INSTRUCTIONS FOR USE

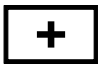
Myco*MEIA*® *Aspergillus* Assay

REF 43-25100

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1. KEY SYMBOLS USED

	Catalog Number		Consult Instructions for Use
	Batch Code		Positive Control
	Use By Date		Negative Control
	Temperature Limitation		Threshold Control
	Caution		Manufacturer
	Contents sufficient for <96> tests		Keep away from Sunlight.
	For In Vitro Diagnostic Use Only		Do not Reuse
	Consult SDS		Causes severe skin burns and eye damage
	Toxic in contact with skin		

2. INTENDED USE

The Myco*MEIA*® *Aspergillus* Assay (Myco*MEIA*) is an enzyme immunoassay (EIA) for the *in vitro* qualitative detection of *Aspergillus* antigens in human urine from adults (≥ 18 years old) with suspected invasive aspergillosis (IA). The assay is not intended for use in lung transplant recipients. The results should be interpreted by trained healthcare professionals, incorporating other diagnostic procedures such as microbiological culture, histological examination of biopsy samples, and radiographic evidence to support the diagnosis of IA.

Rx only

For *in vitro* diagnostic use

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 the 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 or Negative using an optical density (OD) index that is calculated by sample OD relative to the 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 (see Section 6.2)	Component	Description	Quantity
1	Microwell Plate	Plate with 12 strips, each containing 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)	Urine with preservative and *Aspergillus* antigen.	1.0 mL
+	Positive Control (PC) Sample (red)	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 Sulfuric Acid	10 mL
6	30X Column Rinse (pink label)	Buffer containing preservative.	10 mL
NA	Plate Sealers	Adhesive, transparent sheets for	10 sheets

		covering microplate wells during assay incubation.	

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 25102, prior to testing. The columns are **purchased separately** from the kit. Use of each spin column requires two 1.7 – 2.0 mL microcentrifuge tubes (not supplied in kit). One is to be used for collecting the storage buffer/column rinse, and another is used to collect the processed sample.

5. WARNINGS, STORAGE, AND STABILITY

5.1. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For prescription use only.
- Results should be interpreted in the context of the patient's clinical risks and other findings.
- Use with samples other than urine is not validated.
- Controls are manufactured from inactivated fungal antigen in urine. These do not contain any infectious material but should be handled using appropriate lab biosafety practices.
- The Safety Data Sheet (SDS) is available upon request or on PearlDx.com.
- Mix reagents well before use.
- Inadequate strip washing can generate false positive results. Follow instructions for wash parameters and washer use.
- Automated plate washing systems must be periodically decontaminated to remove biological contamination, using directions supplied by the manufacturer.
- Use clean or sterile materials (tubes, pipette tips, containers, etc.) and pipette tips with filters for sample handling to minimize contamination.
- Contaminated urine can produce false-positive results.
- Use polypropylene tubes where indicated – do not use polystyrene.
- Do not mix reagents from different kit lots.
- Do not use the test kit or components after the expiration date.
- Avoid exposing the 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 compromised and should not be used.
- Solutions and samples should be visually free of particulates. Alterations in the physical appearance may indicate instability or deterioration.
- Do not allow the microplate wells to dry between wash cycles and the addition of reagents.

5.2. TEST STORAGE AND STABILITY

- Store all kit components at 2-8°C until the expiration date on the kit label. Do not use the kit past the expiration date. Bring reagents to room temperature (15-30°C) for at least 30 minutes before use.
- 25X Concentrated Wash Solution, Product number 25101, is stable at 15-30°C until the expiration date on the label.
- 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.
- Sample Processing Columns, Product number 25102, are stable when stored at 2-8°C until the expiration date on the label.
- Open kits are stable for 84 days.

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 6 hours.

6. PROCEDURE

6.1. MATERIALS

Materials Provided

- Myco*MEIA*™ *Aspergillus* Assay 96 Test Kit – REF Product number 25100

Materials Required – Provided Separately

- 25X Concentrated Wash Solution – REF Product number 25101
- Sample Processing Columns (4 x 25 columns) – REF Product number 25102
- 5 Microwell Plates, with 12 strips containing 8 wells each – uncoated – REF Product number 25103

Optional Materials – Provided Separately

WS001 - Myco*MEIA* Calculation Worksheet

Materials Required – Not Provided

- Distilled or deionized water
- Absorbent paper or paper towels
- 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
- 2 – 15 mL polypropylene tubes for 1X Working Conjugate Solution and 1X Column Rinse
- 1 – 2 L containers for Working Wash Solution
- 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
- Microcentrifuge capable of 1,500 rcf, with a rotor accommodating 1.7-2 mL microcentrifuge tubes
- Microcentrifuge tubes 1.7-2 mL
- Vortex agitator
- Dry air incubator at 37±1°C
- Automated microwell plate washer
- Microwell plate reader equipped with 450 nm and 620 nm filters
- Disposable gloves
- Sodium hypochlorite solution (0.5%) or commercial liquid bleach (8-10% sodium hypochlorite)
- 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 Microwell Strips

Determine the number of samples to be tested and remove the 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 the 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 25103. These are required when using 96-well automated microwell plate washers.

P 25X Concentrated Wash Solution, Product Number 25101

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 a 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. Aviod exposing the TMB to light in the disposable reagent reservoir for longer than 30 min.

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

6 30X Column Rinse

Prepare 1X Column Rinse by adding one (1) part 30X Column Rinse to 29 parts deionized or distilled water in a clean container or tube. One (1) mL of 1X Column Rinse is required for each spin column used for processing controls or urine samples to be tested.

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 1.7-2 mL microcentrifuge tubes for buffer collection. c. Spin in microcentrifuge at 1,500 x g (rcf) for 2 minutes to remove storage buffer; discard the storage buffer. The spin column resin appears compacted with a tilted top surface and is ready for rinsing.
STEP 4	a. Place the spin columns with compacted resin in the same 1.7-2 mL microcentrifuge tubes as used in STEP 3; take the caps off. b. Pipette 500 µL of 1X Column Rinse onto the center of the compacted resin surface and allow the rinse to be absorbed into the resin. c. Loosely replace screwcaps and spin in a microcentrifuge at 1,500 x g for 2 minutes to remove rinse; discard the rinse. d. Repeat steps 4a – 4c one more time for a total of two rinses.
STEP 5	a. Label a fresh set of sterile 1.7-2 mL microcentrifuge tubes with either control or test sample IDs. b. Place the spin columns with compacted resin in the labeled fresh set of sterile 1.7-2 mL microcentrifuge tubes for sample collection; take the caps off. c. 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 5 minutes for the samples to be absorbed into the resin. d. Spin in a microcentrifuge at 1,500 x g for 2 minutes to collect the processed samples in the sample collection tubes. Discard the spin columns. e. Vortex labeled tubes containing 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. Each 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 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, and two wells for Negative Control.**
STEP 3	Remove the plateholder and microwell strips from the 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 the plate with plate sealer to prevent evaporation and incubate for 60±2 minutes at 37˚C (±1°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 a reagent reservoir; add 50 µL of Conjugate to all wells using a 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 at 15-30°C for 30±1 minutes. Do not use an 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 OD range of 0.331 – 0.770. If the TC mean is outside of the defined range, the assay results are INVALID. The user must review directions and repeat the assay in accordance with laboratory practices.

If the TC falls within the acceptable range defined above, the multiplication factor for the Index calculations is selected for all controls and samples on the assay plate based on TC ranges defined in the Table below.

Multiplication Factor	Mean TC OD
5	0.331-0.440
6	0.441-0.550
7	0.551-0.660
8	0.661-0.770

In the Index calculation, a multiplication factor is used to normalize variations in Threshold Control (TC) values. Choose factors 5, 6, 7, or 8, depending on the mean TC OD, as shown in the Table below. Calculate Negative and Positive Control Index values. The Index values for Positive and Negative controls should fall within the boundaries designated in the Acceptable Range and Validity Criteria

Table below. 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.

The Myco*MEIA* Index values for Positive and Negative Controls are calculated using the equation shown below.

$$\text{Myco}MEIA\ \text{Index} = \left(\frac{\text{Mean Control OD}}{\text{Mean TC OD}}\right) \times \text{Multiplication Factor}$$

The Index calculated for the Positive and Negative Controls must fall within the ranges defined in the Table below.

	Control	Acceptable Range and Validity Criteria
−	Negative Control Mean	EIA Index < 0.6
+	Positive Control Mean	EIA Index > 10.0

Controls outside of the boundaries defined in the Table above indicate that the assay results are INVALID. The user must review directions and repeat the assay in accordance with laboratory practices.

Results for clinical samples are calculated according to the equation below.

$$\text{Myco}MEIA\ \text{Index} = \left(\frac{\text{Sample OD}}{\text{Mean TC OD}}\right) \times \text{Multiplication Factor}$$

Results can be calculated manually using the instructions provided above. PearlDx provides a validated Excel spreadsheet, WS001, Myco*MEIA* Calculation Worksheet, that performs Myco*MEIA* Index calculations.

The worksheet contains a tab with detailed instructions and a tab for entering the data. The worksheet calculates the TC control results, compares the results to the acceptable ranges, determines the multiplication factor, and calculates the Index results for the Positive and Negative controls and for the specimens. If any of the controls are outside of the acceptable range, the worksheet will return the INVALID result, which indicates that the assay must be repeated.

10. CLINICAL SAMPLE INTERPRETATION

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

Sample Myco*MEIA* Index	Interpretation
EIA INDEX < 0.6	NEGATIVE
EIA INDEX ≥ 0.6 to < 0.7	Repeat sample testing
EIA INDEX ≥ 0.7	POSITIVE

It is recommended that the customers repeat the assay on the same sample for index results ranging from 0.6 to 0.7. Upon repeat, the final interpretation for the sample is shown below:

Myco*MEIA* Index Following Sample Repeat	Final Interpretation
EIA INDEX < 0.6	**NEGATIVE**
EIA INDEX ≥ 0.6	**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 = 0.3

Validity Checks:

Negative Control index is < 0.6	PASS
Positive Control index is > 10.0	PASS
Calculate clinical sample Myco*MEIA* index:	
(0.082 / 0.516) x 6 = 1.0	POSITIVE

11. LIMITATIONS OF THE PROCEDURE

- The Myco*MEIA Aspergillus* assay is a qualitative test and does not provide a quantitative result.
- The Myco*MEIA Aspergillus* assay is only validated for use in unpreserved urine specimens. Other specimens or preserved urine are not indicated for use and should not be tested.
- The Myco*MEIA Aspergillus* assay is an aid in the diagnosis of invasive aspergillosis. Tests should be interpreted in the context of clinical information and other indicators of infection.

- There is a risk of false positive results due to the presence of other Ascomycetes fungi in urine specimens. Cross-reactivity has been observed with the Myco*MEIA Aspergillus* Assay with infections caused by other Ascomycetes fungi (Histoplasma, Blastomyces, Fusarium).
- There is a risk of false positive results in the presence of some interferents in urine specimens. Cross-reactivity has been observed at high concentrations of betanin, bilirubin, caffeine, Gyne-Lotrimin cream (clotrimazole), hemoglobin, Lotrimin Ultra cream (butenifine), Monistat 7 cream (miconazole), Vaginal contraceptive gel, Vagisil cream (benzocaine), and Vagistat 1 cream (ticonazole).
- Plasma-Lyte A at 10% v/v may contribute to a false-positive result in the Myco*MEIA Aspergillus* assay. See the Interfering Substances section for all interferents tested.
- Contamination of urine can occur if samples are stored for over 8 hours at room temperature, causing interference with the assay.
- 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.
- The Myco*MEIA Aspergillus* assay has not been validated for use in pediatric patient populations and should not be tested.
- The performance of the assay has not been evaluated in individuals with prior antifungal therapy.
- The performance of the assay has not been evaluated in lung transplant recipients and should not be tested.
- Assays with an index value between 0.6 to 0.7 should be repeated, as approximately 5% of tests can be falsely positive around the 0.6 cut-off.

12. SPECIFIC PERFORMANCE DATA

Performance data were generated using prospectively collected urine samples, and 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). Studies requiring use of contrived, or "mock" samples were performed by spiking *Aspergillus* antigen into urine obtained from healthy donors without infection. 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 are clinically interpreted as "Positive"). The LoD of the assay is 3 ng/mL. The LoD was determined consistent with the guidelines in CLSI document EP17-A2 (10) based on 60 determinations with low target analyte level replicates.

Precision

Within-lab precision and repeatability were determined according to CLSI Guideline EP05-A3 (11). Samples included kit controls (NC, TC, PC), contrived samples with known *Aspergillus* antigen concentrations (negative, low positive, positive), and clinical sample pools. Assays were run twice daily (AM and PM) using three kit lots by two different operators for twenty days. Each sample was tested in duplicate (n=2) for each run. Mean OD and index (IDX) values, standard deviation (SD), % Coefficient of variation (%CV) were calculated to determine repeatability (intra-assay variation) and within-laboratory precision (inter-assay variation). Coefficient of variation results are shown for positive samples only.

Panel Members	Data	N	Mean	Repeatability		Within-Laboratory Precision	
				SD	CV	SD	CV
NC	OD	80	0.019	0.002	--	0.004	--
	IDX	80	0.2	0.023	--	0.047	--
TC	OD	80	0.603	0.048	8%	0.080	13%
	IDX	80	7.1	0.587	8%	0.915	13%
PC	OD	80	1.909	0.090	5%	0.163	9%
	IDX	80	22.6	1.076	5%	2.174	10%
Negative Pool	OD	80	0.022	0.002	--	0.003	--
	IDX	80	0.3	0.023	--	0.038	--
Contrived Low Positive	OD	80	0.065	0.007	10%	0.009	13%
	IDX	80	0.8	0.077	10%	0.106	14%
Contrived Positive	OD	80	0.118	0.006	5%	0.013	11%
	IDX	80	1.4	0.069	5%	0.137	10%
Clinical Sample Pool	OD	80	0.135	0.008	6%	0.013	10%
	IDX	80	1.6	0.094	6%	0.171	11%
Clinical Sample Pool	OD	80	0.238	0.010	4%	0.038	16%
	IDX	80	2.8	0.112	4%	0.515	18%

Reproducibility

Reproducibility was determined according to CLSI Guideline EP05-A3 (11). Samples included kit controls (NC, TC, PC), contrived samples with known *Aspergillus* antigen concentrations (negative, low positive, positive), and clinical sample pools. Assays were run twice daily (AM and PM) using one kit lot by two different operators for five days. Each sample was tested in triplicate (n=3) for each run. Assays were run at three sites. Mean OD and index (IDX) values, standard deviation (SD), % Coefficient of variation (%CV) were calculated to determine repeatability, within-laboratory precision, and reproducibility. Coefficient of variation results are shown for positive samples only.

Results from one site

Panel Members	Data	N	Mean	Repeatability		Within-Laboratory Precision	
				SD	CV	SD	CV
NC	OD	30	0.020	0.002	--	0.005	--
	IDX	30	0.2	0.028	--	0.059	--
TC	OD	30	0.452	0.064	14%	0.094	21%
	IDX	30	5.6	0.833	15%	1.097	20%
PC	OD	30	1.407	0.149	11%	0.171	12%
	IDX	30	17.6	1.873	11%	2.554	15%
Negative Pool	OD	30	0.027	0.011	--	0.017	--
	IDX	30	0.3	0.132	--	0.202	--
Contrived Low Positive	OD	30	0.065	0.016	24%	0.016	24%
	IDX	30	0.8	0.191	24%	0.192	24%

Panel Members	Data	N	Mean	Repeatability		Within-Laboratory Precision	
				SD	CV	SD	CV
Contrived Positive	OD	30	0.125	0.013	10%	0.028	22%
	IDX	30	1.5	0.164	11%	0.332	21%
Clinical Sample Pool	OD	30	0.073	0.014	20%	0.015	20%
	IDX	30	0.9	0.186	20%	0.210	23%
Clinical Sample Pool	OD	30	0.181	0.017	10%	0.026	15%
	IDX	30	2.3	0.218	10%	0.357	16%

Results from all sites

Panel Members	Data	N	Mean	Reproducibility	
				SD	CV
NC	OD	90	0.024	0.011	--
	IDX	90	0.3	0.123	--
TC	OD	90	0.515	0.104	20%
	IDX	90	6.2	1.077	17%
PC	OD	90	1.580	0.213	13%
	IDX	90	19.0	2.470	13%
Negative Pool	OD	90	0.031	0.012	--
	IDX	90	0.4	0.138	--
Contrived Low Positive	OD	90	0.080	0.019	24%
	IDX	90	1.0	0.210	22%
Contrived Positive	OD	90	0.142	0.031	22%
	IDX	90	1.7	0.318	19%
Clinical Sample Pool	OD	90	0.084	0.016	19%
	IDX	90	1.0	0.186	18%
Clinical Sample Pool	OD	90	0.207	0.029	14%
	IDX	90	2.5	0.297	12%

Interfering Substances

Interference of the assay was determined according to CLSI Guideline EP07 (12). Interference was tested using endogenous and 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 with 8 ng/mL antigen, approximately 3X the assay LOD. 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 with 10% v/v Plasma-Lyte A.

Interference is noted here and in the Limitations of the Procedure section. Cross-reactivity was observed with negative samples resulting in false positive results in the absence of *Aspergillus* antigen.

Substances and conditions shown in the table below showed no interference in the Myco*MEIA Aspergillus* assay.

#	Interferent	Concentration / Condition
1	1-Methylnicotinamide (Vitamin B3 metabolite)	0.130 mg/mL
2	Acetaminophen	2.5 mg/mL
3	Acetone (Ketone)	2.4 mg/mL
4	Albumin	10 mg/mL
5	Amoxicillin	1.59 mg/mL
6	Amphotericin B	0.22 mg/mL
7	Ascorbic acid (Vitamin C)	0.5 mg/mL, 1 mg/mL
8	Azithromycin	0.15 mg/mL
9	Biotin (Vitamin B)	0.4 µg/mL
10	Boric Acid	7.89 mg/mL
11	CD14	3 µg/mL
12	Cimetidine	0.9 mg/mL
13	Ciprofloxacin	1.2 mg/mL
14	Erythromycin	0.675 mg/mL
15	Ethanol	30 mg/mL
16	Ganciclovir hydrate	0.9 mg/mL
17	Glucose	6 mg/mL
18	Human chorionic gonadotropin (HCG)	0.3 µg/mL
19	Ibuprofen	0.27 mg/mL
20	Immunoglobulin G	2 mg/mL
21	Itraconazole	0.22 mg/mL
22	Metronidazole	1 mg/mL
23	Mucin	5% w/v
24	Mucin-5B	0.1% w/v
25	Naproxen sodium	0.02 mg/mL
26	Nicotine	0.015 mg/mL
27	Orosomucoid 2	0.2 µg/mL
28	Oxalic acid	0.1 mg/mL, 1 mg/mL
29	Penicillin G sodium salt	3.2 mg/mL
30	Phenazopyridine HCl	1.2 µg/mL
31	Phylloquinone (Vitamin K1)	0.05 µg/mL
32	Piperacillin sodium salt	19 mg/mL
33	Potassium clavulanate	0.65 mg/mL
34	RBC (erythrocytes)	2% v/v
35	Riboflavin (Vitamin B2)	0.24 mg/mL
36	Rifampicin	10 mg/mL

#	Interferent	Concentration / Condition
37	Sodium salicylate	4.73 mg/mL
38	Tazobactam sodium salt	17 mg/mL
39	Triglycerides	0.3 mg/mL, 1 mg/mL
40	Urobilinogen	0.06 mg/mL
41	Vancomycin HCl	3.1 mg/mL
42	Voriconazole N-oxide	0.022 mg/mL
43	Vanillylmandelic acid	0.03 mg/mL
44	Water-based personal lubricant	50 mg/mL
45	WBC (White Blood Cells / Leukocytes)	300,000 cells/mL
46	α1-Acid glycoprotein	0.6 µg/mL
47	α-CEHC (metabolite of Vitamin E)	5 µg/mL
48	β-carotene (parent compound of Vitamin A)	3 mg/mL
49	Contrived alkaline pH	pH 10.0
50	Contrived acidic pH	3.82
51*	Abnormal clinical urine	Protein 30 mg/dL pH 8.0 Hemolyzed blood 200 Ery/µL, Ketones >160mg/dL Bilirubin +++ Glucose 250mg/dL
52*	Abnormal clinical urine	pH 7.51 Bilirubin +++
53*	Abnormal clinical urine	Ketones 80 mg/dL Bilirubin ++, Glucose 250 mg/dL
54*	Acidic pH	pH 5.16
55*	High protein	100 mg/dL
56*	Normal clinical urine	N/A
57*	Normal clinical urine	N/A
58	Betanin	0.05 mg/mL
59	Bilirubin	0.03 mg/mL
60	Caffeine (in urine)	0.16 mg/m
61	Gyne-Lotrimin cream (clotrimazole)	0.14% w/v
62	Hemoglobin	0.15 mg/mL
63	Lotrimin Ultra cream (butenafine)	0.14% w/v
64	Monistat 7 cream (miconazole)	0.14% w/v
65	Nitrites	0.04 mg/mL
66	Vaginal contraceptive gel	0.14% w/v
67	Vagisil cream (benzocaine)	0.14% w/v

#	Interferent	Concentration / Condition
68	Vagistat 1 cream (ticonazole)	0.14% w/v

* Clinical samples with abnormal parameters listed

Substances and conditions shown in the table below showed cross-reactivity at in the Myco*MEIA Aspergillus* assay at or above the concentrations shown below.

Interferent	Concentration
Betanin	0.25% w/v
Bilirubin	0.06 mg/mL
Caffeine	6.75 mg/mL
Gyne-Lotrimin cream	2.5% w/v
Hemoglobin	4.2 mg/mL
Lotrimin Ultra cream	1.25% w/v
Monistat 7 cream	1.25% w/v
Nitrites	0.075 mg/mL
Plasma-lyte A	10% w/v
Vaginal contraceptive gel	1.25% w/v
Vagisil cream	1.25% w/v
Vagistat 1 cream	1.25% w/v

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 virus, bacterial and fungal organisms listed in the table below. Testing was performed in duplicate (n=2). The Myco*MEIA*® assay gave false positive results with Fusarium, an Ascomycetes fungus. No microbial interference was observed in the presence of the organisms and the assay analyte.

Organisms shown in the table below were tested in replicates after spiking in pooled urine to evaluate cross-reactivity in the MycoMEIA Aspergillus assay.

#	Organisms	Concentration	Average Myco*MEIA* Index	Interpretation
1	*Acinetobacter baumanii*	*4.00 x 10^5 CFU/mL*	0.3	Negative
2	*Bacteroides fragilis*	*1.01 x 10^6 CFU/mL*	0.3	Negative
3	*Candida albicans*	*1.73 x 10^6 CFU/mL*	0.3	Negative
4	*Candida glabrata*	*1.91 x 10^6 CFU/mL*	0.3	Negative
5	*Candida parapsilosis*	*3.06 x 10^6 CFU/mL*	0.3	Negative
6	*Candida tropicalis*	*1.57 x 10^6 CFU/mL*	0.3	Negative
7	*Chlamydia trachomatis*	*7.00 x 10^5 CFU/mL*	0.3	Negative
8	*Citrobacter freundii*	*6.7 x 10^5 CFU/mL*	0.2	Negative

#	Organisms	Concentration	Average Myco*MEIA* Index	Interpretation
9	*Clostridia*	*5.28 x 10^5 CFU/mL*	0.2	Negative
10	*Corynebacterium amycolatum*	*2.80 x 10^5 CFU/mL*	0.2	Negative
11	*Cryptococcus neoformans*	*1.25 x 10^6 CFU/mL*	0.2	Negative
12	*Enterobacter cloacae*	*1.65 x 10^6 CFU/mL*	0.2	Negative
13	*Enterococcus faecalis*	*1.4 x 10^6 CFU/mL*	0.3	Negative
14	*Escherichia coli*	*1.15 x 10^6 CFU/mL*	0.2	Negative
15	*Fusarium*	*8.80 x 10^5 CFU/mL*	1.0	Positive
16	*Geotrichum*	*2.80 x 10^5 CFU/mL*	0.3	Negative
17	*Klebsiella pneumoniae*	*6.9 x 10^5 CFU/mL*	0.3	Negative
18	*Lactobacillus crispatus*	*2.80 x 10^5 CFU/mL*	0.2	Negattive
19	*Neisseria gonorrhoeae*	*3.90 x 10^5 CFU/mL*	0.3	Negative
20	Parainfluenza virus 1	*1.00 x 10^6 Virus/mL*	0.2	Negative
21	Parainfluenza virus 2	*5.00 x 10^5 Virus/mL*	0.2	Negative
22	Parainfluenza virus 3	*1.00 x 10^6 Virus/mL*	0.2	Negative
23	*Peptostreptococci*	*6.84 x 10^5 CFU/mL*	0.3	Negative
24	*Prevotella bivia*	*1.84 x 10^6 CFU/mL*	0.2	Negative
25	*Proteus mirabilis*	*1.35 x 10^6 CFU/mL*	0.3	Negative
26	*Pseudomonas aeruginosa*	*1.1 x 10^6 CFU/mL*	0.2	Negative
27	Rhinovirus 2	*2.00 x 10^6 Virus/mL*	0.1	Negative
28	Rhinovirus 83	*4.50 x 10^5 Virus/mL*	0.2	Negative
29	*Serratia marcescens*	*1.1 x 10^6 CFU/mL*	0.3	Negative
30	*Staphylococcus aureus*	*1.37 x10^6 CFU/mL*	0.3	Negative
31	*Staphylococcus epidermidis*	*5.8 x 10^5 CFU/mL*	0.2	Negative
32	*Staphylococcus saprophyticus subsp. saprophyticus*	2.60 x10^5 CFU/mL	0.2	Negative
33	*Stenotrophomonas maltophilia*	*2 x 10^6 CFU/mL*	0.3	Negative
34	*Streptococcus agalactiae*	*1.37 x 10^6 CFU/mL*	0.3	Negative

Cross-reactivity was also tested using urine samples obtained from people with different medical conditions, including fungal, viral, and bacterial infections. Cross-reactivity was detected from samples collected from people with histoplasmosis (n=1), blastomycosis (n=1), candidemia (n=1), streptococcus (n=2) and rhinovirus/parainfluenzavirus (n=1). One sample that showed a positive Myco*MEIA* test result (index 0.6) was identified as possible invasive *Aspergillus*.

Clinical samples shown in the table below were tested in replicates to evaluate for cross-reactivity in the MycoMEIA Aspergillus assay.

Potentially cross-reactive medical conditions	Average Myco*MEIA* Index	Interpretation
UTI Streptococcus spp.	3.9	Both results Positive
UTI Enterococcus spp. , Enterobacter lung / blood	0.5	Both results Negative

Potentially cross-reactive medical conditions	Average Myco*MEIA* Index	Interpretation
UTI Streptococcus spp.	0.3	Both results Negative
UTI Staphylococcus spp.	0.4	Both results Negative
Klebsiella lung/blood	0.3	Both results Negative
E. coli lung / blood	0.3	Both results Negative
Possible IA + UTI Enterococcus, Enterococcus lung / blood*	0.6	Both results Positive
Enterobacter lung / blood, Streptococcus lung / blood	0.2	Both results Negative
Streptococcus lung / blood	0.3	Both results Negative
UTI Mixed gram positive	0.2	Both results Negative
UTI Staphylococcus spp.	0.5	Both results Negative
Acinetobacter junii lung / blood, URI – Rhinovirus, Parainfluenzavirus	0.3	Both results Negative
URI – Rhinovirus, Parainfluenzavirus	0.5	One result Positive, One result Negative
Streptococcus lung / blood	0.4	Both results Negative
MSSA, Staphylococcus lung / blood, URI – Influenza	0.3	Both results Negative
Staphylococcus lung / blood	0.3	Both results Negative
Streptococcus lung / blood	0.6	One result Positive, One result Positive
P. aeruginosa lung / blood	0.3	Both results Negative
MRSA, Staphylococcus lung / blood	0.3	Both results Negative
Candida krusei	0.2	Both results Negative
Candida krusei	0.2	Both results Negative
MRSA, Staphylococcus lung / blood	0.2	Both results Negative
URI – Rhinovirus, Parainfluenzavirus	0.2	Both results Negative
E. coli lung / blood	0.1	Both results Negative
Other IFI lung / blood	0.2	Both results Negative
MRSA, Staphylococcus lung / blood	0.3	Both results Negative
MRSA, Staphylococcus lung / blood	0.4	Both results Negative
Histoplasmosis	0.2	Both results Negative
Candida albicans (Candidiasis)	0.2	Both results Negative
Pneumocystis jirovecii pneumonia	0.3	Both results Negative
Other IFI lung / blood (cryptococcosis)	0.5	Both results Negative
Histoplasmosis	0.3	Both results Negative
Histoplasmosis	0.3	Both results Negative
Histoplasmosis	0.3	Both results Negative
Histoplasmosis	0.4	Both results Negative
Histoplasmosis	18.6	Both results Positive

Potentially cross-reactive medical conditions	Average Myco*MEIA* Index	Interpretation
Pneumocystis jirovecii pneumonia	0.3	Both results Negative
Candidiasis	0.2	Both results Negative
Blastomyces lung / blood	0.6	Both results Positive
Staphylococcus lung / blood	0.3	Both results Negative
P. aeruginosa lung / blood	0.2	Both results Negative
Pneumocystis jirovecii pneumonia	0.3	Both results Negative
Candidiasis	0.6	Both results Positive
MRSA, Stenotrophomonas lung / blood	0.4	Both results Negative
P. aeruginosa lung / blood	0.3	Both results Negative
Fusariosis lung / blood	0.2	Both results Negative
P. aeruginosa lung / blood	0.3	Both results Negative

**Sample identified as possible IA and cannot be evaluated for interference*

Additional cross-reactivity testing was performed using clinical samples associated with episodes of mixed infections and non-*Aspergillus* infections. All subjects were at risk for IA by virtue of having current or recent episodes of neutropenia (e.g., due to cytotoxic therapy), receipt of allogeneic BMT, receipt of solid organ transplant (kidney, heart, liver), receipt of other biologic immunosuppressants for autoimmune disease, inherited severe immunodeficiency or another underlying disease that portends high-risk for IA (e.g., HIV/AIDS, severe malnutrition). Subjects in the clinical study were inpatients and had at least 2 weeks of clinical follow-up to determine clinical diagnosis, with at least 4 urine samples obtained during the clinical evaluation period or at least 1 sample obtained within 1 week of diagnosis.

Of 21 subjects with documented bacterial pneumonia (18 with concurrent bacteremia) no urine samples tested positive with Myco*MEIA*. Cross-reactivity was detected from samples collected from people with mixed bacterial infection (n=1), mixed gastrointestinal complications (n=2), histoplasmosis (n=2), blastomycosis (n=1), candidemia (n=1), and disseminated fusariosis (n=1). Positivity was more frequent in people who had ambiguous diagnoses. These included people considered to have 'possible IA' with specific radiographic findings, but with bacterial cultures positive in cultures other than BAL (e.g., sputum or blood).

These findings show that cross-reactive Myco*MEIA* tests can occur with infections caused by other Ascomycetes fungi.

Adjudicated Diagnosis	Positive / Tested Episode (%)
Bacterial UTI1	0/4
Mixed bacterial infection2	1/4
Bacteremia3	0/2
Bacterial pneumonia4	0/3
Bacterial pneumonia with bacteremia	
Gram – positive5	0/8
Gram – negative6	0/8
Mixed7	0/2
Staphylococcus skin infection	0/1
Viral upper respiratory infection8	0/2
Mixed gastrointestinal complications9	2/2
Fungal infections	
Histoplasmosis	2/9
Candidemia	1/6
Pneumocystis jirovecii (PJP)	0/4
Fusarium spp.	1/2

Adjudicated Diagnosis	Positive / Tested Episode (%)
Blastomycosis	1/1
Lomentospora prolificans	0/1
External otitis, A. fumigatus	0/1
Mixed infection10	0/3
Mixed ambiguous fungal and bacterial infections	
Possible IA + bacteremia11	2/3
Possible IA + possible bacterial pneumonia12	1/1
Possible IA + other13	2/5
Probable IA + bacterial pneumonia14	0/3

[1] *Staphylococcus* spp. (n=2), *Streptococcus* spp. (n=1), Mixed gram-positive bacteria (n=1)
[2] *Coagulase negative Staphylococcus bacteremia and Klebsiella + Lacobacillus* abscess (n=1) and Streptococcal UTI (n=1), *P. aeruginosa*, *E. faecalis*, *E. faecium*, and *C. perfrigens* liver abscess (n=1), Enterococcal UTI and bacteremia (n=1)
[3] *Klebsiella* spp. (n=1), *S. viridans* (n=1)
[4] *S. aureus* (n=1), *S. aureus* complicating Rhinovirus URI (n=1), *Serratia* spp. and *Stenotrophomonas* spp. (n=1)
[5] *S. aureus* (n= 5), *Streptococcus* spp. (n=2), *S. aureus* complicating Influenza URI (n=1)
[6] *E. coli* (n=2); *Klebsiella* spp. (n=1); *P. aeruginosa* (n=4) and *Acinetobacter* spp. complicating Rhinovirus and Parainfluenza URI (n=1)
[7] *Streptococcus* spp. and *Enterobacter* spp. (n=1), *S. aureus* and *Stenotrophomonas* spp. (n=1)
[8] Rhinovirus and Parainfluenza virus (n=2)
[9] Adenovirus colitis with GVHD grade 3 (n=1), HSV with mucositis grade 2 (n=1)
[10] Cryptococcosis and histoplasmosis (n=1), *Candida* spp. and polymicrobial bacteria liver abscess (n=1), *Exerohilum* spp. + *E. coli* bacteremia (n=1)
[11] Possible IA + bacteremia with *P. aeruginosa* and *Enterococcus* spp. (n=1); *Klebsiella* spp. (n=1), *S. viridans* (n=1)
[12] Possible IA + sputum with *S. aureus*, BAL no growth (n=1)
[13] Possible IA + *S. aureus* skin infection (n=1), candidemia (n=1), PJP (n=1), toxoplasmosis (n=1), Streptococcal UTI + coagulase – negative *Staphylococcus* bacteremia (n=1)
[14] Probable IA (+BAL GM EIA) and *Klebsiella pneumoniae* in BAL (n=3 subjects, 5 samples tested)

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 Myco*MEIA* assay result to assess whether the patient had IA, defined by criteria for proven or probable infection, or had no infection or another diagnosis corresponding to the event, timed as within 2 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).

A total of 475 samples from 290 different subjects were evaluated. Of the total 475 samples, 226 samples were tested from 50 subjects with IA that were proven (n = 3) or probable (n = 47).

Prospective Study

A prospective study was performed to analyze the specificity of the assay in fresh samples. Testing of the clinical samples was performed within 6 hours of collection. The study was performed to include all consenting inpatients who had blood for GM EIA testing sent to the lab, regardless of age, underlying disease, or reason for clinical suspicion. Lung transplant recipients and pediatric patients are not indicated for use with the Myco*MEIA* and were excluded from analyses. Because the definition of 'no IA' vs. 'possible IA' is contingent on results of chest CT, subjects who did not have CT performed within 2 weeks of urine sampling were also excluded from analyses. Clinical diagnoses were adjudicated by a reviewer who was blinded to Myco*MEIA* test results. Diagnoses included proven IA, probable IA, and possible IA, as per the 2020 EORTC/MSG definitions (3).

A total of 210 subjects were consented and provided 254 urine samples for testing during 213 different suspected

infection episodes. Of these, 34 subjects had lung transplants and were excluded from IA analysis. 2 pediatric subjects were also excluded, Of 177 remaining episodes of suspected IA, 8 more were excluded. One sample was noted by visual appearance in the laboratory to be likely contaminated prior to testing. Three subjects did not have CT scans performed during the 2-week interval around sample collection. Four subjects were discontinued because samples were collected after receipt of > 3 days of mold-active antifungal therapy. Final analysis was performed with 166 subjects and 169 infectious episodes.

"Possible" IA (n=106) was the most common clinical adjudication in the prospective study and was excluded from the analysis. Thirty-eight (38) subjects were adjudicated as having no invasive fungal infection; these subjects provided 40 urine samples. Twenty-six (26) subjects were adjudicated as having mixed or other (non-IA) infections.

Across the studies, Myco*MEIA* Index values ranged from 0.1 to 45.0. Performance of the assay was estimated as a per-subject sensitivity of 92.4% (95% CI 82.1-97.0%) and per-sample sensitivity of 58.1% (95% 54.2 - 61.9%). The mean Myco*MEIA* Index value for the population with no IA was 0.5. The specificity in the prospective study was 86.1% (95% CI 75.7-92.5%) using the cut-off of 0.6.

	MycoMEIA Positive	MycoMEIA Negative	Sensitivity (95% CI)
Positive Subjects			
Cases, proven and probable IA (N=53)	**49**	**4**	**92.4% (82.1-97.0)**
Cases, proven IA, archived (n=3)	**3**	**0**	**100% (43.8-100)**
Cases, probable IA, archived (n=44)	**41**	**3**	**93.2% (81.8-97.7)**
Cases, probable IA, prospective (n=6)	**5**	**1**	**83.3% (43.6-97.0)**
Positive Samples			
Samples, proven and probable IA (N=636)	**370**	**266**	**58.2% (54.3-61.9)**
Samples, proven IA, archived (n = 72)	**46**	**26**	**63.9% (52.3-74.0)**
Samples, probable IA, archived (n=551)	**318**	**233**	**57.7% (53.5-61.8)**
Samples, probable IA, prospective (n=13)	**6**	**7**	**46.1% (23.2-70.9)**
	MycoMEIA Positive	**MycoMEIA Negative**	**Specificity (95% CI)**
Negative Samples (Prospective Study)			
Prospective (N=65)	**9**	**56**	**86.1% (75.7-92.5)**
No infection (n=39)	**4**	**34**	**89.5% (75.9-95.8)**
Mixed or other infections (n=26)	**5**	**21**	**80.8% (62.1-91.5)**

Clinical Reproducibility

A reproducibility study with clinical samples was conducted at three laboratories. The study utilized blinded urine samples to evaluate lab-to-lab variability of Myco*MEIA*. Results were analyzed qualitatively, by applying established cut-off criteria to define positivity, and quantitatively, to assess reproducibility. Results of the reproducibility study are listed in the table below.

	Site 1	Site 2	Site 3
Samples tested (#)	411	423	475
Mean index (SD)	1.4 (3.5)	1.4 (3.6)	1.5 (4.3)
Index Range	0.1 – 41.0	0.2 – 32.9	0.1 – 45.5
Sensitivity* (95% CI)	90.5% (77.4-97.3%)	86.4% (72.6-94.8%)	92.0% (80.8 - 97.8%)

Specificity* (95% CI)	82.9% (77.2-87.6%)	86.1% (80.8 - 90.4%)	87.6% (82.8-91.4)

*Sensitivity and specificity results using the cut-off of 0.6

High-Dose Hook Effect

No high-dose hook effect was observed with *Aspergillus* antigen ethanol precipitation (EP) concentrations up to 1,000 ng/mL.

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