510(k) Summary
ALPHA Histoplasma Antigen EIA

This 510(k) summary is submitted in accordance with 21 CFR §807.92

Assigned 510(k) No.: K101407

Owner:
Immuno-Mycologics, Inc.
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Prepared: July 7, 2011

Trade Name: ALPHA *Histoplasma* Antigen EIA

Common Name: *Histoplasma* Antigen EIA

Classification Name: None

Regulation: 866.3320

Predicate Device: Bio-Rad's Platelia™ *Aspergillus* EIA, K060641

Intended 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.

Device Description:
The ALPHA *Histoplasma* Antigen EIA is an immunoenzymatic sandwich microplate assay which detects *Histoplasma* antigens in urine. Rabbit anti-*Histoplasma* IgG antibodies bound to microwell plates are used as capture antibodies and biotinylated rabbit anti-*Histoplasma* IgG antibodies are used as detect antibodies.

The positive control is made of buffer spiked with *Histoplasma capsulatum* antigens and the negative control is buffer only. Standards are made of *Histoplasma capsulatum* antigens from culture filtrate. The kit contains the 100 Standard, 30 Standard, 10 Standard, and 2 Standard

that are used to generate a sigmoid calibration curve using a four-parameter fit of the blanked absorbance values versus the assigned EIA Values. R-squared values must be greater than or equal to 0.990.

Urine samples are run untreated and undiluted. The 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. For detection, biotin-streptavidin chemistry reagents including 3,3',5,5' tetramethybenzadine (TMB) and stop solution are used. 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 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 450nm and 640nm or 450nm alone. EIA Units for specimens are calculated using a four-parameter curve-fit generated with the 4 standards supplied in the kit.

Comparison with Predicate:

A comparison of the similarities and differences between the ALPHA *Histoplasma* Antigen EIA and the predicate device is presented in the table below (Table 1).

Table 1. Similarities and Differences between ALPHA *Histoplasma* EIA and Platelia Aspergillus EIA

SIMILARITIES		
Feature	ALPHA *Histoplasma* Ag EIA Device	Platelia Aspergillus EIA K060641
Intended Use		
Intended Use	Antigen detection	Antigen detection
Indication For Use	Aid in the diagnosis	Aid in the diagnosis
Device Description		
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	none	none
Microplate	96-well microplate coated with antibody	96-well microplate coated with antibody
Performance Characteristics		
Precision	Acceptable % CVs	Acceptable % CVs

Linearity	Not applicable	Not applicable
Specificity	Cross-reacts with other fungal organisms	Cross-reacts with other fungal organisms

DIFFERENCES		
Feature	ALPHA Histoplasma Ag EIA Device	Platelia Aspergillus EIA K060641
Intended Use		
Intended Use	Detection of *Histoplasma* antigen in urine samples	Detection of *Aspergillus* galactomannan antigen in adult and pediatric serum samples
Indication For Use	Aid in the diagnosis of histoplasmosis.	Aid in the diagnosis of invasive aspergillosis.
Device Description		
Sample Matrix	Urine	Serum
Controls	*Histoplasma* antigens	*Aspergillus* galactomannan
Output	"EIA Units" as determined from standard curve	"Index" as determined by OD of sample divided by Cut-Off Control OD
Detection Antibody	Biotinylated anti-*Histoplasma* polyclonal antibody	HRP-linked anti-*Aspergillus* monoclonal antibody
Microplate	96-well microplate coated with anti-Histoplasma polyclonal antibody	96-well microplate coated with anti-Aspergillus monoclonal antibody
Assay Time	~3 hours	~2 hours
Performance Characteristics		
Shelf life	1 year	Each kit component different

Analytical Performance Summary

A. Urine Precision Studies

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 5 days, using multiple operators. At the clinical laboratory, each sample was tested in triplicate over the course of 3 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.

Variance was estimated by calculating the mean value of each sample, the standard deviation and percent CV. 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 (≤ 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 2-4 below.

Table 2. 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%
	Day 2 Operator 2	Ave	-0.003	0.039	0.065	0.061	0.612	0.172
		SD	0.006	0.002	0.001	0.002	0.024	0.008
		%CV	183%	4.0%	0.9%	2.5%	3.9%	4.9%
	Day 3 Operator 3	Ave	0.011	0.048	0.072	0.064	0.642	0.182
		SD	0.001	0.003	0.004	0.009	0.078	0.007
		%CV	9.1%	5.2%	4.9%	13.5%	12.2%	3.9%
	Day 4 Operator 4	Ave	-0.001	0.045	0.071	0.077	0.654	0.195
		SD	0.010	0.005	0.003	0.007	0.018	0.005
		%CV	766%	11.8%	4.3%	9.1%	2.7%	2.4%
	Day 5 Operator 4	Ave	0.007	0.042	0.061	0.069	0.620	0.142
		SD	0.001	0.002	0.015	0.002	0.031	0.011
		%CV	17.3%	3.7%	24.5%	2.2%	5.0%	8.0%
IMMY	Day 1 Operator 1	Ave	0.012	0.049	0.070	0.081	0.686	0.204
		SD	0.003	0.010	0.014	0.011	0.039	0.027
		%CV	24.2%	20.6%	19.4%	13.6%	5.7%	13.0%
	Day 2 Operator 1	Ave	0.004	0.039	0.068	0.066	0.623	0.226
		SD	0.002	0.002	0.010	0.005	0.043	0.028
		%CV	57.1%	4.5%	15.1%	7.6%	6.8%	12.5%

	Day 3 Operator 1	Ave	0.003	0.045	0.067	0.076	0.723	0.216
		SD	0.001	0.006	0.009	0.004	0.051	0.004
		%CV	36.4%	13.6%	13.3%	5.3%	7.0%	1.6%
	Day 4 Operator 2	Ave	0.008	0.043	0.064	0.068	0.653	0.202
		SD	0.003	0.006	0.009	0.006	0.025	0.017
		%CV	38.6%	15.1%	14.7%	8.1%	3.8%	8.4%
	Day 5 Operator 2	Ave	0.005	0.042	0.072	0.080	0.663	0.228
		SD	0.002	0.005	0.008	0.008	0.034	0.006
		%CV	31.6%	12.2%	11.3%	10.3%	5.1%	2.7%

Table 3. Inter-Run Reproducibility Analysis

	Inter-Run Analysis - Blanked OD Values						
		Neg. Urine	C5	C50	C95	Moderate Urine	Positive Control
	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%

Table 4. 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%

B. Analytical Sensitivity (Lower limits of the assay)

Analytical sensitivity was determined (according to CLSI EP17-A) by determining the assay's Limit of the Blank and Limit of Detection. Wash buffer was spiked with *Histoplasma* antigens at a concentration range of 1 – 4 EIA Units. To determine the LoB, 82 replicates of wash buffer were assayed. To determine the LoD, 30 replicates of the spiked wash buffer were assayed. The Limit of the Blank is 0.009 OD and the Limit of Detection is 2 EIA Units (0.044 Blanked OD).

C. Analytical Specificity (Cross-Reactivity)

Urine specimens that tested negative for *Histoplasma* antigen were spiked with antigen from *Blastomyces dermatitidis, Coccidioides immitis, Aspergillus* sp., *Paracoccidioides brasilliensis, Candida albicans,* and *Cryptococcus neoformans*, individually at 1ug/ml. The ALPHA *Histoplasma* Antigen EIA is found to be cross-reactive with *Blastomyces dermatitidis, Coccidioides immitis,* and *Paracoccidioides brasilliensis*. The assay is not cross-reactive with *Candida albicans, Cryptococcus neoformans*, or *Aspergillus* sp. in urine. Furthermore, *Aspergillus, Candida, Paracoccidioides, and Penicillium* culture-positive urine specimens were tested in the ALPHA *Histoplasma* Antigen EIA. The results are summarized in Table 5.

Table 5. Specificity Using Culture-Confirmed Urine Specimens

	Percent Positive
Aspergillus	0% (0/20)
Candida spp.	0% (0/12)
Paracoccidioides	9.1% (1/11)
Penicillium	0% (0/2)

D. Analytical Interference

To evaluate substances that could potentially interfere with the ALPHA *Histoplasma* Antigen EIA, urine specimens containing various substances were obtained from a national reference laboratory. Substances included protein, blood, epithelial cells, ketones, mucus, casts, glucose, and bilirubin. Each specimen was spiked with *Histoplasma* antigen and tested. None of these substances were found to interfere with the ALPHA *Histoplasma* Antigen EIA. 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.

E. Carry-over

To examine potential well-to-well carry-over, a positive sample was used in series alternating with a negative sample in a checkerboard pattern. Samples were found not to carry-over when methods described in the package insert were followed.

F. Prozone Effect

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 and tested. All samples remained positive, therefore, prozoning is not seen in the ALPHA *Histoplasma* Antigen EIA.

G. Linearity

N/A

H. Assay cut-off

The assay's cut-off of 2.0 EIA Units was established by Limit of Detection (Section B) experiments and confirmed with the determination of the C_{50}. Receiver Operator Curve (ROC) analysis of culture-proven specimens indicated a 1.3 EIA Unit cutoff. However, this is below the Limit of Detection (2.0 EIA units), and therefore an inappropriate cutoff.

Negative: < 2.0 EIA Units
Positive: ≥ 2.0

I. Single Versus Dual Wavelength Analysis

Eight positive urine samples and the 4 standards were tested in duplicate and according to the package insert. The microwells were read at 450/630 nm and then at 450 nm only. The study confirmed that the *blanked* OD of a sample is not affected by the read method, as indicated by the low percent CVs across both read methods. Furthermore, 450/630 vs. 450 alone can be used with equal accuracy, and data from the two methods can be used interchangeably.

J. 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. While the decrease was not significant, it is still preferable to read the assay as soon after the addition of the Stop Solution as possible to avoid errors.

K. Specimen Acceptance Criteria

In specimen storage studies, reductions in EIA values after 2-week storage at 4°C and after multiple freeze thaws were observed. However, no specimen went from positive to negative (Figures 1 and 2). 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.

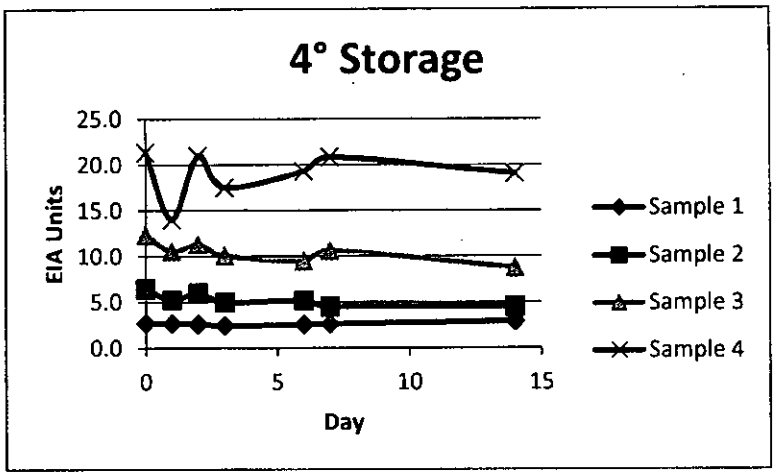

Figure 1. EIA Units Versus Days Stored at 4°C

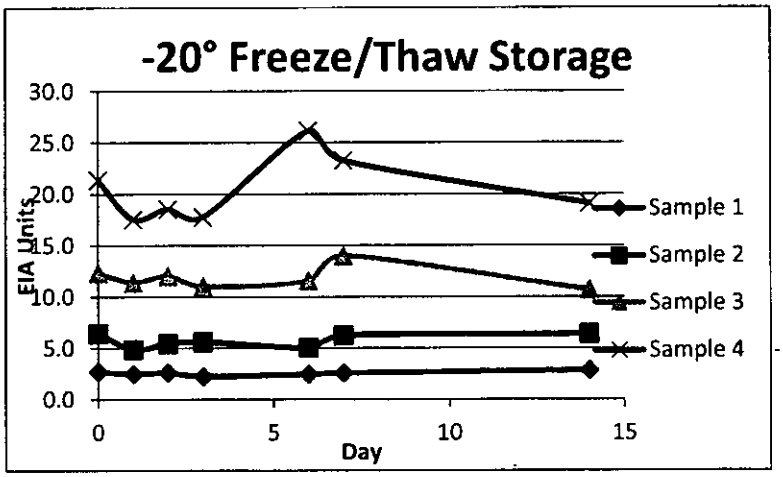

Figure 2. EIA Units Versus Days Stored at -20°C

L. Method Comparison

Comparison to Culture/Histopathology:

The clinical performance of the ALPHA *Histoplasma* Antigen EIA was evaluated using a total of 278 culture- or histopathology-confirmed urine specimens. The results are summarized in Tables 6 and 7.

Table 6. 2x2 Contingency Table Comparing Device to Culture/Histopathology

		Culture/Histopathology	
		Positive	Negative
IMMY ALPHA *Histoplasma* EIA	Positive	38	3
	Negative	9	228

Table 7. Statistical Analysis: Device vs Culture/Histopathology

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

Comparison to Other *Histoplasma* Antigen EIA

Supplemental comparison studies were performed using a non-FDA-cleared *Histoplasma capsulatum* quantitative antigen EIA. The other test has an equivocal range and therefore, analysis was performed by either considering the equivocals positive or negative. The resulting 2x2 contingency tables (Tables 8 and 10) and statistical analysis (Tables 9 and 11) are below.

Table 8. 2x2 Contingency Table Comparing Device to other EIA; Equivocals Assumed Positive

		Other *Histo* Antigen EIA	
		Positive	Negative
IMMY ALPHA	Positive	35	3
Histoplasma EIA	Negative	14	47

Table 9. Statistical Analysis

	Point Estimate	95% CI
% Agree Positive	71.4%	57.6-82.2%
% Agree Negative	94.0%	83.8-97.9%

Table 10. 2x2 Contingency Table Comparing Device to other EIA; Equivocals Assumed Negative

		Other *Histo* Antigen EIA	
		Positive	Negative
IMMY ALPHA	Positive	35	3
Histoplasma EIA	Negative	3	58

Table 11. Statistical Analysis

	Point Estimate	95% CI
% Agree Positive	92.1%	79.2-97.3%
% Agree Negative	95.1%	86.5-98.3%

Conclusion

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

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Immuno-Mycologics, Inc
c/o Sean K. Bauman, Ph.D.
President and CEO
2700 Technology Place
Norman, OK 73071

JUL 19 2011

Re: k101407

Trade/Device Name:	ALPHA *Histoplasma* Antigen EIA
Regulation Number:	21CFR §866.3320
Regulation Name:	*Histoplasma capsulatum* serological reagents.
Regulatory Class:	Class II
Product Code:	MIZ
Dated:	July 11, 2011
Received:	July 12, 2011

Dear Dr. Bauman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050. This letter will allow you to begin marketing your device as described in your Section

510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use Statement

510(k) Number (if known): K101407

Device Name: ALPHA *Histoplasma* Antigen EIA

Indications 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.

Prescription Use ✓ AND/OR Over-The-Counter Use ____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k): K101407