CE

Pearl DIAGNOSTICS

INSTRUCTIONS FOR USE

Myco*MEIA*™ *Aspergillus* Assay

REF 43-25001

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1. KEY SYMBOLS USED

REF	Catalog Number		Consult Instructions for Use
LOT	Batch Code	+	Positive Control
	Use By Date	−	Negative Control
	Temperature Limitation	TC	Threshold Control
	Caution		Manufacturer
	Contents sufficient for <96> tests		Keep away from Sunlight
EC REP	Authorized Representative in the European Community	IVD	For In Vitro Diagnostic Use Only

2. INTENDED USE

The Myco*MEIA*™ Aspergillus Assay (Myco*MEIA*) is an enzyme immunoassay (EIA) for the in vitro qualitative measurement of Aspergillus antigens in human urine. Myco*MEIA* can be used as an aid in the diagnosis of invasive aspergillosis (IA) when clinically suspected in people with immunosuppression. It can also be used to screen for IA in people at high risk, such as in the setting of prolonged neutropenia. Myco*MEIA* is designed for use in a clinical laboratory, and results should be interpreted by trained healthcare professionals, incorporating other diagnostic procedures such as radiography and microbiologic testing.

3. SUMMARY AND EXPLANATION OF THE TEST

Invasive aspergillosis is caused by *Aspergillus* species fungi that live in the environment. Infection develops in people who have impaired defenses to inhaled spores. Disease is frequent in people with prolonged defects in neutrophils, such as with cytotoxic therapy for hematologic malignancies and receipt of blood or bone marrow transplant (BMT) (1, 2). Risks have expanded with contemporary treatments of multiple conditions, and are now accepted to also be high in people with impaired cellular immunity, such as with solid organ transplant (SOT) and treatment of autoimmune conditions. Most recently, high risks are noted in people who have severe lung disease after viral infections, especially influenza and COVID-19 (3-5). Infection is often suspected based on suggestive radiological findings, such as pulmonary nodules. Infrequently, *Aspergillus* species can be recovered by culture from respiratory samples, including sputum and bronchoalveolar lavage (BAL). More often, microbial evidence is supported by detection of fungal components, such as antigens or nucleic acids (3). Given high mortality with late diagnosis and delayed antifungal therapy, prophylactic antifungal therapy and/or frequent screening for early signs of infection are frequently deployed.

Secreted antigens that bear galactofuranose (gal*f*) are present in lungs, blood, and urine of infected animals and people, and form the basis of current tests that utilize blood and BAL (6, 7). The Myco*MEIA*™ *Aspergillus* Assay is an EIA that captures *Aspergillus* antigens in urine using an antibody cocktail. Urine samples are processed prior to testing to optimize antigen recognition, which is measured optically using a spectrophotometer. Positive, Negative, and Threshold Controls

supplied in the kit are tested and must be within a designated range to accept test sample results. Sample results are interpreted as Positive, Low Positive, or Negative using an optical density (OD) index that is calculated by sample OD relative to mean OD of the kit Threshold Control, with the fraction multiplied by a numeric factor calculated based on the value of the Threshold Control.

4. TEST COMPONENTS

Store the kit at 2-8°C, and bring reagents to room temperature (15-30°C) for 30 minutes before use. After use, return to 2-8°C, with unused strips and plates sealed in the desiccant-containing pouch. Reagents are supplied in sufficient quantity to perform 96 tests, with a maximum of 90 specimens (tested in singlicate) and three controls (tested in duplicate) in one run, or up to four runs of three stripwells containing a maximum of 18 specimens per run.

Symbol	Component	Description	Quantity
1	Microwell Plate	Plate with 12 strips, each contains 8 wells coated with anti-*Aspergillus* antigen monoclonal antibodies.	12 strips
−	Negative Control (NC) Sample (green)	Synthetic urine with preservative, no antigen.	1.0 mL
TC	Threshold Control (TC) Sample (blue)	Synthetic urine with preservative and *Aspergillus* antigen.	1.0 mL
+	Positive Control (PC) Sample (red)	Synthetic urine with preservative and *Aspergillus* antigen.	1.0 mL
2	Conjugate (100X) (white label)	100X concentrated peroxidase-labeled monoclonal antibodies containing preservative.	0.1 mL
3	Conjugate Diluent (white label)	Protein solution for diluting conjugate to 1X prior to use.	10 mL
4	Chromogen Solution (yellow label)	3,3′,5,5′-tetramethylbenzidine solution	10 mL
5	Stop Solution (blue label)	2N H_2SO_4	10 mL
6	Plate Sealers	Adhesive, transparent sheets for covering microplate wells during assay incubation.	10 sheets

P Concentrated 25X Wash Solution and Processing Columns are purchased separately from the kit

Component Notes

Samples are processed using Sample Processing Columns, Product number 25024, prior to testing. The columns are **purchased separately**, from the kit. Use of each spin column requires two microcentrifuge tubes (not supplied in kit). One is to be used for collecting the storage buffer, and another used to collect the processed sample.

5. WARNINGS, STORAGE, AND STABILITY

5.1. WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. Results should be interpreted in context of the patient's clinical risks and other findings.
3. Use with samples other than urine is not validated.
4. Controls are manufactured from inactivated fungal antigen in synthetic urine. These do not contain any infectious material but should be handled using appropriate lab biosafety practices.
5. The Safety Data Sheet (SDS) is available upon request or on PearlDx.com.
6. Mix reagents well before use.
7. Inadequate strip washing can generate false positive results. Follow instructions for wash parameters and washer use.
8. Automated plate washing systems must be periodically decontaminated to remove biological contamination, using directions supplied by the manufacturer.
9. Use clean or sterile materials (tubes, pipette tips, containers, etc.) and pipette tips with filters for sample handling to minimize contamination.
10. Use polypropylene tubes where indicated - do not use polystyrene.
11. Do not mix reagents from different kit lots.
12. Avoid exposing Chromogen Solution to strong light during storage or incubation. This solution must be colorless before use. The appearance of a blue color indicates the reagent is contaminated and should not be used.
13. Solutions should be visually free of particulates. Alterations in the physical appearance may indicate instability or deterioration.
14. Do not allow the microplate wells to dry between wash cycles and addition of reagents.

5.2. TEST STORAGE AND STABILITY

1. Store all kit components at 2-8°C until expiration date on the kit label. Bring reagents to room temperature (15-30°C) for at least 30 minutes before use.
2. 25X Concentrated Wash Solution, Product number 25070, is stable at 15-30°C until expiration date on the label.
3. Working Wash Solution (1X solution prepared from the 25X Concentrated Wash Solution) is stable for 5 days at 15-30°C from the date of preparation.
4. Sample Processing Columns, Product number 25024, are stable when stored at 2-8°C until expiration date on the label.

5.3. SPECIMEN COLLECTION AND STORAGE

Collect urine samples aseptically. Specimens in transit between laboratories should be maintained at 2-8°C to minimize microbial overgrowth. If a delay in specimen processing occurs, specimens can be stored at 2-8°C for up to 5 days, or stored for longer periods at -80°C. Specimens should be brought to room temperature (15-30°C) prior to testing and mixed well. Specimens should not remain at room temperature for longer than 8 hours.

6. PROCEDURE

6.1. MATERIALS

Materials Provided

1. Myco*MEIA*™ *Aspergillus* Assay 96 Test Kit – REF Product number 25001

Materials Required – Provided Separately

1. 25X Concentrated Wash Solution – REF Product number 25070
2. Sample Processing Columns (4 x 25 columns) – REF Product number 25024
3. 5 Microwell Plates, with 12 strips containing 8 wells each – uncoated – REF Product number 25025

Materials Required – Not Provided

1. Distilled or deionized water
2. Absorbent paper or paper towels
3. Single-channel and 8- or 12-channel multichannel micropipettes, adjustable or fixed, to measure and dispense 5 µL, 50 µL, 100 µL, 200 µL, and 1000 µL
4. 2 - 15 mL polypropylene tubes for 1X Working Conjugate Solution
5. 1 – 2 L containers for Working Wash Solution
6. Disposable polystyrene V-shaped reagent reservoirs (5 mL preferred for small volumes) capable of accommodating 5-25 mL volume and the width of an 8- or 12-channel micropipette
7. Microcentrifuge capable of 1,500 rcf, with a rotor accommodating 1.5-2 mL microcentrifuge tubes
8. Microcentrifuge tube 1.5-2 mL
9. Vortex agitator
10. Dry air incubator at 37±1°C
11. Automated microwell plate washer
12. Microwell plate reader equipped with 450 nm and 620 nm filters
13. Disposable gloves
14. Sodium hypochlorite solution (0.5%) or commercial liquid bleach (8-10% sodium hypochlorite)
15. Container for 1x Wash Buffer 1.25 L – 2.5 L

6.2. REAGENT PREPARATION AND STORAGE

Prepare all reagents before or during the assay procedure. All reagents and urine specimens should be at ambient room temperature (15-30°C) prior to use.

1 **Micowell Strips**

Determine the number of samples to be tested and remove requisite number of strips. Put the frame and the unused strips back in the original pouch and store sealed at 2-8°C. Ensure unused coated strips are stored with supplied desiccant. If the entire plate is not used for the assay, fill the empty positions in the plate frame with Uncoated Microwell Strips, Product number 25025. These are required when using 96-well automated microwell plate washers.

P **25X Concentrated Wash Solution, Product Number 2070**

Check the 25X Concentrated Wash Solution for the presence of crystals or precipitates. If crystals or precipitates have formed in the concentrate, re-solubilize by warming at 37°C for 30 min. Mix gently before diluting. Prepare 1X Working

Wash Solution by adding one part 25X Concentrated Wash Solution to 24 parts deionized or distilled water in a clean container. Example dilution calculations are shown below.

Preparation of Working Wash Solution

Number of Microwell Strips	Volume of Wash Concentrate	Volume of Purified / DI H2O	Total Volume
WHEN USING AUTOMATED 96-WELL PLATE WASHER			
1-12 (One plate)	50 mL	1,200 mL	1.25 L
>12-24 (Two plates) washed in same assay run	75 mL	1,800 mL	1.875 L
>12-24 (Two plates) washed in 2 assay runs	100 mL	2,400 mL	2.5 L
WHEN USING AUTOMATED STRIPWELL WASHER			
Max. 12 (One plate)	50 mL	1,200 mL	1.25 L
>12-24 (Two plate) washed in same assay run	75 mL	1,800 mL	1.875 L

Estimated total volumes include extra for automated washer priming and dead volumes.

After opening, the 25X Concentrated Wash Solution is stored at room temperature (15-30°C) until the expiration date indicated on the label. The 1X Working Wash Solution can be stored for 5 days at room temperature (15-30°C).

+
TC
−

Kit Controls

Positive, Negative and Threshold Controls must be processed identically to patient urine samples, as detailed below. After opening, these can be stored at 2-8°C until kit expiration. Mix by vortex prior to use without producing excessive foam or bubbles.

2 3 **Conjugate (100X) and Conjugate Diluent**

Clean, disposable **polypropylene** containers or tubes should be used. Do not use polystyrene tubes or containers to prepare 1X Working Conjugate Solution. Prepare 1:100 diluted 1X Working Conjugate Solution according to the table below. Mix Conjugate (100X) and Conjugate Diluent prior to use.

Preparation of Working Conjugate Solution

Number of Test Strips	Volume of Conjugate Concentrate	Volume of Conjugate Diluent	Total Volume
3	20 µL	1980 µL	2.0 mL
6	32 µL	3168 µL	3.2 mL
9	44 µL	4356 µL	4.4 mL
12 (full plate)	60 uL	5940 µL	6.0 mL

Mix vessel or tube by vortexing or by inversion without causing foaming. 1X Working Conjugate Solution is stable for four (4) hours at 15-30°C. For dispensing into test plate, fill a disposable reagent reservoir of appropriate volume capacity for use.

4 Chromogen Solution

This reagent is ready to use. The TMB substrate should be colorless. For dispensing into test plate, fill a disposable reagent reservoir of appropriate volume capacity for use. Once TMB substrate has been added to a reservoir, do not return unused TMB substrate to the original bottle.

5 Stop Solution

This reagent is ready to use. For dispensing into test plate, fill a disposable reagent reservoir of appropriate volume capacity for use, as shown in the table below.

Number of Microwell Strips	Volume of Chromogen
3	2.0 mL
6	3.2 mL
9	4.4 mL
12 (full plate)	6.0 mL

7. TEST PROCEDURE: SAMPLE PROCESSING

Sample Processing by Spin Columns

STEP 1	Prepare working assay reagents as outlined above.
STEP 2	a. Bring kit components and samples to room temperature for 30 minutes. Frozen samples must be fully defrosted prior to processing. b. Mix samples by gently inverting or swirling the container 5 times.
STEP 3	a. Loosen the spin columns' screwcaps by rotating to where the caps sit loosely but do not come off. b. Twist-off bottom closures of the columns and place the columns into 2 mL microcentrifuge tubes for buffer collection. c. Spin in microcentrifuge at 1,500rcf for 1 minute to remove storage buffer; discard the tubes with buffer. The resin appears compacted with a tilted top surface and is ready to use.
STEP 4	a. Place the spin columns with compacted resin in a fresh set of sterile 2 mL microcentrifuge tubes for sample collection; take the caps off. b. Pipette 105 µL of each urine sample or assay control onto the center of the compacted resin surface in a corresponding spin column. Loosely replace screwcaps on columns and allow 2-5 minutes for the samples to be absorbed into the resin. c. Spin in microcentrifuge at 1,500 x g for 2 minutes to collect the processed samples in the sample collection tubes. Discard the spin columns. d. Vortex the controls and samples at low speed for 2 seconds.

Note: The assay requires **50 µL** per well. Positive Control, Threshold Control and Negative Control are tested in duplicate. Patient sample is tested in singlicate. One processing step yields enough volume (>100 µL) for duplicate assay wells (50 µL each).

8. TEST PROCEDURE: SAMPLE TESTING

Myco*MEIA*™ *Aspergillus* Assay Sample Testing

STEP 1	Prime the automated plate washer following manufacturer directions. Assure that the final prime is with wash solution.
STEP 2	Prepare a plate map identification of test samples, controls, and blanks in the microplate. **Each run should contain, in the first strip, two wells for Positive Control, two wells for Threshold Control, two wells for Negative Control.**
STEP 3	Remove the plateholder and microwell strips from pouch and return unused strips to the pouch with desiccant, and reseal.
STEP 4	Add 50 µL of processed urine samples or processed controls into each well.
STEP 5	 Cover plate with plate sealer to prevent evaporation and incubate for 60±2 minutes at 37°C.
STEP 6	Wash plate 3 times with 365 µL of Working Wash Solution per well for each wash using a 20-second soak between wash cycles. Rotate the plate 180°. Wash the plate an additional 3 times with Working Wash Solution for a total of 6 wash cycles. Tap the inverted microplate on absorbent paper to remove excess wash solution.
STEP 7	Place the diluted 1X Working Conjugate Solution in the reagent reservoir; add 50 µL of Conjugate to all wells using multichannel micropipette, taking care to avoid touching walls or rims of wells.
STEP 8	 Cover plate with plate sealer to prevent evaporation and incubate for 60±2 minutes at 37°C (±1°C).
STEP 9	Wash plate 3 times with 365 µL of Working Wash Solution per well for each wash using a 20-second soak between wash cycles. Rotate the plate 180°. Wash the plate an additional 3 times with Working Wash Solution for a total of 6 wash cycles. Tap the inverted microplate on absorbent paper to remove excess wash solution.
STEP 10	Immediately add 50 µL of Chromogen Solution to all wells using a multichannel micropipette.
STEP 11	 Incubate the microplate in the dark at 15-30°C for 30±1 minutes. Do not use adhesive plate sealer during this incubation step.
STEP 12	Immediately add 50 µL of Stop Solution to each well (maintain the same sequence and time intervals used for Chromogen Solution addition) using a multichannel pipettor. Tap the plate gently to mix well.
STEP 13	Within 5±1 minutes of stopping the reaction, read well ODs at 450 nm, with the reference wavelength set at 620 nm. The absorbance from each well is expressed as the difference between A_{450} and A_{620}.

9. MEASURING AND INTERPRETING QC

Controls and patient samples are processed identically. Calculate the mean of 2 Threshold Control (TC) ODs that were run in the assay. The mean should fall within the range specified in the table below.

	Controls	Acceptable Range and Validity Criteria
TC	Threshold Control Mean	OD 0.331-0.660
−	Negative Control	EIA Index < 0.6
+	Positive Control	EIA Index > 10

Using the mean TC OD, calculate Myco*MEIA* index values for Positive and Negative Controls and clinical samples using the equation shown below.

$$\textbf{Myco}\textit{\textbf{MEIA}}\textbf{ Index} = \left(\frac{\text{OD Sample}}{\text{Mean OD Threshold Control}} \right) \textbf{ X Multiplication Factor}$$

In the index calculation, a multiplication factor is used to normalize variations in Threshold Control (TC) values. Choose factors 5, 6, or 7, depending on the mean TC OD, as shown in the Table below. Calculate Negative and Positive Control Index values. The Index values should fall within the boundaries designated in the Acceptable Range and Validity Criteria Table above. Controls that are outside of those boundaries indicate that test results are **INVALID**, and it is advised that the user review directions and repeat the assay. If all instructions have been followed and control results continue to fall out of range, contact Pearl Diagnostics Technical Support.

Multiplication Factor	Mean TC OD
5	0.331-0.440
6	0.441-0.550
7	0.551-0.660

10. CLINICAL SAMPLE INTERPRETATION

Clinical samples are interpreted as Positive, Low Positive and Negative by Myco*MEIA* Index using cut-offs shown below:

Sample Myco*MEIA* Index	Interpretation
EIA INDEX < 0.6	NEGATIVE
EIA INDEX 0.6-0.799	LOW POSITIVE
EIA INDEX ≥ 0.8	POSITIVE

Example Calculations

With OD values presented in the table below, mean TC OD = (0.470 + 0.562) ÷ 2 = 0.516

	Test	OD Values
TC	Threshold Control Mean	0.470, 0.562
−	Negative Control	0.023, 0.029
+	Positive Control	1.355, 1.375
	Clinical Sample	0.082

Validity Check: in range → PASS, choose Factor 6

Calculate Positive and Negative Control indices:

Positive Control mean OD = (1.355 + 1.375) ÷ 2 = 1.365

Positive Control index = (1.365/0.516) x 6 = 15.9

Negative Control mean OD = (0.023 + 0.029) ÷ 2 = 0.026

Negative Control index = (0.026/0.516) x 6 = 0.30

Validity Checks:

Negative Control index is < 0.6	PASS
Positive Control index is >10	PASS
Calculate clinical sample Myco*MEIA* index:	
(0.082 / 0.516) x 6 = 0.953	POSITIVE

11. LIMITATIONS OF THE PROCEDURE

1. Use only with urine.
2. Tests should be interpreted in context of clinical risks and other indicators of infection. Testing in patients without risks for IA may increase likelihood of false results.
3. If used for screening, urine should be tested twice weekly, and interpreted in context.
4. Contamination of urine can occur if samples are stored for over 8 hours at room temperature, causing interference with the assay.
5. The Myco*MEIA Aspergillus* assay has not been validated to aid the diagnosis of non-invasive pulmonary disease, such as allergic bronchopulmonary aspergillosis or sinus infection.
6. Significant hematuria may interfere with results. See Interfering Substances section.

12. SPECIFIC PERFORMANCE DATA

Performance data were generated using previously frozen urine samples from multiple clinical studies performed in the U.S. and Belgium, including people with and without IA, defined by consensus criteria (3). Assays requiring use of contrived, or "mock" samples were performed by spiking *Aspergillus* antigen into urine obtained from healthy donors without infection, or synthetic urine. Results obtained by individual users may differ from these results.

Analytical Sensitivity

LoD

The LoD is the lowest amount of analyte that can be reliably detected (≥ 95% of results greater than the limit of blank). The LoD of the assay is 7.8 ng/mL.

LoQ

The LoQ was determined with an accuracy goal at the LoQ of ≤ 35% total error (Westgard model). The LoQ was equivalent to LoD, 7.8 ng/mL.

Linearity

The linearity of the Myco*MEIA*™ *Aspergillus* Assay was determined according to CLSI guideline EP06-Ed2 (11). ODs of serially diluted contrived samples were plotted against their corresponding concentrations to generate a binding curve using log-linear axes and an XY scatter plot format. The binding curve followed sigmoidal dose-response kinetics with variable slope and was best represented (R^2 0.9994) with a non-linear regression equation, producing best fit values of a Hill Slope at 2.52 (95% CI: 2.4-2.65) and EC50 of 43.4 ng/mL (95% CI: 42.5-44.4). The highest antigen concentration (500 ng/mL) elicited the highest mean OD (±SD) of 3.399 (± 0.038), demonstrating an absence of hook effect. To find the linear portion of the non-linear regression binding curve, middle values from the range of ODs were iteratively plotted against the antigen concentration using linear-linear axes. The slope (±SD) of the best-fit curve was 0.046 (± 0.00064) with an R^2 of 0.9986. The assay was linear between 15.63 and 62.5 ng/mL corresponding to ~OD range 0.3-2.5.

Precision

Within-lab precision and repeatability were determined throughout the measuring range according to CLSI Guideline EP05-A3 (9). Samples included kit controls (NC, TC, PC) and contrived samples with known *Aspergillus* antigen concentrations (+, ++, +++). Assays were run twice daily (AM and PM) using kit lot by two different operators for five days. Mean OD and index (IDX) values, standard deviation (SD), % Coefficient of variation (%CV) were calculated to determine within-run (intra-assay) and between-run (inter-assay) variation. Results are shown for positive samples only. Within-run variation is only shown for sample OD, not index, as only one mean TC OD contributes to within-run index calculations.

Panel Members		N	Mean	Within-Run (intra-assay) SD	%CV	Total (inter-assay) SD	%CV
Healthy neg	OD	10	0.027	0.00127	-	0.00249	-
	IDX		0.32	-	-	0.0358	-
+	OD	10	0.076	0.00297	4.6%	0.01512	20.0%
	IDX		0.87	-	-	0.163922	18.8%
++	OD	10	0.240	0.01824	8.0%	0.04012	16.7%
	IDX		2.76	-	-	0.345146	12.5%
+++	OD	10	0.798	0.03295	4.3%	0.07058	8.8%
	IDX		9.21	-	-	0.523205	5.7%
NC	OD	10	0.019	0.00113	-	0.00321	-
	IDX		0.22	-	-	0.046511	-
TC	OD	10	0.558	0.02828	5.1%	0.10866	19.5%
	IDX	-	NA	-	-	-	-
PC	OD	10	1.638	0.08895	5.2%	0.31543	19.3%
	IDX		18.82	-	-	2.812988	14.9%

Interfering Substances

Interference of the assay was tested using exogenous substances spiked into pooled healthy urine, and by testing clinical samples from patients with known endogenous conditions, as indicated by abnormal urinalysis results. Interferents and conditions tested are shown in the table below. Contrived samples were prepared to contain enough antigen to elicit an OD index three-times that of the positivity cut-off of 0.8. OD was tested before and after addition of *Aspergillus* antigen to assess potential cross-reactivity caused by the exogenous or the endogenous substances. Among the substances and conditions tested, cross-reactivity was observed after addition of exogenous ascorbic acid, and in a urine sample obtained from a patient with pyuria. Interference in a positive assay was elicited in a urine sample spiked with whole blood (5%), indicating that high-grade hematuria interferes with positive test results.

#	Interferent	Concentration / Condition
1	Amphotericin B	0.22 mg/mL
2	Ascorbic acid (vit C)	1 mg/mL
3	Caffeine	15 mg/mL
4	Blood	5% (v/v)
5	Human albumin	10 mg/mL
6	Itraconazole	0.22 mg/mL
7	Oxalic acid	0.01% (w/v)
8	Vaginal contraceptive gel	5% (w/v)
9	Water-based personal lubricant	5% (w/v)

#	Interferent	Concentration / Condition
10	WBC (Buffy Coat)	105 cells / mL
11*	acdidic pH	pH 5
12*	neutral pH	pH 7
13*	high protein	>100 mg/dL
14*	low protein	<30 mg/dL
15*	trace protein	<15 mg/dL
16*	leukocyte esterase and/or nitrite	+
17*	bilirubin	>0.4 mg/dL

* Clinical samples with abnormal parameters listed

Cross-Reactivity

Antibodies used in the Myco*MEIA*™ *Aspergillus* assay detect galactofuranose-containing antigens, typically produced in high quantities by *Aspergillus* spp., other Ascomycetes fungi, and select other microorganisms, but not by mammals. Cross-reactivity was tested using healthy urine containing bacterial and fungal organisms, listed in the table below, grown to $1x10^7$ colony forming units. Among the isolates tested, one of three urine samples spiked with *S. aureus* elicited a positive response with an index just above the positive cut-off of 0.8.

#	Organisms	# Isolates	#	Organisms	# Isolates
1	Escherichia coli	3	10	Staphylococcus epidermidis	3
2	Serratia marcescens	2	11	Citrobacter freundii	1
3	Proteus mirabilis	1	12	Stenotrophomonas maltophilia	1
4	Acinetobacter Baumanii	3	13	Corynebacterium amycolatum	1
5	Enterobacter cloacae	3	14	Candida glabrata	1
6	Pseudomonas aeruginosa	3	15	Candida krusei	1
7	Klebsiella pneumoniae	3	16	Candida albicans	1
8	Klebsiella oxytoca	1	17	Candida tropiicalis	1
9	Staphlyococcus aureus	3	18	Candida parapsilosis	1

Cross-reactivity was also tested using urine samples obtained from people having different medical conditions, including fungal, viral, and bacterial infections. Samples were collected within one week of established diagnosis. Of the 38 samples collected from 38 patients, four (10.5%) Myco*MEIA* tests showed low-positive results (index 0.6-0.8). Cross-reactivity was detected from samples collected from people with histoplasmosis. Cross-reactivity was observed in samples obtained from people with cryptococcosis (n=2), blastomycosis (n=1), and candidemia (n=1). One candidemia case was caused by *C. krusei*; this patient also had pulmonary nodules of undetermined etiology. One of two samples from people with bacteremia (*Serratia* spp.) was also positive. This cross-reactivity pattern is generally consistent with other assays that rely on detection of galactofuranose-containing antigens (8).

Potentially cross-reactive medical conditions	# urine samples	# Positive (≥ 0.8)	# Low Positive (0.6-0.799)
Histoplasmosis (probable)	5	0	2
Histoplasmosis (proven)	5	3	0

Cryptococcosis (probable)	5	0	2
Cryptococcosis (proven)	5	0	0
Candidemia	2	0	1
Fusariosis (proven)	1	0	0
Blastomycosis (proven)	1	0	1
PCP (probable)	3	0	0
Viral infections[1]	2	0	0
Bacteremia (*E. coli*)	1	0	0
Bacteremia (*Serratia*)	1	1	0
MSSA pneumonia + bacteremia	1	0	0
Legionella pneumonia	1	0	0
Pseudomonas pneumonia	2	0	0
Stenotrophomonas pneumonia	2	0	0
Mycobacterium chelonae pneumonia	1	0	0

[1] Influenza (n=1), Respiratory synctial virus (n=1)

Clinical Performance as an Aid to Diagnose Invasive Aspergillosis

Clinical performance was characterized using urines collected from studies in which hospitalized patients were suspected to have invasive fungal disease (IFD). Clinical records were reviewed blinded to assay result to assess whether the patient had IA, defined by criteria for proven, probable, or possible infection, or had no infection or another diagnosis corresponding to the event, timed as within 6 weeks of sample collection. Definitions of proven or probable IA, and other IFD (histoplasmosis, cryptococcosis, mucormycosis, fusariosis, *Pneumocystis* pneumonia) were derived from the most recent consensus definitions recommended by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG) (3). To better capture diagnostic criteria that are actionable by clinicians, diagnostic criteria for probable IA also included tree-in-bud radiographic abnormalities. Results of Platelia serum or BAL galactomannan tests (GM EIA) contributed to probable IA diagnosis as defined by consensus criteria (3).

To test clinical performance as an aid to diagnose IA, 920 specimens from 310 people with suspected IFD were evaluated. Index values ranged from 0.14 to 38.1. Assay cut-offs were first defined in a validation cohort that included people who had proven or probable IA with minimal (<3 days) receipt of mold-active antifungal therapy. In the validation cohort of 21 people with proven or probable IA, and 161 controls, sensitivity was 90.5% (95% CI: 69.6-98.8) and specificity was 91.9% (95% CI: 86.6-95.6%) using the low index cut-off (0.6). Using the high (0.8) index cut-off, sensitivity was 85.7% (95% CI: 63.7-97%) and specificity was 96.3% (95% CI 92.1-98.6%). Myco*MEIA* receiver operator characteristic (ROC) area under the curve was 0.97 (95% CI: 0.93-1).

In the larger cohort of 920 samples from 310 people enrolled with suspected infection, index values ranged from 0.14 to 38.1. Per-subject performance of the assay estimated a sensitivity of 90.5% (95% CI 70-90%) and specificity of 89.2% (95% CI 81-94%) at the low index cut-off (0.6). This analysis omitted cases who had received mold-active antifungal therapy for >3 days unless they showed evidence of progressive infection, people with ambiguous diagnosis (possible IFD) and people with documented pulmonary aspergillosis involving the airway or sinuses only., Among 5 children with IFD enrolled in the cohort (age 6-21), 4/4 with probable IA had a positive Myco*MEIA*; 1 with candidemia had a negative test result.

Clinical Performance as a Screening Assay for Invasive Aspergillosis

To evaluate performance as a screening assay, sequential urine samples obtained from people with hematologic malignancies and/or receipt of allogeneic BMT were tested. Patients had undergone screening with serum GM EIA and bronchoalveolar lavage (BAL). Urine samples were collected twice weekly and stored frozen at -80˚C. Myco*MEIA* was tested blind to infection diagnosis, adjudicated using EORTC/MSG definitions (8). Results of urine tests obtained within the 2-week window prior to clinical diagnosis were considered for screening performance. Results were interpreted using a low index cut-off (0.6) to optimize sensitivity and negative predictive value (NPV). Eligible urine samples (n=64) from 18 cases (proven/probable IA) and controls were analyzed. Myco*MEIA* results had to be positive before diagnosis was established using other commercial assays and/or before start of empiric mold-active therapy in order to be a "true positive" for screening sensitivity. With this conservative definition, sensitivity for use as a screening test was 64.5% (95% CI: 23-77%) for Myco*MEIA* and 50% (95% CI: 35-90%) for serum GM EIA. Assuming IA pre-test probability of 10%, a negative Myco*MEIA* result (at index <0.6) generated 92.7% (95% CI: 79-98%) NPV. Urine was positive by Myco*MEIA* in 5/7 cases with positive serum GM EIA and 7/7 cases with positive BAL GM EIA. Myco*MEIA* positive cases pre-dated diagnosis established with the aggressive clinical screening by a mean 16.4 (range -35 to -2) days.

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MANUFACTURER INFORMATION

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