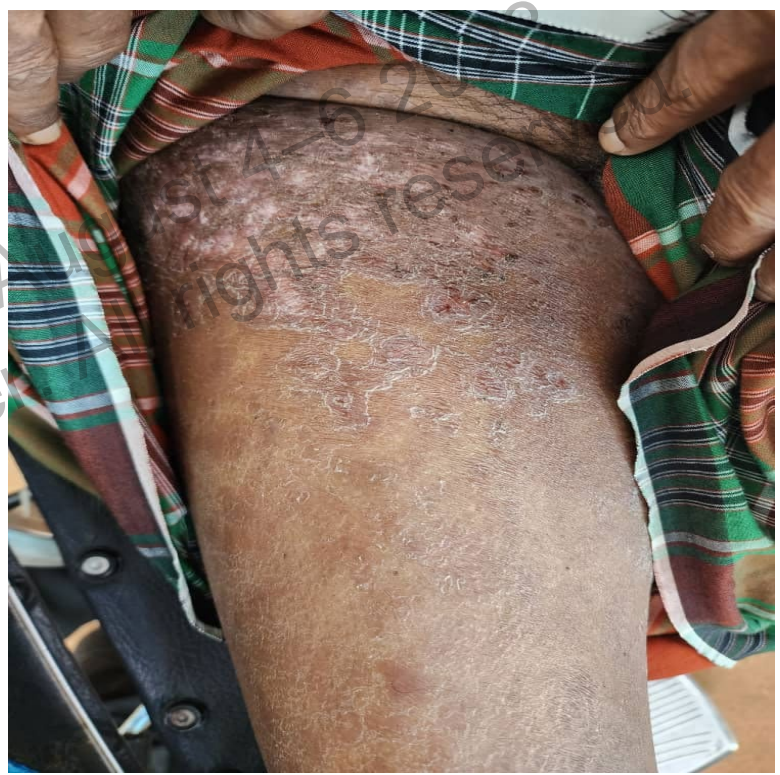
Hevey MA, George IA, Rauseo AM, et al. Performance of the lateral flow assay and the latex agglutination serum cryptococcal antigen test in cryptococcal disease in patients with and without HIV. J Clin Microbiol **2020**

FilmArray meningitis/encephalitis panel (Biofire, Salt Lake City, UT)

- A multiplex PCR assay that detects 14 meningitis-causing pathogens (bacteria, viruses, and fungi), including *Cryptococcus*.
- FilmArray PCR detected *Cryptococcus* in 96% of CSF when there were >100 yeasts CFU per ml of CSF, and specificity was 100%

Histoplasmosis

HPE : yeast cell seen (GMS / PAS)
Tissue (SKIN) : PCR : *Histoplasma capsulatum*
Tissue (skin) culture: *Histoplasma capsulatum*



Histoplasmosis

- Culture from clinical specimen is the gold standards methods for diagnosis.
- It may take > 2 weeks to grow, with higher sensitivity in patients disseminated histoplasmosis (74%) than patients with acute pulmonary histoplasmosis (42%)
- Higher sensitivity culture obtained from bone marrow

Histopathological identification :

- It is usually intracellular, poorly seen on Gram stain
- 2–4 µm narrow-based budding yeast when methenamine silver or PAS stains are used.
- HPE: granulomatous inflammation

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Vetter et al. *J. Clin. Microbiol.* **2001**, *39*, 4380–4386.
Hage CA et al. Semin Respir Crit Care Med 36:729 –745.

Histoplasmosis : Antigen detection

Antigen detection :

- Most sensitive
- Rapid result
- Does not require invasive procedure

Assay	Specimen	Sensitivity/ Specificity	
MiraVista EIA (4th gen) MiraVista Diagnostics, Indianapolis, IN, USA	Serum, plasma, Urine, CSF, BAL, other body fluids	Disseminated H: 92% Acute Pul H : 83% Subacute Pul H: 30% Chronic Pul H : 88%	Samples are required to be shipped to the company's facilities
IMMY *Histoplasma* GM EIA kit IMMY, Norman, OK, USA).	Urine	98% / 97-98%	FDA approved

- Sensitivity of Serum Ag and Urine Ag highest in disseminated disease in HIV patients, comparing to non-immunocompromised hosts.
- Persistent antigenuria for >12 weeks during successful treatment with amphotericin B or itraconazole
- Both tests : Cross-reactivity with other dimorphic fungi *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *T. marneffei, Coccidioides immitis*

Zhang et al. Med Mycol. 2015;53 (8):868–873
Falci et al. Open Forum Infect Dis. 2019;6(4): ofz073. doi:10.1093/ofid/ofz073
Hage et al. Clin Vaccine Immunol. 2011;18(4):661–666. doi:10.1128/CVI.00389-10

Histoplasma antibody

	Antibody
IMMY/Immunodiffusion Fungal Antibody Test Kit (Ref. ID1001)	Commercial: CE label http://www.immy.com/products/immunodiffusion-reagents-and-plates/#1473453177095-9d6fbd00-fc50
Meridian/Immunodiffusion reagents (Ref: 100201 and 100601)	Commercial: approved by the United States Food and Drug Administration https://www.meridianbioscience.com/human-condition/other/fungals/histoplasma-antigen-for-fungal-immunodiffusion
IMMY/Complement Fixation Reagents (Ref. H10150, H20110, H30150 and H40110)	Commercial: CE and in vitro diagnostic label http://www.immy.com/complement-fixation-reagents/#1473453809831-e685dea0-e154
IMMY. LA-Histoplasma (Ref. HL1001)	Commercial: approved by the United States Food and Drug Administration and CE label. https://www.immy.com/latex
Gibson Bioscience. Fungal serology.	Commercial: The ATCC Licensed Derivative Emblem. http://www.gibsonbioscience.com/GB-Products/Fungal-Serology/

Histoplasma antibody

- In meta-analysis, the overall sensitivity was 58% and specificity was 100%
- Immunoblot and ELISA methods are more sensitive comparing to antibody detection by immunodiffusion (ID) or complement fixation (CF).
- Anti-*Histoplasma* antibodies emerge 4 to 8 weeks after exposure and may persist for years after infection
- Sensitivity is limited in immunocompromised hosts who are unable to mount antibody response
- Antibody testing is most useful for non-immunosuppressed patients with chronic or subacute pulmonary histoplasmosis
- In endemic areas, unable to differentiate from previous exposure
- Cross reaction with other fungi

Caceres et al. Fungi (Basel). 2019 Aug 18;5(3):76. doi: 10.3390/jof5030076.
Wheat J. Endemic mycoses in AIDS: a clinical review. Clin Microbiol Rev. 1995;8: 146–159.

TABLE 1 Summary of diagnostic test for histoplasmosis[a]

Test	% histoplasmosis result by type			
	Acute pulmonary	Subacute pulmonary	Chronic pulmonary	Progressive disseminated
Culture	0–20	53.8	66.7	74.2
Pathology	0–42	42.1	75.0	76.3
Antigen	82.8–83.3	30.4	87.5	91.8
Serology	64.3–66.7	95.1	83.3	75

[a]See references 14 and 16

HIV

- In acute Pulmonary histoplasmosis : combined Se and urine Ag, may increase sensitivity Or
- to consider Histo Ag in BAL

Swartzentruber S, Rhodes L, Kurkjian K, et al. Diagnosis of acute pulmonary histoplasmosis by antigen detection. Clin Infect Dis **2009** ; 49(12):1878-82.
Azar et al. Laboratory diagnostics for histoplasmosis. J Clin Microbiol. 2017. 55:1612–1620

Molecular method

- PCR method : no standardized protocol or gene targets
- PCR-enzyme immunoassay-based method was only 18.5% sensitive
- A nested PCR detected 86% of cases with elevated *H. capsulatum*-specific antibodies
- Role of PCR is identification of organisms isolated from culture

Tang et al. Diagn Microbiol Infect Dis. 2006;54(4):283–287

Others

1) Histoplasma Ag Lateral flow assay (MiraVista) in serum:

- Manual reading: sensitivity was 96% and specificity was 90%.
- Automated reader: sensitivity was 92% and specificity was 94%

2) Loop-mediated isothermal amplification (LAMP) rests on the use of a DNA polymerase with high displacement strand activity and a set of specifically designed primers to amplify targeted DNA

- Sensitivity of 67-83%

Carceres et al, Mycoses. 2020, 63(2), 139-144
Zetti et al. *PLoS Negl. Trop. Dis.* 13:e0007692. doi: 10.1371/journal.pntd.0007692

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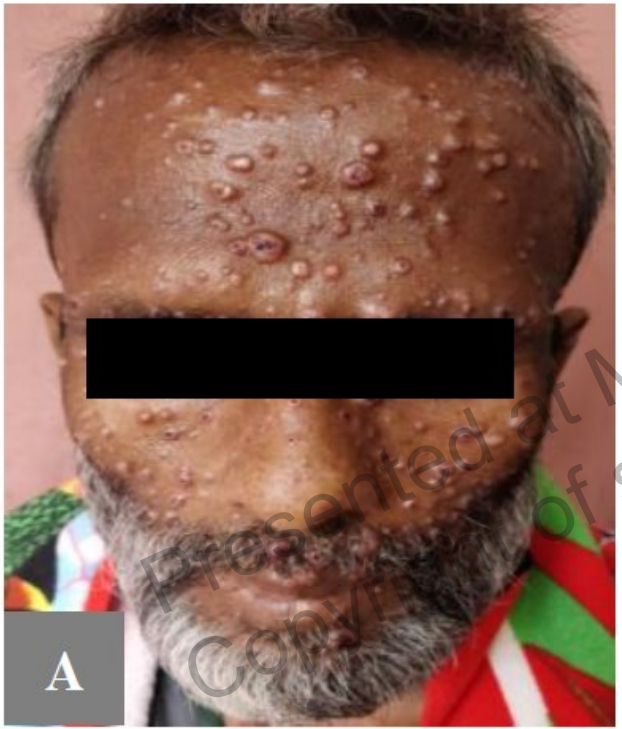

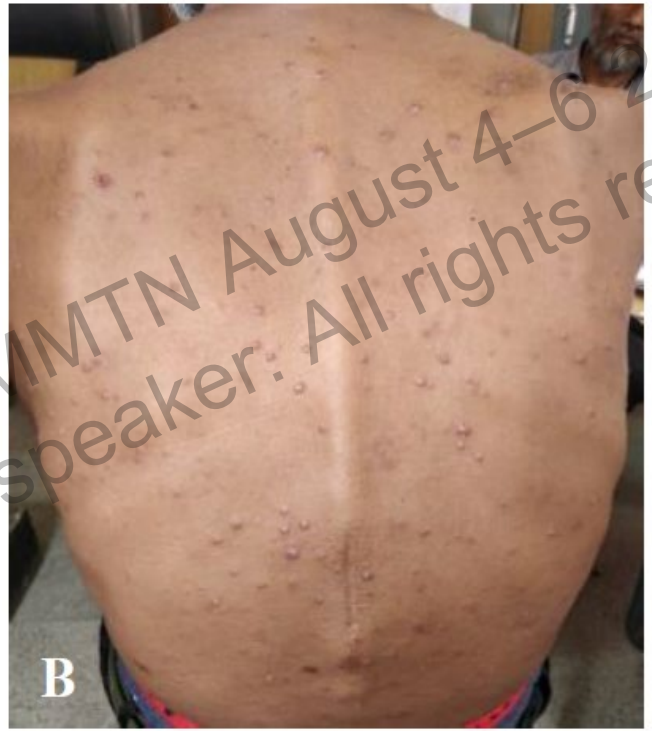

- RVD with CD4 < 100 cells/UL

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Devi B et al. Int J Res Dermatol. 2020 Sep;6(5):676-679

Talaromycosis

- Culture remains the mainstay of diagnosis for talaromycosis.

An antigen detection EIA targeting the *Talaromyces marneffei*–specific cell wall mannoprotein Mp1p:

- sensitive (86%) and specific (98%)

Others

- *Blastomyces* antigen

To detect the cell wall polysaccharide (galactomannan) of *Blastomyces dermatitidis* (Mira Vista Laboratories, Indianapolis, IN, USA) in urine, serum, or body fluids

- Paracoccidioidomycosis: serology test with specific (>95%) and sensitive (~80%)
- Coccidiodomycosis : serology in Serum and CSF

PCR in BAL sample

THANK YOU