510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K243496

B Applicant

Pearl Diagnostics, Inc.

C Proprietary and Established Names

Myco*MEIA Aspergillus* Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
NOM	Class I	21 CFR 866.3040 - *Aspergillus* Spp. Serological Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain substantial equivalence determination for the Myco*MEIA Aspergillus* Assay.

B Measurand:

Aspergillus galactofuranose (gal*f*)-containing antigens

C Type of Test:

Enzyme immunoassay (EIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

B Indication(s) for 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.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

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), Plasma-Lyte, Vaginal contraceptive gel, Vagisil cream (benzocaine), and Vagistat 1 cream (ticonazole).

D Special Instrument Requirements:

The assay is for use with the Myco*MEIA* Calculation Worksheet, an Excel spreadsheet to manually enter sample values to determine test results.

IV Device/System Characteristics:

A Device Description:

The Myco*MEIA Aspergillus* Assay (Myco*MEIA*) is an enzyme immunoassay (EIA) that detects *Aspergillus* galactofuranose (gal*f*)-containing antigens in urine. The urine sample is first processed through Sample Processing Columns to eliminate an inhibitor of antibody-antigen recognition. The processed urine samples eluted from the columns are added to the assay plate wells coated with the antibodies and incubated. The bound antigen is then incubated with antibodies linked to horseradish peroxidase (HRP) conjugates. Wash steps between each reaction remove unbound material. Next, the peroxidase substrate solution is added, which reacts with the complexes bound to the well to form a blue color reaction. The enzyme reaction is stopped by the addition of acid, which also changes the blue color to yellow. The absorbance (optical density) of specimens and controls is determined with a spectrophotometer set at 450 and 620 nm wavelengths.

External quality controls are required and provided in the kit, to validate assay results and determine the index values that are calculated to determine test results. The amount of antigen in the clinical sample is determined by optical density (OD) using a spectrophotometer and interpreted as an OD index relative to the mean OD of the threshold control. The index values are determined manually or by entering the test sample and control spectrophotometer readings

into the Myco*MEIA* Calculation Worksheet, an Excel spreadsheet that is provided to users and calculates the index values to determine the qualitative test result, positive or negative. If any of the controls are outside of the acceptable range, the test is invalid. For positive results with index values of ≥ 0.6 to < 0.7 and invalid results, the user is instructed to repeat the testing.

The assay kit includes 96-well microtiter plates with pre-coated mAbs, column washing solution, HRP-conjugated mAbs, conjugate diluent, a peroxidase TMB substrate (chromogen), a stopping solution, and three controls, Negative, Positive and Threshold. 25X Concentrated Wash Solution and Sample Processing Columns are required to perform the assay and are provided separately from the kit.

B Principle of Operation:

The Myco*MEIA Aspergillus* Assay (Myco*MEIA*) is based on a sandwich enzyme immunoassay (EIA) technology. The assay uses two mouse monoclonal antibodies (mAbs) that recognize galactofuranose (gal*f*) epitopes and are coated to 96-well microplates. Both mAbs are used as capture and reporter antibodies that bind the antigen, and the bound antigen is detected with the same two antibodies conjugated to horseradish peroxidase.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Platelia *Aspergillus* EIA Model 62793

B Predicate 510(k) Number(s):
K093678

C Comparison with Predicate(s):

Device & Predicate Device(s):	K243496 (This device)	K093678 (Predicate)
Device Trade Name	Myco*MEIA Aspergillus* Assay	Platelia *Aspergillus* EIA
Intended Use/Indications For 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	The Platelia *Aspergillus* Ag EIA is an immunoenzymatic sandwich microplate assay for the detection of *Aspergillus* galactomannan antigen in adult and pediatric serum and Bronchoalveolar Lavage (BAL) fluid samples. The Platelia *Aspergillus* EIA is a test which, when used in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples and radiographic evidence, can be used as an aid in the diagnosis of Invasive Aspergillosis.

	support the diagnosis of IA.	
General Device Characteristic Similarities		
Test Design	Sandwich antigen capture assay	Same
Test Principle	Enzyme immunoassay (EIA), 96-well plate coated with mAbs	Same
Required Equipment	96-well plate EIA optical density (OD) reader	Same
Quality Controls	Positive, negative and threshold (TC) controls	Positive, negative and cut-off controls
Repeat Testing	Repeat testing for index values of ≥ 0.6 to < 0.7 is recommended	Repeat testing of positive patients recommended
Storage Requirements	2-8°C	Same
General Device Characteristic Differences		
Measurand	Galactofuranose (gal*f*)-containing antigens	Galactomannan antigen
Specimen Type	Urine	Serum and BAL
Sample Preparation	Sample elution through Sample Processing Columns	EDTA pretreatment boil at 120°C and centrifugation
Test Output	Index values calculated by dividing by TC OD and multiplying by multiplication factor. Positive (≥ 0.6), Negative (< 0.6). Repeat testing is recommended for index values of ≥ 0.6 to < 0.7.	Index value calculated by dividing sample OD by Cut-off control OD. Positive results ≥ 0.5.

VI Standards/Guidance Documents Referenced:

Document #	Title
CLSI EP05-A3	Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline —Third Edition
CLSI EP07 3rd Edition	Interference Testing in Clinical Chemistry
CLSI EP12 3rd Edition	Evaluation of Qualitative Binary Output Examination Performance
CLSI EP17-A2	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition
CLSI EP25-A	Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

VII Performance Characteristics (if/when applicable):

Analytical Performance:

1. Precision/Reproducibility:

Precision/reproducibility was conducted according to CLSI EP05-A3[1] to assess the performance of the Myco*MEIA Aspergillus* Assay (Myco*MEIA*).

Multi-Site Precision

A multisite precision study was conducted at three sites (one internal and two external) on a panel of eight samples: three assay controls, one negative sample consisting of pooled human urine, two samples of pooled human urine spiked at 1x and 2x the limit of detection (LoD) with *Aspergillus* antigen, and two positive clinical samples consisting of pooled human urine. Testing was conducted on one lot of the assay and accessories and one set of instruments. Ninety (90) data points were generated for each sample: 3 replicates per run × 2 runs/operators per day × 5 days × 3 sites = 90 data points. Acceptance criteria are ≤ 0.025 SD for negative samples and ≤ 25% CV for positive samples, and results were as expected. Results are summarized in **Table 1** below.

Table 1. Summary Results of Multisite Precision Study for All Sites (N=90)

Sample	Optical Density Precision			Myco*MEIA* Index Precision		
	Mean OD	SD	%CV	Mean MI	SD	%CV
1 Positive Control	1.580	0.213	13	19.0	2.470	13
2 Threshold Control	0.515	0.104	20	6.2	1.077	17
3 Negative Control	0.024	0.011	NA	0.3	0.123	NA
4 Negative Sample	0.031	0.012	NA	0.4	0.138	NA
5 Contrived Low Positive Sample	0.080	0.019	24	1.0	0.210	22
6 Contrived Positive Sample	0.142	0.031	22	1.7	0.318	19
7 Pooled Positive Sample	0.084	0.016	19	1.0	0.186	18
8 Pooled Positive Sample	0.207	0.029	14	2.5	0.297	12

Sample 1= Assay Positive Control, Sample 2= Assay Threshold Control, Sample 3= Assay Negative Control, Sample 4= Pooled Negative Sample, Sample 5= Contrived Low Positive Sample containing 2.5 ng/mL *Aspergillus* antigen (~1x LoD), Sample 6= Contrived Positive Sample containing 5 ng/mL *Aspergillus* antigen (~2x LoD), Sample 7= Pooled Positive Sample, Sample 8= Pooled Positive Sample, SD= Standard Deviation, %CV= Percent Coefficient of Variation.

Repeatability and Within-Laboratory Precision

A single-site precision study was conducted at one internal site on a panel of eight samples including three assay controls, one negative sample consisting of pooled human urine, two samples of pooled human urine spiked at 1x and 2x the limit of detection (LoD) with *Aspergillus* antigen, and two positive clinical samples consisting of pooled human urine. Testing was conducted on three lots of the assay. Eighty (80) data points were generated per lot: 2 replicates per run × 2 runs/operators per day × 20 days = 80 data points for a total of 240 data points. Acceptance criteria are ≤ 0.010 SD for negative samples and ≤ 10% CV for positive samples for Repeatability, and ≤ 0.025 SD for negative samples and ≤ 25% CV for

[1] CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

positive samples for Within-Lab Precision. Results were as expected except for Sample 3 in Lot 2, which was outside the acceptance criteria for both Repeatability and Within-Lab Precision and Sample 5 in Lot 2, which was outside the acceptance criteria for Repeatability. The data are acceptable. Results are summarized in **Tables 2 and 3** below.

Table 2. Optical Density (OD) Values and Repeatability Results by Lot (N=80)

Sample	Lot 1			Lot 2			Lot 3		
	Mean OD	SD	%CV	Mean OD	SD	%CV	Mean OD	SD	%CV
1 PC	1.801	0.0603	3	1.645	0.0447	3	1.909	0.0899	5
2 TC	0.620	0.0547	9	0.493	0.0454	9	0.603	0.0484	8
3 NC	0.018	0.0016	NA	0.025	0.0316	NA	0.019	0.0019	NA
4 Negative Sample	0.025	0.0029	NA	0.024	0.0014	NA	0.022	0.0020	NA
5 Contrived Low Positive Sample	0.080	0.0056	7	0.069	0.0118	17	0.065	0.0066	10
6 Contrived Positive Sample	0.158	0.0131	8	0.128	0.0096	8	0.118	0.0058	5
7 Pooled Positive Sample	0.116	0.0069	6	0.126	0.0081	7	0.135	0.0081	6
8 Pooled Positive Sample	0.256	0.0109	4	0.225	0.0135	7	0.238	0.0095	4

Sample 1= Assay Positive Control, Sample 2= Assay Threshold Control, Sample 3= Assay Negative Control, Sample 4= Pooled Negative Sample, Sample 5= Contrived Low Positive Sample containing 2.5 ng/mL *Aspergillus* antigen (~1x LoD), Sample 6= Contrived Positive Sample containing 5 ng/mL *Aspergillus* antigen (~2x LoD), Sample 7= Pooled Positive Sample, Sample 8= Pooled Positive Sample, SD= Standard Deviation, %CV= Percent Coefficient of Variation.

Table 3. Optical Density (OD) Values and Within-Lab Precision Results by Lot (N=80)

Sample	Lot 1			Lot 2			Lot 3		
	Mean OD	SD	%CV	Mean OD	SD	%CV	Mean OD	SD	%CV
1 PC	1.801	0.1597	9	1.645	0.1143	7	1.909	0.1625	9
2 TC	0.620	0.0965	16	0.493	0.0747	15	0.603	0.0798	13
3 NC	0.018	0.0039	NA	0.025	0.0318	NA	0.019	0.0035	NA
4 Negative Sample	0.025	0.0051	NA	0.024	0.0023	NA	0.022	0.0031	NA
5 Contrived Low Positive Sample	0.080	0.0125	16	0.069	0.0140	20	0.065	0.0085	13
6 Contrived Positive Sample	0.158	0.0214	14	0.128	0.0166	13	0.118	0.0130	11
7 Pooled Positive Sample	0.116	0.0109	9	0.126	0.0137	11	0.135	0.0133	10
8 Pooled Positive Sample	0.256	0.0430	17	0.225	0.0494	22	0.238	0.0382	16

Sample 1= Assay Positive Control, Sample 2= Assay Threshold Control, Sample 3= Assay Negative Control, Sample 4= Pooled Negative Sample, Sample 5= Contrived Low Positive Sample containing 2.5 ng/mL *Aspergillus* antigen (~1x LoD), Sample 6= Contrived Positive Sample containing 5 ng/mL *Aspergillus* antigen (~2x LoD), Sample 7= Pooled Positive Sample, Sample 8= Pooled Positive Sample, SD= Standard Deviation, %CV= Percent Coefficient of Variation.

2. Linearity:
   This study is not applicable as the test device is a qualitative assay.

3. Analytical Specificity/Interference:
   The Myco*MEIA Aspergillus* Assay (Myco*MEIA*) was evaluated for analytical specificity/ cross-reactivity and interference in accordance with CLSI EP07[2].

   1. *Cross-Reactivity and Microbial Interference (Analytical Specificity)*
      The Myco*MEIA* was evaluated for microbial cross-reactivity. Clinical urine samples were obtained from individuals diagnosed with infectious diseases but known to be free of *Aspergillus* infection. Additional samples were contrived from pooled human urine that was negative for *Aspergillus* infection and spiked with other infectious agents. Acceptance criteria are that all samples should yield the expected negative results, with Myco*MEIA* index values < 0.6. **Table 4** shows the results for microbial cross-reactivity from replicate testing. Cross-reacting positive results are shown in red font. Cross-reactivity is observed with Ascomycetes fungi (*Histoplasma*, *Blastomyces*, *Fusarium*). The observed cross-reactivity is noted in the device labeling.

Table 4. Summary Results of Microbial Cross-reactivity Study

Sample Description	Organism / Concentration Tested	Myco*MEIA* Index Value		Interpretation	
		Replicate 1	Replicate 2	Replicate 1	Replicate 2
Clinical urine sample	UTI *Enterococcus* spp., *Enterobacter* lung / blood	0.5	0.5	Negative	Negative
Clinical urine sample	UTI *Streptococcus* spp.	0.3	0.3	Negative	Negative
Clinical urine sample	UTI *Staphylococcus* spp.	0.3	0.4	Negative	Negative
Clinical urine sample	*Klebsiella* lung / blood	0.3	0.2	Negative	Negative
Clinical urine sample	*E. coli* lung / blood	0.3	0.3	Negative	Negative
Clinical urine sample	Possible IA + UTI *Enterococcus*, *Enterococcus* lung / blood	0.6*	0.6*	Positive*	Positive*
Clinical urine sample	*Enterobacter* lung / blood, *Streptococcus* lung / blood	0.2	0.2	Negative	Negative
Clinical urine sample	*Streptococcus* lung / blood	0.3	0.2	Negative	Negative
Clinical urine sample	UTI Mixed gram positive	0.3	0.2	Negative	Negative
Clinical urine sample	UTI *Staphylococcus* spp.	0.5	0.5	Negative	Negative
Clinical urine sample	UTI *Streptococcus* spp.	3.9*	3.8*	Positive*	Positive*
Clinical urine sample	*Acinetobacter junii* lung / blood, URI – Rhinovirus, Parainfluenzavirus	0.2	0.3	Negative	Negative
Clinical urine sample	*Streptococcus* lung / blood	0.4	0.4	Negative	Negative
Clinical urine sample	MSSA, *Staphylococcus* lung / blood; URI – Influenza	0.3	0.3	Negative	Negative
Clinical urine sample	*Staphylococcus* lung / blood	0.2	0.3	Negative	Negative
Clinical urine sample	*P. aeruginosa* lung / blood	0.3	0.3	Negative	Negative
Clinical urine sample	MRSA, *Staphylococcus* lung / blood	0.2	0.3	Negative	Negative
Clinical urine sample	*Candida krusei*	0.2	0.2	Negative	Negative
Clinical urine sample	*Candida krusei*	0.2	0.2	Negative	Negative
Clinical urine sample	MRSA, *Staphylococcus* lung / blood	0.2	0.2	Negative	Negative
Clinical urine sample	URI – Rhinovirus, Parainfluenzavirus	0.2	0.3	Negative	Negative
Clinical urine sample	*E. coli* lung / blood	0.1	0.1	Negative	Negative

[2] CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

Sample Description	Organism / Concentration Tested	Myco*MEIA* Index Value		Interpretation	
		Replicate 1	Replicate 2	Replicate 1	Replicate 2
Clinical urine sample	Other IFI lung / blood	0.2	0.2	Negative	Negative
Clinical urine sample	MRSA, *Staphylococcus* lung / blood	0.3	0.3	Negative	Negative
Clinical urine sample	MRSA, *Staphylococcus* lung / blood	0.3	0.5	Negative	Negative
Clinical urine sample	Histoplasmosis	0.2	0.2	Negative	Negative
Clinical urine sample	*Candida albicans* (candidiasis)	0.2	0.2	Negative	Negative
Clinical urine sample	*Pneumocystis jirovecii* (pneumonia)	0.2	0.3	Negative	Negative
Clinical urine sample	Other IFI lung / blood (cryptococcosis)	0.5	0.5	Negative	Negative
Clinical urine sample	Histoplasmosis	0.3	0.3	Negative	Negative
Clinical urine sample	Histoplasmosis	0.3	0.3	Negative	Negative
Clinical urine sample	Histoplasmosis	0.3	0.3	Negative	Negative
Clinical urine sample	Histoplasmosis	0.5	0.4	Negative	Negative
Clinical urine sample	*Pneumocystis jirovecii* (pneumonia)	0.3	0.3	Negative	Negative
Clinical urine sample	Candidiasis	0.2	0.2	Negative	Negative
Clinical urine sample	*Staphylococcus* lung / blood	0.3	0.3	Negative	Negative
Clinical urine sample	*P. aeruginosa* lung / blood	0.2	0.2	Negative	Negative
Clinical urine sample	*Pneumocystis jirovecii* (pneumonia)	0.3	0.3	Negative	Negative
Clinical urine sample	MRSA, *Stenotrophomonas* lung / blood	0.4	0.4	Negative	Negative
Clinical urine sample	*P. aeruginosa* lung / blood	0.4	0.3	Negative	Negative
Clinical urine sample	Fusariosis lung / blood	0.2	0.2	Negative	Negative
Clinical urine sample	*P. aeruginosa* lung / blood	0.2	0.3	Negative	Negative
Pooled, spiked urine	*Acinetobacter baumanii* 4.00×10^5 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Bacteroides fragilis* 1.01×10^6 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Candida albicans* 1.73×10^6 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Candida glabrata* 1.91×10^6 CFU/mL	0.3	0.2	Negative	Negative
Pooled, spiked urine	*Candida parapsilosis* 3.06×10^6 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Candida tropicalis* 1.57×10^6 CFU/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	*Chlamydia trachomatis* 7.00×10^5 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Citrobacter freundii* 6.70×10^5 CFU/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	*Clostridia* 5.28×10^5 CFU/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	*Corynebacterium amycolatum* 2.80×10^5 CFU/mL	0.1	0.2	Negative	Negative
Pooled, spiked urine	*Cryptococcus neoformans* 1.25×10^6 CFU/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	*Enterobacter cloacae* 1.65×10^6 CFU/mL	0.1	0.2	Negative	Negative
Pooled, spiked urine	*Enterococcus faecalis* 1.40×10^6 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Escherichia coli* 1.15×10^6 CFU/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	*Geotrichum* 2.80×10^5 CFU/mL	0.2	0.3	Negative	Negative
Pooled, spiked urine	*Klebsiella pneumoniae* 6.90×10^5 CFU/mL	0.2	0.3	Negative	Negative
Pooled, spiked urine	*Lactobacillus crispatus* 2.80×10^5 CFU/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	*Neisseria gonorrhoeae* 3.90×10^5 CFU/mL	0.4	0.2	Negative	Negative
Pooled, spiked urine	Parainfluenza virus Type 1 1.00×10^6 virus/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	Parainfluenza virus Type 2 5.00×10^5 virus/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	Parainfluenza virus Type 3	0.2	0.2	Negative	Negative

Sample Description	Organism / Concentration Tested	Myco*MEIA* Index Value		Interpretation	
		Replicate 1	Replicate 2	Replicate 1	Replicate 2
	1.00 x 10^6 virus/mL				
Pooled, spiked urine	*Peptostreptococci* 6.84 x 10^5 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Prevotella bivia* 1.84 x 10^6 CFU/mL	0.2	0.1	Negative	Negative
Pooled, spiked urine	*Proteus mirabilis* 1.35 x 10^6 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Pseudomonas aeruginosa* 1.10 x 10^6 CFU/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	Rhinovirus 2 2.00 x 10^6 virus/mL	0.1	0.1	Negative	Negative
Pooled, spiked urine	Rhinovirus 83 4.50 x 10^5 virus/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	*Serratia marcescens* 1.10 x 10^6 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Staphylococcus aureus* 1.37 x 10^6 CFU/mL	0.3	0.3	Negative	Negative
Pooled, spiked urine	*Staphylococcus epidermidis* 5.80 x 10^5 CFU/mL	0.1	0.2	Negative	Negative
Pooled, spiked urine	*Staphylococcus saprophyticus subsp. saprophyticus* 2.60 x 10^5 CFU/mL	0.2	0.2	Negative	Negative
Pooled, spiked urine	*Stenotrophomonas maltophilia* 2.00 x 10^6 CFU/mL	0.2	0.3	Negative	Negative
Pooled, spiked urine	*Streptococcus agalactiae* 5.80 x 10^5 CFU/mL	0.2	0.3	Negative	Negative
Cross-reactants for which cross-reactivity was observed					
Clinical urine sample	URI – Rhinovirus, Parainfluenzavirus	0.6	0.5	Positive	Negative
Clinical urine sample	Streptococcus lung / blood	0.6	0.67	Positive	Positive
Clinical urine sample	Histoplasmosis	18.3	18.9	Positive	Positive
Clinical urine sample	Blastomyces lung / blood	0.6	0.6	Positive	Positive
Clinical urine sample	Candidiasis	0.6	0.6	Positive	Positive
Pooled, spiked urine	*Fusarium* 8.80 x 10^5 CFU/mL	1.0	1.0	Positive	Positive

* Two clinical samples were identified as possible invasive aspergillosis.

Microbial interference was also evaluated. Samples were prepared using pooled negative urine that was spiked with *Aspergillus* to a final concentration of 8 ng/mL (prepared from a stock of ethanol precipitate (EP) of whole organism) and spiked with other infectious agents. Acceptance criteria are that all samples should yield the expected positive results, with Myco*MEIA* index values ≥ 0.6. No microbial interference was observed.

2. *Endogenous/Exogenous Interference and Cross-reactivity*

An interfering substances study was conducted to assess the performance of the Myco*MEIA* in the presence of potentially interfering substances. Samples were contrived from pooled human urine that was negative for *Aspergillus* and spiked with endogenous and exogenous materials. In addition, clinical urine samples were obtained from individuals with other disorders but known to be free of *Aspergillus* infection. Both cross-reactivity and interference were evaluated in the presence or absence of *Aspergillus* antigen. Acceptance criteria are that all samples should yield the expected results. Negative samples should yield Myco*MEIA* index values of < 0.6, which is the expected result for samples where *Aspergillus* antigen is absent. Positive samples should yield index values of ≥ 0.6 which is the expected result for samples where *Aspergillus* antigen is present. **Table 5** lists the interferents and concentrations for which no cross-reactivity or interference was observed.

Table 5. Summary Results of Interfering Substances Testing

Potential Interferent	Concentration Tested	*Aspergillus* Ag Added	MycoMEIA Index Value		Interpretation	
			Replicate 1	Replicate 2	Replicate 1	Replicate 2
1-Methylnicotinamide (Vitamin B3 metabolite)	0.130 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.8	1.5	Positive	Positive
Acetaminophen	2.5 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	3.0	2.7	Positive	Positive
Acetone (Ketone)	2.4 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.9	1.9	Positive	Positive
Albumin	10 mg/mL	No	0.2	0.2	Negative	Negative
		Yes	1.7	1.7	Positive	Positive
Amoxicillin	1.59 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.9	2.1	Positive	Positive
Amphotericin B	0.22 mg/mL	No	0.4	0.3	Negative	Negative
		Yes	3.0	3.0	Positive	Positive
Ascorbic acid 1 (Vitamin C)	0.5 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.5	1.5	Positive	Positive
Ascorbic acid 2 (Vitamin C)	1 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.6	1.8	Positive	Positive
Azithromycin	0.15 mg/mL	No	0.4	0.5	Negative	Negative
		Yes	1.7	1.8	Positive	Positive
Biotin (Vitamin B)	0.4 µg/mL	No	0.4	0.3	Negative	Negative
		Yes	1.6	1.7	Positive	Positive
Boric Acid	7.89 mg/mL	No	0.4	0.3	Negative	Negative
		Yes	2.0	1.8	Positive	Positive
CD14 (contrived for RA)	3 µg/mL	No	0.2	0.2	Negative	Negative
		Yes	2.0	1.8	Positive	Positive
Cimetidine	0.9 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.8	1.6	Positive	Positive
Ciprofloxacin	1.2 mg/mL	No	0.4	0.4	Negative	Negative
		Yes	2.3	2.0	Positive	Positive
Erythromycin	0.675 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	6.5	7.0	Positive	Positive
Ethanol	30 mg/mL	No	0.5	0.5	Negative	Negative
		Yes	2.3	2.4	Positive	Positive
Ganciclovir hydrate	0.9 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	2.0	2.0	Positive	Positive
Glucose	6 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.5	1.5	Positive	Positive
Human chorionic gonadotropin (HCG)	0.3 µg/mL	No	0.2	0.2	Negative	Negative
		Yes	1.7	1.8	Positive	Positive
Ibuprofen	0.27 mg/mL	No	0.4	0.3	Negative	Negative
		Yes	2.4	2.3	Positive	Positive
Immunoglobulin G (contrived for hypergammaglobulinemia)	2 mg/mL	No	0.4	0.4	Negative	Negative
		Yes	1.4	1.4	Positive	Positive
Itraconazole	0.22 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	2.1	2.2	Positive	Positive
Metronidazole	1 mg/mL	No	0.2	0.3	Negative	Negative
		Yes	2.0	2.0	Positive	Positive
Mucin	5% w/v	No	0.4	0.4	Negative	Negative
		Yes	0.7	0.7	Positive	Positive
Mucin-5B	0.1% w/v	No	0.3	0.3	Negative	Negative
		Yes	2.4	2.7	Positive	Positive
Naproxen sodium	0.02 mg/mL	No	0.4	0.3	Negative	Negative
		Yes	2.0	1.9	Positive	Positive
Nicotine (contrived for tobacco)	0.015 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	2.4	2.4	Positive	Positive
Orosomucoid 2 (contrived for RA)	0.2 µg/mL	No	0.3	0.2	Negative	Negative
		Yes	2.1	2.2	Positive	Positive
Oxalic acid 1 (contrived for	0.1 mg/mL	No	0.3	0.2	Negative	Negative

Potential Interferent	Concentration Tested	*Aspergillus* Ag Added	Myco*MEIA* Index Value		Interpretation	
			Replicate 1	Replicate 2	Replicate 1	Replicate 2
spinach)		Yes	1.4	1.3	Positive	Positive
Oxalic acid 2 (contrived for spinach)	1 mg/mL	No	0.2	0.2	Negative	Negative
		Yes	1.4	1.6	Positive	Positive
Penicillin G sodium salt	3.2 mg/mL	No	0.3	0.2	Negative	Negative
		Yes	1.4	1.5	Positive	Positive
Phenazopyridine HCl (Pyridium)	1.2 µg/mL	No	0.2	0.2	Negative	Negative
		Yes	1.7	1.5	Positive	Positive
Phylloquinone (Vitamin K1)	0.05 µg/mL	No	0.3	0.3	Negative	Negative
		Yes	2.1	2.0	Positive	Positive
Piperacillin sodium salt	19 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	2.2	2.8	Positive	Positive
Potassium clavulanate (contrived for Augmentin)	0.65 mg/mL	No	0.2	0.2	Negative	Negative
		Yes	1.6	1.6	Positive	Positive
RBC (Red Blood Cells / Erythrocytes)	2% v/v	No	0.5	0.5	Negative	Negative
		Yes	2.7	3.2	Positive	Positive
Riboflavin (Vitamin B2)	0.24 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	2.3	2.2	Positive	Positive
Rifampicin	10 mg/mL	No	0.4	0.3	Negative	Negative
		Yes	2.1	1.8	Positive	Positive
Sodium salicylate (metabolite of acetylsalicylic acid)	4.73 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.4	1.4	Positive	Positive
Tazobactam sodium salt	17 mg/mL	No	0.4	0.4	Negative	Negative
		Yes	2.2	1.8	Positive	Positive
Triglycerides 1	1 mg/mL	No	0.2	0.2	Negative	Negative
		Yes	1.5	1.6	Positive	Positive
Triglycerides 2	0.3 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.4	1.3	Positive	Positive
Urobilinogen	0.06 mg/mL	No	0.2	0.2	Negative	Negative
		Yes	1.4	1.7	Positive	Positive
Vancomycin HCl	3.1 mg/mL	No	0.4	0.3	Negative	Negative
		Yes	1.8	1.4	Positive	Positive
Voriconazole N-oxide	0.022 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	2.4	2.4	Positive	Positive
Vanillylmandelic acid	0.03 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	1.8	1.8	Positive	Positive
Water-based personal lubricant	50 mg/mL	No	0.4	0.3	Negative	Negative
		Yes	1.6	1.4	Positive	Positive
WBC (White Blood Cells / Leukocytes)	300,000 cells/mL	No	0.2	0.3	Negative	Negative
		Yes	1.4	1.4	Positive	Positive
α1-Acid glycoprotein (contrived for RA)	0.6 µg/mL	No	0.2	0.2	Negative	Negative
		Yes	2.2	2.0	Positive	Positive
α-CEHC (metabolite of Vitamin E)	5 µg/mL	No	0.3	0.3	Negative	Negative
		Yes	2.0	2.0	Positive	Positive
β-carotene (parent compound of Vitamin A)	3 mg/mL	No	0.3	0.3	Negative	Negative
		Yes	2.1	2.1	Positive	Positive
High pH urine	Contrived by adding NaOH to normal urine (pH 10.01)	No	0.2	0.2	Negative	Negative
		Yes	1.4	1.5	Positive	Positive
Low pH urine	Contrived by adding HCl to normal urine (pH 3.82)	No	0.2	0.2	Negative	Negative
		Yes	1.7	1.8	Positive	Positive
Clinical urine	Protein 30 mg/dL, pH 8.0, Hemolyzed blood 200 Ery/µL, Ketones > 160mg /dL, Bilirubin +++, Glucose 250mg/dL	No	0.4	0.3	Negative	Negative
		Yes	1.6	1.5	Positive	Positive
Clinical urine	pH 7.51 Bilirubin +++	No	0.4	0.4	Negative	Negative
		Yes	1.9	1.8	Positive	Positive
Clinical urine	pH 5.16	No	0.4	0.4	Negative	Negative
		Yes	2.3	2.6	Positive	Positive
Clinical urine	Protein 100 mg/dL	No	0.2	0.2	Negative	Negative

Potential Interferent	Concentration Tested	*Aspergillus* Ag Added	Myco*MEIA* Index Value		Interpretation	
			Replicate 1	Replicate 2	Replicate 1	Replicate 2
		Yes	1.3	1.3	Positive	Positive
Clinical urine	Ketones 80 mg/dL, Bilirubin ++, Glucose 250 mg/dL	No	0.2	0.2	Negative	Negative
		Yes	1.4	1.8	Positive	Positive
Clinical urine	N/A	No	0.4	0.4	Negative	Negative
		Yes	6.9	6.6	Positive	Positive
Clinical urine	N/A	No	0.2	0.2	Negative	Negative
		Yes	1.7	2.1	Positive	Positive

Potential cross-reacting interferents that gave positive results in the absence of *Aspergillus* antigen at the initial concentrations tested were further investigated, and additional testing determined that the assay yields the expected negative result when the substance was tested at lower concentrations. Results are shown in **Table 6.** Results for concentrations that still show cross-reactivity at lower dilutions are noted in red font indicating the expected negative result was not obtained. The observed interference at high concentrations is noted in the device labeling.

Table 6. Results of Additional Interfering Substances Testing

Potential Interferent / Sample	Concentration Tested	Mean Myco*MEIA* Index Value	Interpretation
Betanin	0.00% w/v	0.2	Negative
	0.05 mg/mL	0.2	Negative
	0.25% w/v	1.1	Positive
	0.50% w/v	2.0	Positive
	0.75% w/v	2.9	Positive
	1.00% w/v	4.0	Positive
Bilirubin	0.00 mg/mL	0.2	Negative
	0.03 mg/mL	0.2	Negative
	0.06 mg/mL	0.6	Positive
	0.09 mg/mL	0.8	Positive
	0.12 mg/mL	1.1	Positive
Caffeine (in urine)	0.00 mg/mL	0.2	Negative
	0.16 mg/mL	0.2	Negative
	3.375 mg/mL	0.5	Negative
	6.750 mg/mL	0.8	Positive
	10.125 mg/mL	1.3	Positive
	13.50 mg/mL	1.7	Positive
Gyne-Lotrimin cream (clotrimazole)	0.00% w/v	0.2	Negative
	0.14% w/v	0.2	Negative
	1.25% w/v	0.5	Negative
	2.50% w/v	0.8	Positive
	3.75% w/v	1.3	Positive
	5.00% w/v	1.9	Positive
Hemoglobin	0.00 mg/mL	0.2	Negative
	0.15 mg/mL	0.4	Negative
	1.05 mg/mL	0.4	Negative
	2.10 mg/mL	0.5	Negative
	3.15 mg/mL	0.5	Negative
	4.20 mg/mL	0.7	Positive
Lotrimin Ultra cream (butenifine)	0.00% w/v	0.3	Negative
	0.14% w/v	0.2	Negative
	1.25% w/v	0.8	Positive
	2.50% w/v	0.9	Positive
	3.75% w/v	1.3	Positive
	5.00% w/v	1.8	Positive
Monistat 7 cream (miconazole)	0.00% w/v	0.3	Negative
	0.14% w/v	0.2	Negative

Potential Interferent / Sample	Concentration Tested	Mean Myco*MEIA* Index Value	Interpretation
	1.25% w/v	0.7	Positive
	2.50% w/v	1.0	Positive
	3.75% w/v	1.3	Positive
	5.00% w/v	1.9	Positive
Nitrites	0.00 mg/mL	0.3	Negative
	0.04 mg/mL	0.4	Negative
	0.0750 mg/mL	0.8	Positive
	0.1125 mg/mL	1.0	Positive
	0.1500 mg/mL	1.3	Positive
Plasma-lyte A	0.0% v/v	0.3	Negative
	7.5% v/v	0.5	Negative
	10% v/v	0.7	Positive
	22.5% v/v	2.3	Positive
	45.0% v/v	5.4	Positive
	67.5% v/v	9.3	Positive
	90.0% v/v	14.0	Positive
Vaginal contraceptive gel	0.00% w/v	0.3	Negative
	0.14% w/v	0.2	Negative
	1.25% w/v	0.9	Positive
	2.50% w/v	1.5	Positive
	3.75% w/v	1.5	Positive
	5.00% w/v	2.1	Positive
Vagisil cream (benzocaine)	0.00% w/v	0.2	Negative
	0.14% w/v	0.2	Negative
	1.25% w/v	0.6	Positive
	2.50% w/v	1.1	Positive
	3.75% w/v	2.1	Positive
	5.00% w/v	2.8	Positive
Vagistat 1 cream (ticonazole)	0.00% w/v	0.3	Negative
	0.14% w/v	0.2	Negative
	1.25% w/v	1.0	Positive
	2.50% w/v	1.8	Positive
	3.75% w/v	3.3	Positive
	5.00% w/v	5.0	Positive
Clinical urine (High Nitrites)	NA	0.2	Negative

3. High-dose Hook Effect

A high-dose hook effect study was conducted to evaluate if a hook effect occurs for the Myco*MEIA Aspergillus* Assay (Myco*MEIA*) when high levels of the target analyte are present in the test sample. Samples were prepared from pooled human urine spiked with *Aspergillus* antigen (Ethanol Precipitate (EP)) at 0 ng/mL, 125 ng/mL, 250 ng/mL, 500 ng/mL, and 1000 ng/mL; the LoD for the Myco*MEIA* is 3 ng/mL. No high-dose hook effect was observed.

4. Assay Reportable Range:

This study is not applicable as the test device is a qualitative assay.

5. Traceability, Stability and Expected Values (Controls, Calibrators, or Methods):

Sample Stability

Sample stability studies were conducted to demonstrate the stability of urine after refrigerated storage and after freezing and serial freeze-thaw cycles. One hundred twenty-four (124) hospitalized subjects with suspected aspergillosis were consented to provide urine,

and 148 fresh samples were prospectively collected, aliquoted and tested in duplicate. Urines received in the clinical lab were tested fresh, within 6 hours of receipt, refrigerated for 5 days, and retested to compare results with that of fresh samples. Of 129 evaluable samples from 104 subjects that underwent paired testing of refrigerated storage over 5 days, 118 gave concordant positive or negative test results (118/129, 91%). From these samples, 13 positive samples (confirmed by Myco*MEIA* testing) were frozen at -70°C and underwent 5 freeze-thaw cycles. All remained positive through each freeze-thaw cycle (13/13, 100%). Additional testing was performed with 2 positive samples and 10 negative samples each from the different urine collection methods (clean-catch, catheter-collected, and non-sterile collection devices (e.g., bedpans, urine hats)). Samples were assessed at timepoints after 6 days of refrigeration followed by 4 freeze-thaw cycles. All samples showed ≥ 90% agreement compared to Day 0 results. These results demonstrate that freezing does not impact results and supports the use of refrigerated and frozen samples in analytical and clinical validations.

Quality Controls

The Myco*MEIA Aspergillus* Assay (Myco*MEIA*) contains three external quality controls, Negative, Positive and Threshold Control. The Threshold Control (TC) mean optical density (OD) is calculated and should fall between 0.331 and 0.770. A TC mean OD outside the range defined above indicates that the assay results are invalid, and the user is instructed to repeat the assay. The TC determines the Index calculation used to normalize the assay results by utilizing a multiplication factor based on the TC mean OD values.

Multiplication Factor	Mean Threshold OD
5	0.331 – 0.440
6	1.441 – 0.550
7	0.551 – 0.660
8	0.661 – 0.770

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

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

The Myco*MEIA* Index value of the Negative Control must be less than 0.6. The Myco*MEIA* Index of the Positive Control must be greater than 10.0. Negative and Positive Controls outside of the boundaries defined above indicate that the assay results are invalid, and the user is instructed to repeat the assay. Failure of any of the controls to meet the validity criteria described above upon repeat testing renders the assay invalid, and patient specimen results should not be reported. The controls were run as part of the analytical and clinical validations according to the Instructions for Use.

6. Detection Limit:

A study was conducted according to the CLSI EP17-A2[3] to evaluate the limit of blank (LoB) and limit of detection (LoD) of the Myco*MEIA Aspergillus* Assay (Myco*MEIA*).

[3] CLSI. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition. CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

LoB

LoB refers to the highest measurement result that is expected to be observed for a blank sample containing no analyte with a probability of 95%. LoB was confirmed by testing 30 clinical samples known to be free of *Aspergillus* infection (by prior testing with Myco*MEIA* and by clinical determination). Samples were pre-tested, aliquoted into single-use aliquots, and frozen. Each sample was tested on two different days with each of three assay lots, generating 60 results for each assay lot. Results were interpreted independently per assay lot and judged based on positive or negative clinical interpretations according to the Myco*MEIA* Index (Negative: Myco*MEIA* Index < 0.6, Positive: ≥ 0.6). One sample (Sample 26, Lot 2, Day 1) gave a false positive result. The LoB of 0 is confirmed.

LoD

LoD refers to the lowest concentration of target analyte that can be detected with a probability of 95%. A 7-dilution series contrived of pooled urine spiked with *Aspergillus* antigen (Ethanol Precipitate (EP)) were used for the testing. Dilutions were tested with three lots of Myco*MEIA* over 5 days. Each day of testing, 4 replicates of each dilution were tested generating a total of 20 results for each sample dilution with each assay lot per day. Results were interpreted independently per assay lot and judged based on positive or negative clinical interpretations according to the Myco*MEIA* Index (Negative: Myco*MEIA* Index < 0.6, Positive: ≥ 0.6). LoD was verified using the Probit approach with an assigned Type II (β) error of 0.05 (5%). The maximum experimentally determined LoD is 2.869 ng/mL; the claimed LoD is 3 ng/mL.

7. Assay Cut-Off:

   An Myco*MEIA* Index result assay cut-off of 0.6 for positivity was established by the receiver operating characteristic (ROC) curve based on the results of urine samples obtained from a cohort of 21 cases with proven or probable Invasive Aspergillosis (IA) and 161 controls with no infectious diagnosis. The ROC AUC of 0.96 (95% CI 0.92–1.00) demonstrated good performance and predicted an optimal index result cutoff of 0.6 to define positivity, where per subject sensitivity of 90.48% (95% CI 71.09–98.31%) and specificity 92.55% (95% CI 87.42–95.02%) were determined at index cutoff of 0.6. Sensitivity of approximately 85.71% (95% CI 65.36–95.02%) and specificity 96.27% (95% CI 92.11–98.28%) at index cut-off of 0.8 was also observed. Index results ranging from ≥ 0.6 to < 0.7 will be recommended to be retested.

Comparison Studies:

1. Method Comparison with Predicate Device:

   Please refer to Section VI.C (Clinical Studies) below for the clinical validation, regarding the method comparison studies.

2. Matrix Comparison:

   A matrix equivalency study was conducted according to CLSI EP35[4] to evaluate equivalence of the Myco*MEIA Aspergillus* Assay (Myco*MEIA*) with the different types of urine specimen sources in the clinical study. Negative matrices were prepared from pooled human urine

[4] CLSI. Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures. 1st ed. CLSI guideline EP35. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.

obtained from the different specimen sources (clean-catch, catheter-collected, and non-sterile collection devices (e.g., bedpans, urine hats)) and served as negative samples (0x LoD). Samples were prepared by spiking negative matrix with *Aspergillus* antigen (Ethanol Precipitate (EP)) at high negative (0.3x LoD), low positive (1x LoD), and positive (3x LoD) analyte levels. Acceptance criteria are that all samples should yield the expected results, with ≥ 95% agreement (PPA and NPA) of catheter and dirty collection methods compared to clean-catch. Results showed 100% agreement of samples.

Clinical Studies:

The clinical performance of the Myco*MEIA Aspergillus* Assay (Myco*MEIA*) was evaluated in a multi-site clinical evaluation.

Study Sites
Archived, retrospective samples were sourced from patients enrolled in three separate clinical studies at a total of four geographically diverse locations in the United States and one in Belgium. Fresh, prospective samples were obtained and tested from one study site.

Study 1: Sites 1, 2 and 3 (Retrospective, Archived). Urine samples from patients with hematological malignancies at risk for invasive aspergillosis (IA) were collected from adults (> 18 years) at three, geographically diverse locations within the United States. Patients were enrolled and samples were collected into sterile containers and aliquoted into cryovials within 12 hours of collection and stored frozen at -70°C. Samples were collected twice weekly, providing multiple samples per subject. Clinical data were collected, and independent clinical reviewers adjudicated cases as proven IA, probable IA, possible IA, or no infection.

Study 2: Site 4 (Retrospective, Archived). Urine samples were collected from adults being treated for hematological malignancies at one hospital in Belgium. These samples were aliquoted and stored frozen. Twice weekly serial samples were collected, and clinical data were evaluated to adjudicate diagnoses, by independent clinical reviewers.

Study 3: Site 5 (Retrospective, Archived, Prospective). Urine samples were collected from adults and children with suspected and confirmed invasive fungal disease (IFD) and multiple underlying immunosuppressive conditions, including hematological malignancies, organ transplantation (excluding lung transplantation) and receipt of immunosuppressive biologic therapy for autoimmune conditions at one site within the US. This study was performed in two stages:

- Stage 1: Prospective Collection and Retrospective Testing. Patients suspected of having an IFD were consented for collection of urine samples and clinical data. Consenting subjects at high-risk for IFD with hematologic malignancies and/or allogeneic blood or marrow transplantation (BMT) had urine samples collected twice weekly. Urine samples were collected in sterile containers, frozen immediately after collection at -70°C. Samples were stored frozen in aliquots. Clinical data were collected, and an independent clinical reviewer adjudicated cases as proven IA, probable IA, possible IA, or no infection.
- Stage 2: Prospective Collection and Testing. Samples were collected as part of the Myco*MEIA* fresh, prospective testing to evaluate specificity. Subjects recognized as having suspected IA were identified after lab receipt of clinical specimens (e.g., serum or

BAL) for BioRad Platelia galactomannan (GM) enzyme immunoassay (EIA) testing, and urine was collected from those who provided consent. An independent clinical reviewer adjudicated cases as proven IA, probable IA, possible IA, or no infection.

Enrollment Criteria

Selection and Inclusion: Subjects were selected who were at high risk of IA due to hematologic malignancies, and/or receipt of allogenic BMT, solid organ transplant (kidney, heart, liver; excluding lung), having current or recent neutropenia (e.g., due to cytotoxic therapy), or immunosuppressed people with suspected IA (e.g., those given immunosuppressants for autoimmune disease, inherited severe immunodeficiency or another underlying disease).

Subjects were inpatients that had at least two weeks of clinical follow-up to determine clinical diagnosis, with at least four urine samples obtained during the clinical evaluation period or at least one sample obtained within one week of diagnosis.

Exclusion:
1. Lung transplant subjects were excluded.
2. Subjects were excluded if radiographic evaluation was not available.
3. Subjects who had received more than three days of exposure to mold-active antifungal therapy at the time of collection were excluded, unless there was mycologic and clinical evidence of antifungal failure (e.g., radiographic and microbiologic test positivity).

Subjects and Samples (Archived Samples)
In some cases, subjects provided more than one sample over time for analysis. A total of 475 samples were collected from 290 different subjects: 226 samples were tested from 50 subjects with IA that was proven (n = 3) or probable (n = 47) per 2020 EORTC/MSG consensus definitions. Most subjects had received therapy for hematologic malignancies or an allogeneic BMT and were neutropenic, with or without other biologic immunosuppression. Three subjects (6.0%) had proven IA, with a biopsy of the lung (n=1), skin (n=1), or bone (n=1). Positive subject demographics, including clinical characteristics and IA diagnostic evidence, are shown in **Table 7**.

Table 7. Demographic Distribution of Positive Subjects (Retrospective, Archived)

	Number (%)
Age, mean (range)	53.4 years (14-74 years)
Gender, male (%)[1]	27 (55.1)
Underlying risk for IA	
Hematologic malignancy, no BMT[2]	27 (54.0)
Allogeneic BMT	19 (38.0)
Solid organ transplant[3]	2 (4.0)
Other[4]	2 (4.0)
Biologic immunosuppression[5]	
Neutropenia	24 (48.0)
Corticosteroids or other immunosuppression	15 (30.0)
Both neutropenia and other immunosuppression	11 (22.0)
Infection diagnosis	
Probable IA	47 (94.0)
Proven IA	3 (6.0)

Microbiologic evidence[6]	
GM EIA +, BAL	29 (58.0)
GM EIA +, serum	25 (50.0)
GM EIA +, both BAL & serum	8 (16.0)
***Aspergillus* culture positive**	10 (20.0)

[1] Gender was not available for one subject.
[2] Diagnoses included acute myelogenous leukemia or myelodysplastic syndrome (n=20), acute lymphocytic leukemia (n=3), chronic myelomonocytic leukemia (n=1), lymphoma (n=1), hematologic malignancy NOS (n=2).
[3] Solid organ transplants included heart (n=1) and kidney + liver (n=1).
[4] Diagnoses included severe malnutrition (n=1) and autoimmune disease (n=1).
[5] Of 49 people. 2 people who had received allogeneic BMT and 1 person with severe malnutrition were not listed as currently receiving biologic immunosuppression.
[6] **Percentage is based on 50 cases. Multiple diagnostics can be positive in subjects.** Culture positivity included 7 BAL *A. fumigatus*, 1 sputum *A. fumigatus*, 1 sputum *A. ustus* and 1 bone biopsy *A. fumigatus*.

Subjects and Samples (Prospective Study)

A prospective study was performed (Study 3, Site 5, Stage 2 as described above) to analyze specificity of the assay using fresh samples. All samples were tested within 6 hours of collection. A total of 210 subjects were consented and provide 254 urine samples for testing. Clinical adjudication was performed by an independent reviewer who was blinded to Myco*MEIA* test results based on pre-established acceptance criteria, i.e., per 2020 EORTC/MSG consensus definitions. Thirty-four (34) subjects had lung transplants and were excluded from analysis. One sample was excluded due to likely contamination prior to testing. Three subjects that did not have CT scans performed were excluded. Four subjects were discontinued because samples were collected after receipt of > 3 days of mold-active antifungal therapy. Six (6) subjects (13 samples) with probable IA were obtained. Of the enrolled negative cohort, 171 samples from 168 subjects were evaluable, 39 no IA, 102 possible IA, and 23 with mixed or other (non-IA) infections. Negative subject demographics are shown in **Table 8** below.

Table 8. Demographic Distribution of Negative Subjects (Prospective)

	Number (%)
Age, median (range)	64 years (5-89 years)
Gender, male (%)	112 (66.7)
Underlying risk for IA[1]	
Hematologic malignancy	132 (78.6)
Solid organ transplant (SOT)	28 (16.7)
Solid tumor	18 (10.7)
Other	8 (4.7)
Biologic immunosuppression	
Neutropenia	54 (32.1)
Corticosteroids	39 (23.2)
Infection diagnosis[2]	
Possible IA	102 (59.6)
No infection	39 (22.8)
Proven or Probable IA (Cases)	7 (4.1)
Mixed or other infections	23 (13.4)
Microbiologic evidence of IA[3]	
GM EIA +, BAL	4 (14.8)
GM EIA +, serum	3 (1.5)
Aspergillus culture positive	0
β-D glucan serum	14 (7.4)

[1] Of 168 subjects. 8 had multiple risks: hematologic malignancy + solid tumor (n=8); hematologic malignancy + HIV/AIDS (n=2); hematologic malignancy + kidney transplant (n=2); hematologic malignancy + solid tumor + rheumatologic disease (n=2); rheumatologic disease + kidney transplant (n=1); hematologic malignancy + liver transplant (n=1); hematologic malignancy + rheumatologic disease (n=1); solid tumor + rheumatologic disease (n=1). SOTs include liver (n=14), kidney (n=7), heart (n=2) and kidney + liver (n=3), kidney + heart (n=1), kidney + pancreas (n=1). Other include rheumatologic or connective tissue disease (n=5), HIV/AIDS (n=2) and Idiopathic CD4 T cell deficiency (n=1).
[2] Positive tests from 171 episodes shown. Possible IA included 6 episodes with positive β-D glucan assays and 1 with positive Karius cfDNA assay.
[3] Positive tests shown from 27 BAL performed, 203 sera sent for GM EIA, 187 sent for β-D glucan assays, and 2 Karius cfDNA assays.

Sample Testing and Reference Method

Criteria for the determination of proven, probable, and possible IA follow the consensus definitions of invasive fungal disease from the EORTC/MSGERC Consortium as described in Donnelly *et al.*[5] Information regarding clinical diagnoses were blinded to the operators performing the testing. True positive subjects include those with proven or probable IA. Negative subjects include a true negative group consisting of randomly selected subjects at risk for IA but who did not have proven, probable or possible IA, or other infections, or receipt of antifungal therapy, and those with other infections but who did not have proven, probable or possible IA. Subjects with "possible" IA were not included in the performance analysis.

Performance and Data Analysis

Each sample obtained at study sites were tested at three CLIA-certified clinical laboratories within the U.S. according to the Myco*MEIA* Instructions for Use (IFU). An additional 6 pediatric subjects were excluded from the clinical performance analysis since the Myco*MEIA* is only for use in adults. Sensitivity was established with 47 evaluable subjects who provided 623 archived, retrospective samples from the three studies and 6 evaluable subjects who provided 13 fresh, prospective samples from a single site. Sensitivity was calculated by sample and by subject (diagnoses of proven or probable IA) as not all samples from patients are expected to be positive. High sensitivity was observed compared to patient diagnosis based on established criteria. Specificity was analyzed by sample from 65 fresh, prospective samples obtained from a single study site. Possible IA samples (n=106) were excluded from the analysis; however, mixed infections adjudicated as no IA were included into the analysis as negative samples. Overall sensitivity of the Myco*MEIA* was determined as 92.4% (95%CI: 82.1-97.0) by subject and 58.1% (95%CI: 54.2-61.9) by sample, and specificity was determined to be 86.1% (95%CI: 75.7-92.5) by sample. A summary of results is shown in **Table 9** below.

Table 9. Clinical Performance of the Myco*MEIA*

	Myco*MEIA* Positive	**Myco*MEIA* 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)

[5] Donnelly, J Peter et. al. 2020. Revision and Update of the Consensus Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin.Infect.Dis. 71:1367-1376]

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)
	Myco*MEIA* Positive	**Myco*MEIA* 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 Cut-Off:

Not applicable.

Expected Values/Reference Range:

Invasive aspergillosis, caused by *Aspergillus* species, is an infection that develops in patients who have impaired immune defenses or other underlying conditions. The occurrence of the disease occurs in groups at risk due to factors such as hematologic malignancy, hematopoietic or solid organ transplant, granulocytopenia, immunosuppressive medications, lung disease or other infections, and the stage of infection.

F Other Supportive Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The premarket notification requires additional information to complete the substantive review and support a substantial equivalence decision.