

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k101407

B. Purpose for Submission:

To obtain a substantial equivalence determination for ALPHA *Histoplasma* Antigen EIA from urine specimens

C. Measurand:

Histoplasma capsulatum antigens

D. Type of Test:

Qualitative Enzyme Immunoassay (EIA)

E. Applicant:

Immuno-Mycologics Inc.

F. Proprietary and Established Names:

ALPHA *Histoplasma* Antigen EIA

G. Regulatory Information:

1. Regulation section:

21 CFR Part 866.3320

2. Classification:

Class II

3. Product code:

MIZ

4. Panel:

83 - Microbiology

H. Intended Use:

1. Intended use(s):

The ALPHA *Histoplasma* Antigen EIA is an immunoenzymatic sandwich microplate assay for the detection of *Histoplasma* antigens in urine samples.

2. Indication(s) for use:

The ALPHA *Histoplasma* Antigen EIA is an immunoenzymatic sandwich microplate assay for the detection of *Histoplasma* antigens in urine samples.

The ALPHA *Histoplasma* Antigen 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 histoplasmosis.

3. Special conditions for use statement(s):

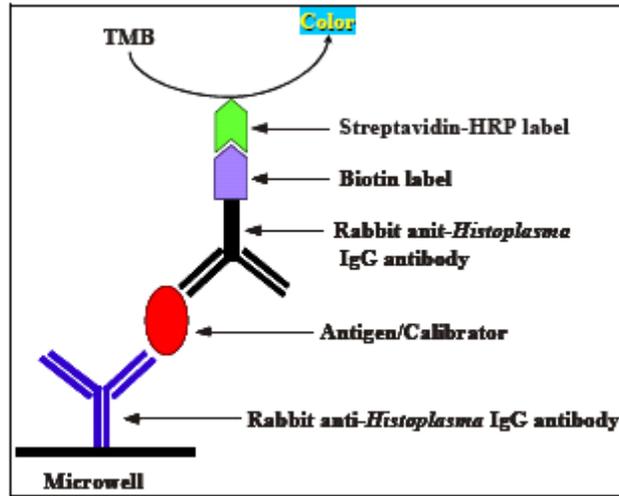
Prescription use

4. Special instrument requirements:

Microplate reader equipped with 450 nm and/or 450/630 nm filters

I. Device Description:

The ALPHA *Histoplasma* Antigen EIA is a two-stage immunoenzymatic sandwich microplate assay which detects *Histoplasma* antigens in urine. Rabbit polyclonal anti-*Histoplasma* IgG antibodies bound to microwell plates are used as capture antibodies and biotinylated rabbit polyclonal anti-*Histoplasma* IgG antibodies are used as detect antibodies. Urine samples are run untreated and undiluted.



The prepared samples are added to microwells coated with capture antibody and incubated. If the patient specimen contains *Histoplasma* antigens that are recognized by the capture antibody, those antigens will become bound to the microwell. The wells are washed to remove unbound patient material and biotinylated detection antibody is added to the wells. If *Histoplasma* antigens are bound to the microwell by the capture antibody, then the detect antibody will also become bound to the microwell. The wells are then washed to remove any unbound detect antibody. Streptavidin conjugated to horseradish peroxidase (HRP) is added to the microwells. In the presence of the biotinylated detect antibody, streptavidin-HRP will become bound to the plate. The plate is then washed to remove any unbound streptavidin-HRP, and 3,3',5,5' tetramethylbenzidine (TMB) substrate solution is added to the microwells. A blue color develops in the presence of the HRP enzyme. The reaction is stopped by the addition of a stop solution. The optical density (absorbance) is determined with a microplate reader at 450 nm alone or at 450nm and 630 nm. EIA Units for specimens are calculated using a four parameter curve-fit generated with the 4 standards supplied in the kit.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Bio-Rad Platelia *Aspergillus* EIA
2. Predicate 510(k) number(s):
k060641

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Antigen Detection	Antigen Detection
Assay Principle	EIA	EIA
Assay components	96-well microplate coated antibody, wash buffer, positive control, negative control, enzyme conjugate, TMB substrate, stop solution	96-well microplate coated antibody, wash buffer, positive control, negative control, enzyme conjugate, TMB substrate, stop solution
Detection Chemistry	HRP + TMB	HRP + TMB
Controls/Standard	Antigen	Antigen
Instruments	Microplate reader equipped with 450 nm and 620/630 nm filters – none specified.	Microplate reader equipped with 450 nm and 620/630 nm filters – none specified.
Microplate	96-well microplate coated with antibody	96-well microplate coated with antibody
Differences		
Item	Device	Predicate
Intended use	Detection of <i>Histoplasma</i> antigen in urine samples	Detection of <i>Aspergillus</i> galactomannan antigen in adult and pediatric serum samples
Indication for Use	Aid in the diagnosis of Histoplasmosis	Aid in the diagnosis of Aspergillosis
Sample Matrix	Urine	Serum
Controls	<i>Histoplasma</i> antigens	<i>Aspergillus</i> galactomannan
Detection Antibody	Biotinylated anti- <i>Histoplasma</i> polyclonal antibody	HRP-linked anti- <i>Aspergillus</i> monoclonal antibody
Output	“EIA Units” as determined from standard curve	“Index” as determined by OD of sample divided by Cut-Off Control OD
Microplate	96-well microplate coated with anti- <i>Histoplasma</i> antibody	96-well microplate coated with anti- <i>Aspergillus</i> antibody
Assay time	~ 3 hours	~2 hours
Shelf life	1 year	Each kit component different

K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition
- CLSI EP12-A2 User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline— Second Edition
- CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

The ALPHA *Histoplasma* Antigen EIA is a two-stage immunoenzymatic sandwich microplate assay which detects *Histoplasma* antigens in urine. Rabbit polyclonal anti-*Histoplasma* IgG antibodies bound to microwell plates are used as capture antibodies and biotinylated rabbit polyclonal anti-*Histoplasma* IgG antibodies are used as detect antibodies. Urine samples are run untreated and undiluted. The prepared samples are added to the microwells coated with the capture antibody and incubated. If the patient specimen contains *Histoplasma* antigens that are recognized by the capture antibody, those antigens will become bound to the microwell. The wells are washed to remove unbound patient material and biotinylated detection antibody is added to the wells. If *Histoplasma* antigens are bound to the microwell by the capture antibody, then the detect antibody will also become bound to the microwell. The wells are then washed to remove any unbound detect antibody. Streptavidin conjugated to horseradish peroxidase (HRP) is added to the microwells. In the presence of the biotinylated detect antibody, streptavidin-HRP will become bound to the plate. The plate is then washed to remove any unbound streptavidin-HRP, and 3,3',5,5' tetramethylbenzidine (TMB) substrate solution is added to the microwells. A blue color develops in the presence of the HRP enzyme. The reaction is stopped by the addition of a stop solution. The optical density (absorbance) is determined with a microplate reader at 450 nm alone or at 450nm and 630 nm. EIA Units for specimens are calculated using a four parameter curve-fit generated with the 4 standards supplied in the kit.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

- i. Study Design: Three sites, a reference laboratory (RL) (Western US), a clinical laboratory (CL) (Upper Mid-Western US), and IMMY (Central US), were used to assess the assay's reproducibility. The panel consisted of urine samples at five levels: negative, high negative (C_5), cut-off (C_{50}), low positive (C_{95}), and moderately positive. At the reference laboratory and at IMMY, each sample was tested in triplicate over the course of five

days, using multiple operators. At the clinical laboratory, each sample was tested in triplicate over the course of three days, using a single operator. Throughout the study, reagent lots and instrument calibrations were held constant. No runs were removed from analysis due to failed runs.

- ii. Results/Acceptance Criteria: Variance was estimated by calculating the mean value of each sample, the standard deviation and percent CV, according to EP5-A2. The data was analyzed separately to evaluate any inter-assay, intra-assay, and inter-site variation. Overall, no major source of variability was identified. Intra-run, Inter-Run, and Inter-site percent CVs are within acceptable limits ($\leq 20\%$), with the exception of the negative urine and low negative urine samples, which is expected when testing beyond the limit of detection. A summary of the data is reported in the tables below.

Intra-Run Reproducibility Analysis

Intra-Run Analysis - Blanked OD Values									
			Neg. Urine	C5	C50	C95	Moderate Urine	Positive Control	
Clinical Laboratory	Day 1	Operator 1	Ave	0.010	0.064	0.078	0.092	0.810	0.218
			SD	0.003	0.001	0.002	0.003	0.032	0.008
			%CV	26.5%	1.8%	2.0%	3.3%	3.9%	3.4%
	Day 2	Operator 1	Ave	0.006	0.071	0.088	0.108	0.900	0.246
			SD	0.003	0.002	0.003	0.003	0.032	0.004
			%CV	50.0%	2.9%	3.4%	2.8%	3.6%	1.5%
	Day 3	Operator 1	Ave	0.007	0.062	0.074	0.086	0.783	0.211
			SD	0.002	0.002	0.004	0.007	0.041	0.009
			%CV	31.2%	3.4%	5.1%	8.1%	5.3%	4.3%
Reference Laboratory	Day 1	Operator 1	Ave	0.006	0.035	0.054	0.057	0.523	0.136
			SD	0.003	0.002	0.001	0.007	0.011	0.008
			%CV	39.7%	4.3%	1.9%	11.3%	2.1%	5.6%

Inter-Run Reproducibility Analysis

		Inter-Run Analysis - Blanked OD Values					
		Neg. Urine	C5	C50	C95	Moderate Urine	Positive Control
Clinical Laboratory	Ave	0.008	0.066	0.080	0.095	0.831	0.225
	Std Dev	0.003	0.005	0.007	0.011	0.061	0.017
	% CV	38.7%	7.0%	8.6%	11.0%	7.4%	7.6%
Reference Laboratory	Ave	0.004	0.042	0.064	0.066	0.610	0.165
	Std Dev	0.007	0.005	0.009	0.009	0.059	0.025
	% CV	187.0%	12.7%	13.9%	13.1%	9.6%	14.9%
IMMY	Ave	0.006	0.043	0.068	0.074	0.670	0.215
	Std Dev.	0.004	0.006	0.009	0.009	0.048	0.020
	% CV	64.2%	14.9%	13.2%	11.7%	7.2%	9.1%

Inter-Site Reproducibility Analysis

		Inter-Site Analysis - Blanked OD Values					
		Neg. Urine	C5	C50	C95	Moderate Urine	Positive Control
All	Ave	0.006	0.048	0.069	0.076	0.684	0.198
	Std Dev	0.005	0.011	0.010	0.014	0.101	0.034
	% CV	95.9%	23.7%	14.8%	19.1%	14.8%	17.0%

- iii. **Carry-Over Studies:** To examine potential well-to-well carry-over, a positive sample (1x Wash Buffer spiked to 300 EIA Units) was used in series alternating with a negative sample (1x Wash Buffer) in a checkerboard pattern across 56 wells of plate. The test was repeated over the course of five days. Acceptance Criteria was defined as % CVs less than 20% and all 1x WB ODs less than 0.100.

Carry-Over Analysis Summary

		Negative Sample	Positive Sample
Day 1	Ave	0.052	1.243
	Std Dev	0.009	0.036
	% CV	18.3	2.9
Day 2	Ave	0.054	1.097
	Std Dev	0.006	0.099
	% CV	11.0	9.0
Day 3	Ave	0.049	1.202
	Std Dev	0.006	0.063
	% CV	12.2	5.2
Day 4	Ave	0.035	1.223
	Std Dev	0.002	0.054
	% CV	7.1	4.4

- iv. Prozone Studies:** To detect the prozone effect in the ALPHA *Histoplasma* Antigen EIA, negative urine was spiked with *Histoplasma* antigen to 2000, 3000, and 4000 EIA units (the top of the standard curve is 100 EIA units). Each sample was tested in duplicate. All samples remained strongly positive. The results are found in table below:

Prozone Study Results

Sample	Blanked OD Rep 1	Blanked OD Rep 2
2000 EIA Units	1.141	1.096
3000 EIA Units	1.123	0.991
4000 EIA Units	0.928	0.957

- v. Single Versus Dual Wavelength Study:** Eight positive urine samples and the four standards in the kit were tested in duplicate and according to the package insert. The microwells were read at 450/630 nm and then at 450 nm only. Line data is provided in the below. The average, standard deviation, and percent CV was calculated for each sample using all four data points (both replicates using both reading protocols). The study confirmed that the blanked OD of the samples is not affected by the read method, as indicated by the low percent CVs. Furthermore, 450/630 vs. 450 alone can be used with equal accuracy, and data from the two methods can be used interchangeably. Acceptance criteria were defined as % CV

less than 20%.

Single Versus Dual Wavelength Line Data

	Blanked OD						
	Rep 1		Rep 2		Average	Std. Dev.	% CV
	450/630 nm	450 nm	450/630 nm	450 nm			
Sample 1	1.578	1.595	1.487	1.510	1.542	0.052	3.4%
Sample 2	1.271	1.286	1.261	1.282	1.275	0.012	0.9%
Sample 3	0.324	0.327	0.309	0.313	0.318	0.009	2.7%
Sample 4	0.253	0.257	0.251	0.255	0.254	0.003	1.1%
Sample 5	0.592	0.593	0.594	0.600	0.595	0.004	0.6%
Sample 6	0.198	0.198	0.192	0.195	0.196	0.003	1.5%
Sample 7	1.364	1.375	1.351	1.358	1.362	0.010	0.8%
Sample 8	1.077	1.077	1.085	1.097	1.084	0.010	0.9%
100 Std	1.040	1.065	0.936	0.940	0.995	0.067	6.7%
30 Std	0.566	0.564	0.483	0.484	0.524	0.047	9.0%
10 Std	0.253	0.255	0.234	0.235	0.244	0.011	4.6%
2 Std	0.062	0.061	0.051	0.050	0.056	0.006	11.4%
1x WB	0.005	0.005	-0.005	-0.005	0.000	0.005	Na

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

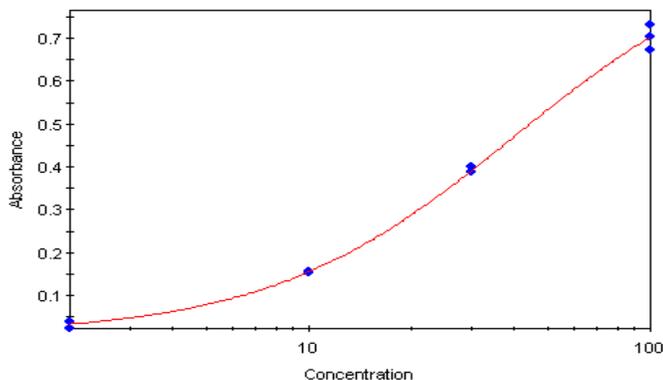
- i. Standard Curve Methods and Acceptance Criteria: The acceptable blanked OD ranges were determined by testing each standard on multiple lots for a total of 83 replicates. The acceptable OD was calculated by averaging all replicates and rounding up to an even number. The ranges were calculated by rounding 3x the standard deviations and adding to the rounded average. The table below summarizes the data.

Acceptable Blanked OD Ranges for Standards

	100 Std	30 Std	10 Std	2 Std
	n=83	n=83	n=83	n=83
Ave	0.775	0.387	0.169	0.038
Std. Dev	0.110	0.059	0.030	0.009
3x Std Dev	0.330	0.176	0.091	0.027
Acceptable OD	0.800	0.400	0.200	0.040
Acceptable OD Ranges +/-	0.300	0.150	0.100	0.020

An assay is considered valid when the standards fall within the acceptable, blanked OD ranges as defined below and $R^2 \geq 0.990$.

Standard	Acceptable Blanked OD
100	0.80 ± 0.3 (1.1-0.5)
30	0.40 ± 0.15 (0.55-0.25)
10	0.20 ± 0.1 (0.3-0.1)
2	0.04 ± 0.02 (0.06-0.02)
Positive Control	0.20 ± 0.1 (0.3-0.1)
Negative Control	< 0.02



4 Parameter ($y = (A - D) / (1 + (x/C)^B) + D$)
 A=0.9284 B=-1.2141 C=39.6866 D=0.0095, R-Square = 0.9976

The users are instructed to run all specimens and controls in duplicate until the user becomes familiar with the kit performance. The Positive Control, Negative Control, and EIA standards must be assayed with each batch of patient specimens to provide quality assurance of the reagents. The Positive and Negative Controls are intended to monitor for substantial reagent failure. The Positive Control should not be used as an indicator of EIA standards precision and only ensures reagent functionality. The

Positive Control EIA units should be between 7 and 13. The Negative Control EIA units should be less than 2.

If the EIA units of the Positive Control and/or Negative Control are not within these parameters, patient test results should be considered invalid, and the assay should be repeated.

- ii. **Specimen Stability Studies:** In specimen stability studies, negative urine was spiked at four concentrations: Low-positive (C95), low-positive, and two moderate-positives. Specimens were divided and then stored at 4°C and -20°C. The set stored at 4°C was followed daily for stability over the course of one week. One set stored at -20°C went through multiple freeze/thaw cycles and was tested after each cycle. A second set was stored for one week without multiple freeze/thaw cycles, and a third set was stored for 2 weeks without multiple freeze/thaw cycles. When tested, each sample was run in duplicate. While there were reductions in EIA values after a 2-week storage at 4°C and after multiple freeze thaws, no specimen went from positive to negative. Since a reduction in EIA values was observed, it is possible that a fresh, very low-positive specimen (near 2.0 EIA units) could become negative if it is stored for several days. Results of the studies (averages of Blanked OD and EIA Units) are summarized below.

Blanked OD of Specimens Stored at 4°C

Blanked OD (4°C)							
	Day 0 (Fresh)	Day 1	Day 2	Day 3	Day 6	Day 7	Day 14
Sample 1	0.050	0.047	0.053	0.045	0.044	0.045	0.062
Sample 2	0.103	0.092	0.119	0.096	0.091	0.073	0.095
Sample 3	0.172	0.161	0.196	0.167	0.148	0.142	0.160
Sample 4	0.260	0.198	0.303	0.242	0.242	0.229	0.274

EIA Units of Specimens Stored at 4°C

EIA Units (4°C)							
	Day 0 (Fresh)	Day 1	Day 2	Day 3	Day 6	Day 7	Day 14
Sample 1	2.70	2.68	2.61	2.47	2.55	2.65	2.96
Sample 2	6.48	5.28	6.03	5.03	5.21	4.57	4.60
Sample 3	12.38	10.57	11.33	10.09	9.50	10.66	8.77

3							
Sample 4	21.40	14.04	21.00	17.55	19.33	20.88	19.10

Blanked OD of Specimens Stored at -20°C

Blanked OD (-20°)									
	Day 0 (Fresh)	Freeze/Thaw						Long Term Storage	
		Day 1	Day 2	Day 3	Day 6	Day 7	Day 14	1 Week	2 Weeks
Sample 1	0.050	0.043	0.053	0.040	0.042	0.044	0.060	0.042	0.051
Sample 2	0.103	0.086	0.109	0.106	0.089	0.094	0.125	0.084	0.100
Sample 3	0.172	0.171	0.206	0.177	0.170	0.173	0.185	0.150	0.174
Sample 4	0.260	0.232	0.280	0.244	0.293	0.245	0.275	0.214	0.278

EIA Units of Specimens Stored at -20°C

EIA Units (-20°)									
	Day 0 (Fresh)	Freeze/Thaw						Long Term Storage	
		Day 1	Day 2	Day 3	Day 6	Day 7	Day 14	1 Week	2 Weeks
Sample 1	2.70	2.48	2.61	2.25	2.46	2.58	2.85	2.43	2.47
Sample 2	6.48	4.89	5.48	5.64	5.08	6.24	6.44	5.44	4.88
Sample 3	12.38	11.41	12.06	11.03	11.59	14.00	10.70	11.61	9.80
Sample 4	21.40	17.57	18.59	17.80	26.16	23.28	19.13	19.22	19.39

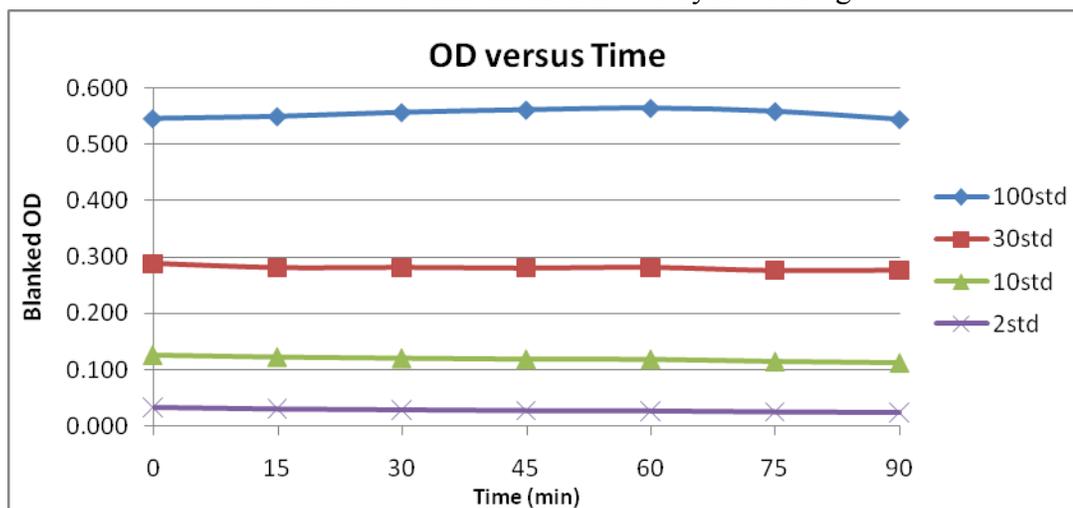
iii. **Reagent/Kit Stability Studies:** The following stability studies were performed to validate claims for the storage of reconstituted reagent.

Stability of Opened / Reconstituted Reagents	Microplate stable for up to five weeks after opening when stored at 2-8°C. Wash Solution stable for up to 15 days after dilution when stored at 2-8°C. Controls stable for up to five weeks after reconstitution when stored at -20°C. Chromogen / Substrate Buffer solution stable for up to six hours when stored at room temperature, in the dark.
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Real Time Kit Stability	The kit is stable for 1 year after date of manufacture.
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- iv. Time Parameter Studies for Reading the Test: To demonstrate that the test should be read within 15 minutes after the addition of the Stop Solution, all the standards were run in triplicate according to the package insert. The test was then read immediately after the addition of the Stop Solution, and then at 15, 30, 45, 60, 75, and 90 minutes. The signal began to decrease after 15 minutes and continued to decrease over the course of 90 minutes.

Blanked OD of Standards after Delayed Reading



Delayed Reading Line Data

	Blanked OD																				
	T=0			T=15			T=30			T=45			T=60			T=75			T=90		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
100std	0.563	0.570	0.503	0.575	0.569	0.504	0.586	0.572	0.511	0.593	0.574	0.515	0.599	0.576	0.517	0.593	0.568	0.515	0.581	0.553	0.499
30std	0.310	0.279	0.275	0.302	0.278	0.265	0.302	0.279	0.264	0.302	0.277	0.262	0.305	0.277	0.263	0.299	0.274	0.259	0.295	0.285	0.254
10std	0.136	0.131	0.112	0.134	0.126	0.109	0.133	0.122	0.107	0.131	0.121	0.104	0.131	0.121	0.104	0.128	0.117	0.101	0.127	0.116	0.096
2std	0.034	0.035	0.032	0.034	0.032	0.027	0.032	0.031	0.025	0.031	0.029	0.023	0.030	0.029	0.023	0.029	0.027	0.022	0.028	0.028	0.019
1x WB	0.001	0.001	0.000	0.001	0.001	0.000	0.001	0.001	-0.001	0.001	0.000	-0.001	0.002	0.001	-0.002	0.002	0.001	-0.001	0.003	0.001	-0.002
1x WB	0.000	0.000	-0.002	0.000	0.000	-0.001	0.000	-0.001	-0.001	0.000	-0.001	-0.001	0.000	-0.001	-0.001	0.001	0.000	-0.001	0.002	0.000	-0.003

d. Detection limit:

- i. Analytical Sensitivity Methods: Analytical sensitivity was estimated at IMMY according to Clinical and Laboratory Standards Institute (CLSI) EP-17A. The limit of the blank (LoB) was estimated by running 82 blank measurements and the limit of detection (LoD) was estimated by running 30 measurements of 9 low standards (1.0 Std, 1.25 Std, 1.5 Std, 1.75 Std, 2.0 Std, 2.25 Std, 2.5 Std, 3.0 Std, 4.0 Std) all made by diluting the *Histoplasma* Antigen Standard (HAG100) in 1x Wash Buffer (HAGWB1).

ii. Results: Line data for LoB and LoD can be found below.

LoB Line Data

Rep 1	0.046	Re 21	0.044	Rep 41	0.058	Rep 61	0.046	Rep 81	0.048
Rep 2	0.042	Re 22	0.044	Rep 42	0.043	Rep 62	0.046	Rep 82	0.047
Rep 3	0.061	Re 23	0.044	Rep 43	0.042	Rep 63	0.049		
Rep 4	0.044	Re 24	0.044	Rep 44	0.044	Rep 64	0.048		
Rep 5	0.045	Re 25	0.044	Rep 45	0.053	Rep 65	0.046		
Rep 6	0.043	Re 26	0.043	Rep 46	0.049	Rep 66	0.049		
Rep 7	0.042	Re 27	0.043	Rep 47	0.047	Rep 67	0.044		
Rep 8	0.042	Re 28	0.046	Rep 48	0.049	Rep 68	0.048		
Rep 9	0.042	Re 29	0.044	Rep 49	0.046	Rep 69	0.045		
Rep 10	0.044	Re 30	0.044	Rep 50	0.047	Rep 70	0.045		
Rep 11	0.045	Re 31	0.044	Rep 51	0.047	Rep 71	0.046		
Rep 12	0.044	Re 32	0.044	Rep 52	0.047	Rep 72	0.044		
Rep 13	0.074	Re 33	0.045	Rep 53	0.046	Rep 73	0.047		
Rep 14	0.044	Re 34	0.043	Rep 54	0.044	Rep 74	0.043		
Rep 15	0.05	Re 35	0.042	Rep 55	0.046	Rep 75	0.045		
Rep 16	0.043	Re 36	0.041	Rep 56	0.047	Rep 76	0.045		

2.0std	0.093	2.25std	0.095	2.5std	0.106	3.0std	0.118	4.0std	0.135
2.0std	0.093	2.25std	0.095	2.5std	0.107	3.0std	0.117	4.0std	0.131
2.0std	0.094	2.25std	0.096	2.5std	0.111	3.0std	0.115	4.0std	0.132
2.0std	0.092	2.25std	0.101	2.5std	0.102	3.0std	0.112	4.0std	0.132
2.0std	0.096	2.25std	0.095	2.5std	0.110	3.0std	0.105	4.0std	0.136
2.0std	0.096	2.25std	0.093	2.5std	0.120	3.0std	0.114	4.0std	0.134
2.0std	0.101	2.25std	0.095	2.5std	0.111	3.0std	0.116	4.0std	0.132
2.0std	0.089	2.25std	0.105	2.5std	0.101	3.0std	0.129	4.0std	0.150
2.0std	0.094	2.25std	0.096	2.5std	0.105	3.0std	0.117	4.0std	0.137
2.0std	0.097	2.25std	0.093	2.5std	0.104	3.0std	0.116	4.0std	0.136
2.0std	0.096	2.25std	0.094	2.5std	0.104	3.0std	0.119	4.0std	0.136
2.0std	0.094	2.25std	0.096	2.5std	0.105	3.0std	0.115	4.0std	0.137
2.0std	0.094	2.25std	0.096	2.5std	0.102	3.0std	0.111	4.0std	0.143
2.0std	0.092	2.25std	0.092	2.5std	0.101	3.0std	0.115	4.0std	0.133
2.0std	0.093	2.25std	0.093	2.5std	0.103	3.0std	0.115	4.0std	0.139
2.0std	0.087	2.25std	0.104	2.5std	0.105	3.0std	0.127	4.0std	0.151
2.0std	0.090	2.25std	0.094	2.5std	0.100	3.0std	0.118	4.0std	0.138
2.0std	0.089	2.25std	0.091	2.5std	0.102	3.0std	0.117	4.0std	0.140
2.0std	0.091	2.25std	0.089	2.5std	0.101	3.0std	0.114	4.0std	0.147
2.0std	0.088	2.25std	0.093	2.5std	0.100	3.0std	0.114	4.0std	0.142
2.0std	0.089	2.25std	0.091	2.5std	0.100	3.0std	0.116	4.0std	0.141
2.0std	0.090	2.25std	0.094	2.5std	0.097	3.0std	0.122	4.0std	0.142
2.0std	0.100	2.25std	0.095	2.5std	0.104	3.0std	0.121	4.0std	0.149
2.0std	0.091	2.25std	0.097	2.5std	0.098	3.0std	0.133	4.0std	0.154
2.0std	0.089	2.25std	0.090	2.5std	0.106	3.0std	0.120	4.0std	0.146
2.0std	0.088	2.25std	0.085	2.5std	0.110	3.0std	0.118	4.0std	0.141
2.0std	0.089	2.25std	0.089	2.5std	0.101	3.0std	0.118	4.0std	0.139
2.0std	0.088	2.25std	0.093	2.5std	0.103	3.0std	0.117	4.0std	0.150
2.0std	0.087	2.25std	0.084	2.5std	0.094	3.0std	0.118	4.0std	0.155

Analytical Sensitivity Analysis:

Limit of the Blank Calculations:

$$\text{LoB} = \mu_{\beta} + 1.645\sigma_{\beta}$$

where μ_{β} is the mean of the blank measurements and σ_{β} is the standard deviation of the blank measurements.

$$\text{LoB} = 0.001427 + 1.645(0.004469)$$

LoB = 0.009 OD

Limit of Detection Calculations:

$$\text{LoD} = \text{LoB} + c_{\beta}\text{SD}_s$$

where SD_s is the standard deviation of the sample measurements and c_{β} is derived from the 95th percentile of the standard Gaussian distribution:

$$c_{\beta} = 1.645/(1-1/(4 \times f)), \text{ where } f \text{ is the degrees of freedom from the } \text{SD}_s.$$

$$f = N_s - K \text{ where } N_s \text{ is the total number of measurements (9} \\ \times 30) \text{ and } K \text{ is number of sources of measurements (9)}$$

$$\text{LoD} = 0.009 + (1.645/(1-1/(4 \times (270-9))))(0.021)$$

$$\text{LoD} = \mathbf{0.044 \text{ OD}}$$

Analysis shows that the LoB is 0.009 RAW OD and LoD is 0.044 BLANKED OD. Our 2.0 EIA units calibrator is approximately equivalent to this Limit of Detection (0.044 OD).

e. Analytical specificity:

- i. Cross-Reactivity Studies: Urine specimens that tested negative for *Histoplasma* antigen were spiked with antigen from *Blastomyces dermatiditis*, *Coccidioides immitis*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Paracoccidioides brasiliensis* and *Candida albicans*, individually, at 1 µg/mL. The ALPHA Histoplasma Antigen EIA was found to be cross-reactive with *Blastomyces dermatiditis*, *Coccidioides immitis*, and *Paracoccidioides brasiliensis* in urine. The assay was not cross-reactive with *Candida albicans*, *Cryptococcus neoformans*, or *Aspergillus spp.* in urine. Source/Strain information of culture filtrates used in cross-reactivity testing are shown in table below:

Organism	Source and Strain
<i>Blastomyces dermatiditis</i>	Marano – Clinical Isolate from Marshfield Clinic
<i>Coccidioides immitis</i>	CDC B-475
<i>Paracoccidioides brasiliensis</i>	CDC B-339
<i>Aspergillus spp.</i>	ATCC 64026 (<i>A. fumigatus</i>) ATCC64028 (<i>A. niger</i>) ATCC 64025 (<i>A. flavus</i>) ATCC 28301 (<i>A. terreus</i>)
<i>Candida albicans</i>	ATCC 32354
<i>Cryptococcus neoformans</i>	184A – Clinical Isolate from New Orleans/Tulane*

Analytical Specificity Line Data and Analysis

Spiked at 1ug/ml	Blanked OD			Statistical Analysis			EIA Units			Statistical Analysis		
	Rep 1	Rep 2	Rep 3	Ave	Std. Dev.	% CV	Rep 1	Rep 2	Rep 3	Ave	Std. Dev.	% CV
<i>Blastomyces</i>	0.534	0.532	0.530	0.532	0.002	0.4%	33.2	33.0	32.7	33.0	0.263	0.8%
<i>Coccidioides</i>	1.136	1.158	1.198	1.164	0.031	2.7%	170.1	180.4	200.4	183.6	15.396	8.4%
<i>Paracoccidioides</i>	1.388	1.367	1.371	1.375	0.011	0.8%	347.2	326.4	330.4	334.7	11.043	3.3%
<i>Aspergillus sp.</i>	0.014	0.012	0.010	0.012	0.002	16.7%	<2	<2	<2	<2	n.a.	n.a.
<i>Cryptococcus</i>	0.000	0.004	0.001	0.002	0.002	83.3%	<2	<2	<2	<2	n.a.	n.a.
<i>Candida</i>	0.014	0.013	0.014	0.014	0.001	4.2%	<2	<2	<2	<2	n.a.	n.a.

- ii. Interfering Substances: Urine specimens containing various potential interfering substances were obtained from a national reference laboratory. Each specimen was tested in duplicate using the IMMY ALPHA *Histoplasma* Antigen EIA to assess the potential for false positive reactivity in the assay. Additionally, each specimen was spiked with *Histoplasma* antigen and tested in the assay. None of these substances were found to interfere with the ALPHA *Histoplasma* Antigen EIA. The data from this study is summarized in the table below. The “Interfering Value” is given as reported by the reference laboratory.

Interference Line Data

Protein	Unspiked EIA Units		Spiked EIA Units		Interfering Value (mg/dl)
	Rep 1	Rep 2	Rep 1	Rep 2	
#4	0	0	3.856	3.574	30
#6	0	0	3.735	3.979	100
#7	0	0	3.109	3.694	30
#8	0	0	2.848	2.959	30
#9	0	0	6.685	6.786	30

Blood					Cells/High-Powered Field
#1	0	0	2.147	2.631	Moderate
#2	0	0	3.574	4.021	Moderate
#3	0	0	4.146	4.616	Large
#4 (Blue)*	0	0	8.491	6.736	Large
#6	0	0	5.196	4.315	Moderate
Epithelial Cells					Cells/High-Powered Field
#2	0	0	5.755	4.529	2-5
#3	0	0	3.185	2.812	2
#4	0	0	5.105	4.97	6
#6	0	0	0	0	10-20
#7	0	0	3.456	3.574	6
Ketones					(mg/dl)
#1	0	0	6.939	6.189	≥80
#3	0	0	4.146	4.357	60
#4	0	0	0	0	10
#5	0	0	0	2.015	40
#6	0	0	3.034	3.378	10
Mucus					Cells/High-Powered Field
#1	0	0	4.009	4.05	Many
#2 (Blue)*	0	0	8.004	7.322	Moderate
#3	0	0	5.188	4.297	Many
#4	0	0	5.893	5.626	Many
#5	0	0	2.37	2.37	Moderate
Cast					Cells/Low-Powered Field
#1	0	0	2.333	2.04	5-Hyal
#2 (Blue)*	0	0	7.608	6.898	Granular
#3	0	0	2.975	2.86	5-Hyal
#4	0	0	2.556	2.185	19-Hyal
#5	0	0	2.594	2.594	0-2 Hyal
Glucose					(mg/dl)
#1	0	0	3.525	3.725	250
#2	0	0	2.898	2.86	250
#3	0	0	5.538	5.319	500
#4	0	0	4.548	5.714	100
#5	0	0	4.132	3.846	500

Bilirubin					Cells/High-Powered Field
#1	0	0	5.362	5.45	Moderate
#2	0	0	2.975	3.326	Moderate
#3	0	0	2.86	3.366	Moderate
#4	0	0	5.319	5.937	Moderate
#5	0	0	5.275	5.102	Moderate

*Specimen was blue in color, reason unknown

Vaginal cream urines and foods which produce color in urine were not tested. Additionally, drugs, such as itraconazole, amphotericin B, acetaminophen, acetylsalicylic acid, ascorbic acid, and caffeine were not tested for interference.

f. Assay cut-off:

- i. Study Design: The assay cut-off was established following CLSI EP12-A2 and EP17-A. The 2 EIA units standard was created to be equivalent to the LoD. Using the 2 EIA units standard as the cut-off, the C_5 - C_{95} Interval and C_{50} were determined by running 30 replicates of 9 samples spiked with *Histoplasma* antigen around the 2 EIA units standard. Percent positive was determined by the number of samples that ran equal to or greater than the average blanked OD of the 2 Standard. The percentage of replicates that ran positive was calculated for each sample in the table below. The Receiver Operator Curve (ROC) analysis of culture-proven specimens indicated a lower cut-off of 1.3 EIA units (figure below). The ROC value is below the Limit of Detection, and therefore considered an inappropriate cutoff. As such, an equivocal zone was not created, as all data firmly supported a cut-off of 2.0 EIA units.

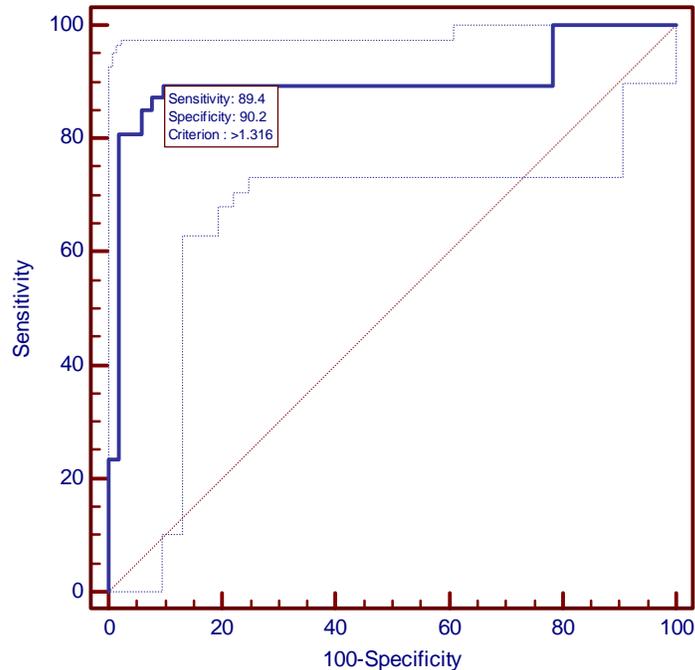
Cut-Off Analysis:

Percent Positives for 11 samples near the LoD

Sample Concentration (EIA Units)	% Positive	
1.00	3.3%	
1.25	0.0%	
1.50	6.7%	C_5
1.75	23.3%	
2.00	53.3%	C_{50}
2.25	62.5%	
2.50	92.5%	C_{95}
2.75	100%	
3.00	100%	
3.50	100%	

4.00	100%	
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ROC analysis of culture-proven specimens



The C_5 is 1.50 and the C_{95} is 2.50 EIA Units while the C_{50} is 2.00 EIA Units. C_{50} is a good indicator of an assay's cut-off. However, ROC analysis indicated a lower cut-off of 1.3 EIA units. Since this is below the calculated LoD of the assay, the C_{50} was used to establish the cut-off. Since analytical sensitivity (LoD) was more conservative than the clinical sensitivity (ROC analysis), an equivocal zone was not created.

The assay cut-offs are defined as follows:

Negative: < 2.0 EIA Units (Limit of Detection)
 Positive: \geq 2.0 EIA Units

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable

b. *Matrix comparison:*

Not applicable; urine only.

3. Clinical studies:

a. *Clinical Sensitivity*

- i. Study Design: The clinical performance of the ALPHA *Histoplasma* Antigen EIA was evaluated using a total of 278 culture- or histopathology-confirmed urine specimens (47 culture/histopathology-positive and 231 culture/histopathology-negative). Specimens were obtained and tested at three geographically diverse hospitals/reference laboratories. All specimens were obtained from patients suspected of experiencing active disease by a physician and confirmed through culture or histopathology. Clinical sensitivity and specificity were calculated according to CLSI EP12-A2.
- ii. Results: The data from this study demonstrated that the ALPHA *Histoplasma* Antigen EIA has an 80.9 % sensitivity (CI: 67.5-89.6%) and 98.7% specificity (CI 96.3-99.6%), when compared to culture and/or histopathology, the current “gold standard” for diagnosis of histoplasmosis. The results are summarized in the tables below.

Histoplasma Ag EIA Versus Culture/Histopathology 2x2 Contingency Table

		Culture/Histopathology	
		Positive	Negative
IMMY ALPHA <i>Histoplasma</i> EIA	Positive	38	3
	Negative	9	228

Statistical Analysis Comparing Device to Culture

	Point Estimate	95% CI
Sensitivity	80.9%	67.5-89.6%
Specificity	98.7%	96.3-99.6%

iii. Supplemental Studies:

- a) An independent comparison between culture/histopathology and the ALPHA *Histoplasma* Antigen EIA Kit was presented at the College of American Pathologists 2008 Annual meeting. A poster titled “Utility of a *Histoplasma capsulatum* Enzyme Immunoassay for Diagnosis of Disseminated Histoplasmosis and Correlation with Disease Activity” was presented *. In the IRB approved study, Cox, et. al. reported that

during the course of one year, they enrolled 177 patients with suspected histoplasmosis and tested their urine specimens with the ALPHA Histoplasma Antigen EIA. Of those, only six were actually diagnosed with histoplasmosis through culture and/or histopathology. Below are tables showing the results of the study, including a 2x2 contingency table and a table of relevant statistics.

* Cox, M., G. Pesek, D. Phan, and G. Woods. 2008. Utility of a *Histoplasma capsulatum* Enzyme Immunoassay for Diagnosis of Disseminated Histoplasmosis and Correlation with Disease Activity. Arch. Pathol. Lab Med 132:1512.

Cox, et. al. Disease vs. IMMY EIA 2x2 Contingency Table

	Disease (By Culture and/or Histopathology)	
	Positive	Negative
IMMY Positive	6	2
IMMY Negative	0	171

Statistical Analysis

	95% CI	
Sensitivity	100%	54%-100%
Specificity	99%	96%-100%
Prevalence	3.3%	1.3%-7.2%
Positive Predictive Value	75%	35%-96%
Negative Predictive Value	100%	98%-100%

- b) Additional studies were performed using a non-FDA cleared *Histoplasma capsulatum* quantitative antigen EIA and resulted in a positive percent agreement of 71.4% (CI: 57.6-82.2%) and negative percent agreement of 94.0% (CI: 83.8-97.9%) when the non-FDA cleared test's equivocal results were assumed positive. When the equivocal results were assumed negative, positive percent agreement was 92.1% (CI: 79.2-97.3%) and negative percent agreement was 95.1% (CI: 86.5-98.3%).

b. Clinical specificity:

Clinical specificity was tested using non-*Histoplasma*, fungal culture-positive urines. The results from this study are summarized in the table below:

Specificity Testing Using Fungal Culture Positive Urines

	No. Positive	No. Negative	% Specificity	95% CI
Aspergillus	0	20	0% (0/20)	83.9-100%
Candida spp.	0	12	0% (0/12)	75.8-100%
Paracoccidioides	1	11	9.1% (1/11)	62.3-98.4%

- c. Other clinical supportive data (when a. and b. are not applicable): Not applicable

4. Clinical cut-off:

The clinical cut-off is the same as the assay cut-off. (Also see Section 3.f.)

Negative: EIA Units $x < 2.0$

Positive: EIA Units $x \geq 2.0$

The clinical performance of the ALPHA *Histoplasma* Antigen EIA was evaluated using a total of 278 culture- or histopathology-confirmed urine specimens (47 culture/histopathology-positive and 231 culture/histopathology-negative). Specimens were obtained and tested at three geographically diverse hospitals/reference laboratories. All specimens were obtained from patients suspected of experiencing active disease by a physician and confirmed through culture or histopathology.

The clinical cut-off values are interpreted as follows:

- Negative results do not rule out the diagnosis of disease as the specimen may be drawn before detectable antigen is present.
- A positive result implies the presence of antigen to *Histoplasma*; however, all test results should be reviewed in light of other clinical data by the physician.
- The magnitude of the measured result, above the cutoff, is not indicative of the total amount of antigen present.
- Specimens should be repeated if the results are low-positive and inconsistent with clinical findings.
- Specimens should be repeated if the results are indeterminate or low-positive and inconsistent with clinical findings.

5. Expected values/Reference range:

The frequency of histoplasmosis is dependent on several factors including: patient population, type of institution, and epidemiology. In this study, 80.9% of true positives determined by culture and/or histopathology were detected.

N. Proposed Labeling:

The proposed labeling is sufficient and satisfies the requirements of 21 CFR section 809.10

O. Conclusion:

The submitted information in the premarket notification is complete and supports substantial equivalence decision.