Urine Aspergillus Antigen Detection as an Aid to Diagnose Invasive Aspergillosis

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Background

- Urine is reliable, easy substrate for multiple diagnostics, but no test has been optimized for IA
  - Animal models of IV 'galactomannan' injection demonstrated some excreted into urine[1]
  - Platelia galactomannan (EBA2) relatively insensitive in clinical studies[2-3]
- Galactomannan tests use β1,5 galactofuranose (gal*f*)as antigenic moiety (long chains)
- Novel gal*f* specific antibody detects urine antigen in animals and humans
  - Rapid localization in bladder with pulmonary IA[4]
  - Urine lateral flow prototype described[5]
- MycoMEIA™ is an ELISA uses 2 mAb with variable epitope gal*f* specificities
  - Optimized against patient samples to recognize small mw glycans excreted in urine

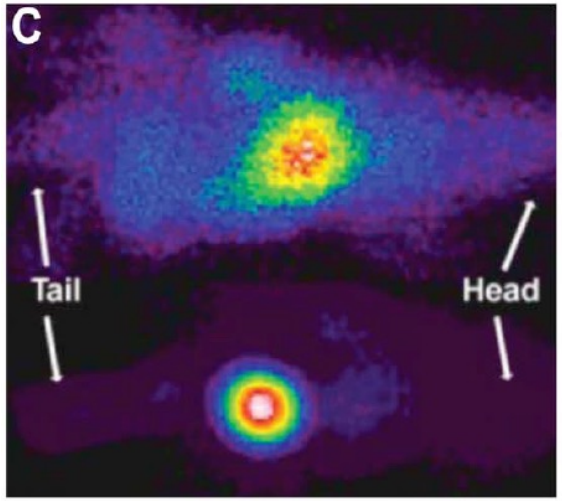

Figure 1. MAb476 recognizes *in vivo* Gal*f* antigen in a murine model of IA and localizes in the bladder of infected mice. *A,B* Sandwich ELISA detection of Gal*f*-containing antigen in BAL and lung homogenates (panel A; N = 5–6) and serum (panel B; N = 2–6) of neutropenic mice, 2 days after aerosol infection with *A. fumigatus*. *C*, Organ distribution of ^{99m}Tc-MAb476 2 days after infection; *Top*, control mouse: ^{99m}Tc-label localization in liver and spleen; *Bottom*, infected mouse: ^{99m}Tc-label localized in the bladder, representative of 2 independent experiments (N = 3 per group).
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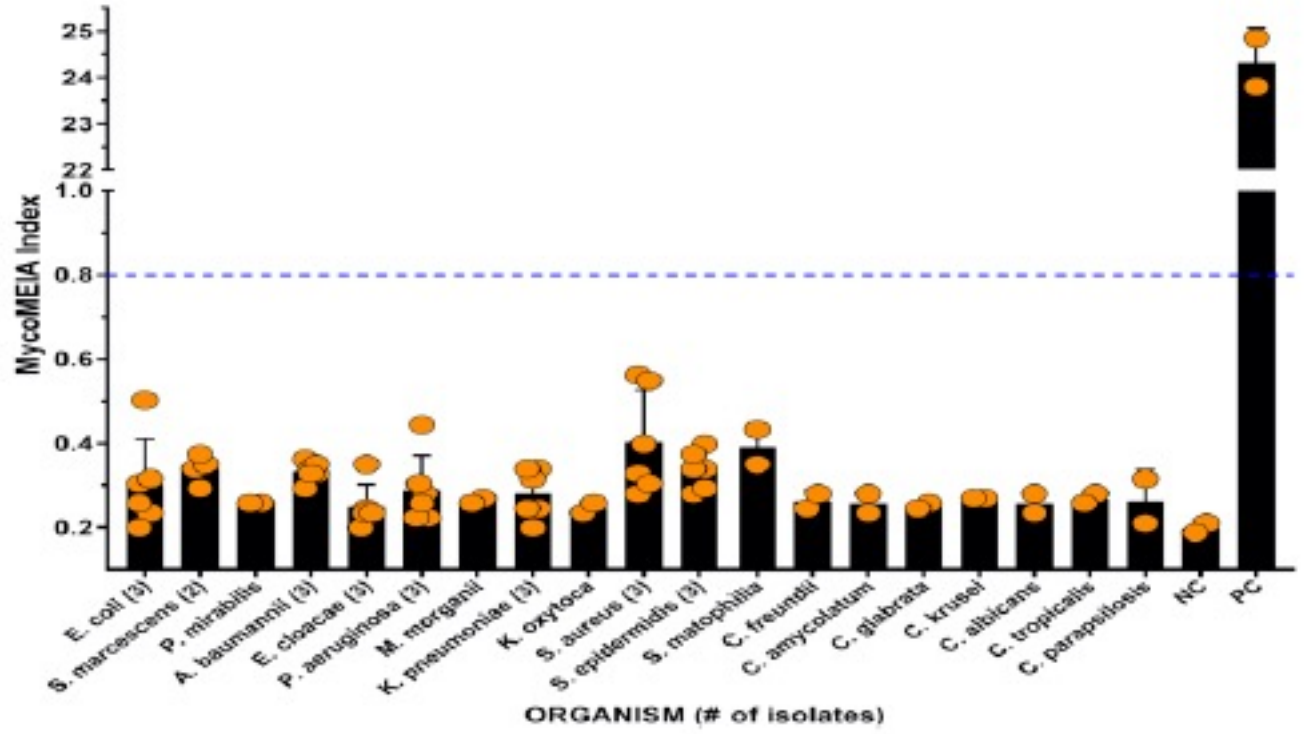
Bennett et al. *J Infect Dis* 1987 155(5)[1]
Klont et al. *Clin Infect Dis* 2004 39(1)[2]
Reischies et al. *J Clin Microbiol* 2016 54(3)[3]
Dufresne et al. *PloSOne* 2012 7(8)[4]
Marr et al. *Clin Infect Dis* 2018 67(11)[5]

MycoMEIA™

- Results of assay interpreted relative to a threshold control
  - EIA index (OD sample / mean OD threshold control)
- In vitro specificity for *Aspergillus* spp. and related Ascomycetes[1]
- In vitro studies show no cross-reactivity with bacteria or yeasts common in urine environment



Datta K, et al. ECCMID 2018 [1]

Performance Data from Multiple Clinical Studies Provided Frozen Urine Samples

MycoMed Technologies
PRODUCTS TO PREVENT INFECTION

- JHU urine sample collections active since 2011
  - Volunteer urine study enrolled people with suspected or confirmed invasive fungal infections
  - Prospective screening study that enrolled people at risk for infection (heme / BMT), sequential samples
- ASTEC repository study from Univ. Florida consortium (Wingard)
- Belgian screening study heme/BMT[1] (Maertens)
- 920 specimens from 310 different people
- Diagnoses independently adjudicated per EORTC/MSG definitions

Aerts et al, TIMM 2021[1]

Analysis in Validation Cohort to Derive Cut-offs

- People with no IFI (controls) and proven / probable IA with receipt of ≤3 days antifungal therapy (cases)
  - 21 cases, 161 controls
  - ROC AUC 0.97 (95% CI 93-100%), p<0.001
- 2 cut-offs derived
  - Low positive (index ≥ 0.6)
    - 90.5% (95% CI 69.6-98.8%) sensitivity
    - 91.9% (95% CI 92.1-98.6) specificity
  - Positive (index ≥ 0.8)
    - 85.7% (95%CI 63.7-97%) sensitivity
    - 96.3% (95% CI 92.1-98.6%) specificity

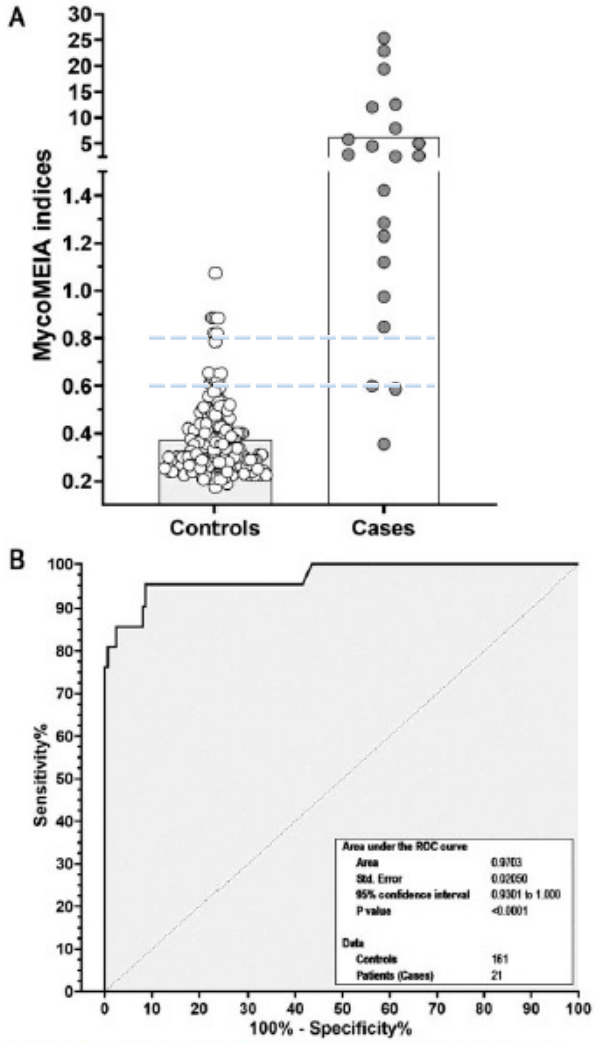


Figure 4. A. Dot-plot distribution and B. ROC curve of MycoMEIA™ performance in validation subset

Cohort Demographics and Clinical Characteristics

- Of 310 people with suspected or diagnosed IFI
  - 63% male, 72% Caucasian
  - 38% hematologic malignancy, 27% undergone BMT, other immunosuppressed
  - Adjudicated diagnoses variable
    - 61 (20%) proven-probable IA
    - 108 (35%) possible IA
    - 45 (14.5%) no IFI

Variable		N	%
Gender (M, %)		194	62.6%
Age, median (range)		58	(6–88)
Race			
	Caucasian	223	71.9%
	Black / African American	53	17.1%
	Asian	13	4.2%
	Latinx	8	2.6%
	Not specified, Other	13	4.2%
Ethnicity, Hispanic (%)		13	4.2%
Clinical Risk for Aspergillosis			
	Hematologic malignancy (no BMT)[1]	117	37.74%
	BMT[2]	83	26.77%
	Malignancy / tumor	28	9.03%
	Solid Organ Transplant, lung	23	7.42%
	Solid Organ Transplant, other[3]	19	6.13%
	Autoimmune[4]	11	3.55%
	HIV/AIDS	7	2.26%
	Other[5]	15	4.84%
	None	10	3.23%
Diagnosis			
	Proven IA	6	1.94%
	Probable IA	55	17.74%
	Possible IFI	108	34.84%
	Airway Aspergillosis (Proven / Probable)[6]	21	6.77%
	Fungal sinusitis	3	0.97%
	Other IFI[7]	46	14.84%
	No Fungal infection[8]	45	14.52%
	Other infections[9]	13	4.19%
	Mixed infections[10]	16	5.16%

MycoMEIA index result distribution

- From 920 samples, MycoMEIA indices ranged from 0.14 – 38.1
- Results from people with adjudicated IA aligned with degree of infection certainty
  - Mean Possible, Probable, Proven greater than control
- Substantial number of possible IA with + MycoMEIA (n=46, 42.6%)

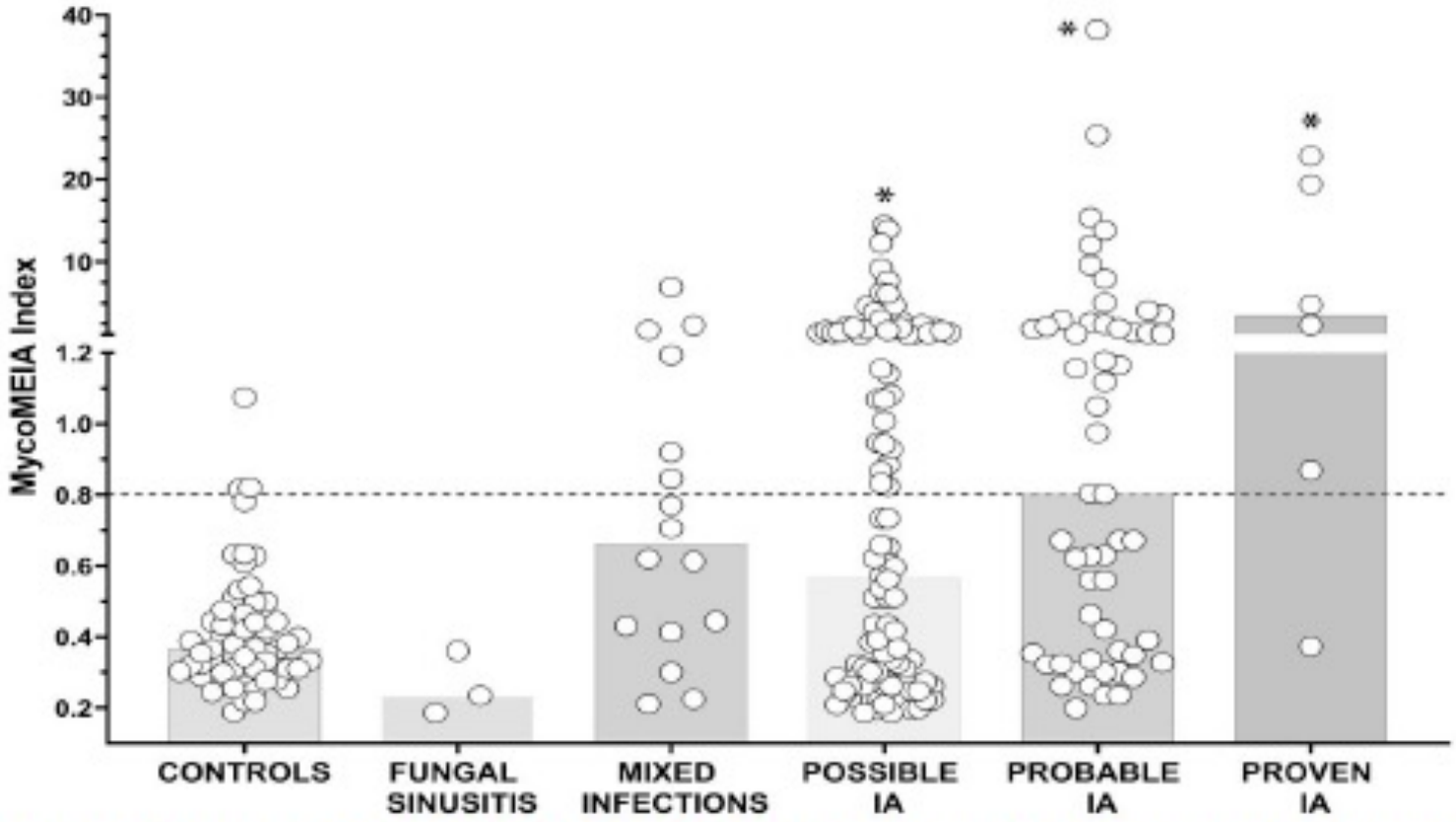


Figure 5. Dot-plot distribution of index values in all cohort subjects (n = 246) according to diagnosis; excludes cases with "Other IFI" (N = 45) and airway disease (N = 19), which are analyzed separately. Bars represent medians. Asterisks indicate significantly different medians compared to the No IFI Control group, $p<0.05$, Kruskal-Wallis test, with Dunn's correction.

Performance

- Entire cohort, independent of antifungals
  - Sensitivity 54.1% (95% CI 41-67%)
  - Specificity 92.8% (95% CI 83.9-97.6%)
- Strong impact of mold-active antifungals
  - When limited to people with ≤3 days mold-active antifungal therapy, sensitivity 82.5% (95% CI 67.2-92.7%)
- 5/6 people with proven IA +MycoMEIA
  - One false negative had vertebral osteomyelitis, no pulmonary IA
- Assuming pre-test probability high (40%), PPV 82% (95% CI 70-89%) and NPV 89.5% (95% CI 80-95%)

Diagnosis	N	Positive (%)	Negative (%)
Controls – No IFI	45	3 (6.7)	42 (93.3)
Controls – non-IFI infections[1]	13	0	13 (100)
Controls – Other IFI[2]	11	2 (18.2)	9 (81.8)
Controls – Total	**69**	**5 (7.2)**	**64 (92.8)**
Cases – Proven IA[3]	6	5 (83.3)	1 (16.7)
Cases – Probable IA[4]	55	28 (50.9)	27 (49.1)
Cases, Total	**61**	**33 (54.1)**	**28 (45.9)**
Cases, no Antifungals[5]	**40**	**33 (82.5)**	**7 (17.5)**
Other – Possible IA (all)[6]	108	46 (42.6)	62 (57.4)
Other – Mixed diagnoses[7]	16	6 (37.5)	10 (62.5)
Ambiguous cases – Total	**124**	**52 (41.9)**	**72 (58.1)**

Special Populations

- 5 children (age 6 – 21) enrolled
  - One candidemia with negative MycoMEIA
  - 4/4 probable IA with positive MycoMEIA
- 22 people with ‘airway’ aspergillosis (CF/lung transplant)
  - 9 (41%) with positive MycoMEIA

Diagnosis	N	Positive (%)	Negative (%)
Controls – No IFI[1]	7	0	7 (100)
Control Total	7	0	11 (100)
Cases – Proven airway aspergillosis	1	0	1 (100)
Cases – Probable airway aspergillosis[2]	17	7 (41)	10 (59)
Cases – Probable IA[3]	3	2 (67)	1 (33)
Other – Mixed diagnoses[4]	1	0	1 (100)
Cases Total	22	9 (41)	13 (59)

Special Populations

- Positive results seen in some people with other Ascomycetes
  - *Histoplasma* spp.
  - Blastomycosis
  - Fusariosis
- One person with candidemia (C. krusei) + undefined pulmonary nodules with 1/8 + MycoMEIA
- No positives in 8 people with proven bacterial infections, PCP

Pathology	+ MycoMEIA / specimens (%)	+ subjects / total (%)
Candidemia	1/8 (12.5)	1/3 (33.3)
Histoplasmosis	9/21 (42.9)	9/18 (50)
Blastomycosis	1/1 (100)	1/1 (100)
Fusariosis	1/1 (100)	1/1 (100)
PCP pneumonia	0/4	0/3
Bacterial pneumonia	0/12	0/5
Mycobacterial pneumonia	0/2	0/2
Pseudomonas bacteremia	0/1	0/1

Conclusions

- MycoMEIA - urine glycan test in final stages of development
- Sensitivity 82.5% to detect proven/probable IA (no antifungal therapy)
  - High number of ‘possible IA’ with positive MycoMEIA
- Like galactomannan tests (gal*f*), cross reactivity with other Ascomycetes likely
- High screening sensitivity, early positivity in people with hematologic malignancies (Aerts, TIMM2021)
  - When combined with serum galactomannan, >95% negative predictive value as screening test
- Promising aid to diagnose and screen for IA in high-risk people

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Questions?

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