510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K120946

B. Purpose for Submission:

To obtain substantial equivalence for an original 510(k) for a device which detects Cryptococcal antigen.

C. Measurand:
Capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*)

D. Type of Test:
Qualitative and semi-quantitative enzyme immunoassay

E. Applicant:
Immuno-Mycologics, Inc.

F. Proprietary and Established Names:

ALPHA Cryptococcal Antigen EIA

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
GMD	II	866.3165	83-Microbiology

H. Intended Use:

1. Intended use:

The ALPHA Cryptococcal Antigen enzyme immunoassay (CrAg EIA) is a qualitative or semi-quantitative (titration) test system for the detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebrospinal fluid (CSF). The ALPHA Cryptococcal Antigen Enzyme Immunoassay is an assay which can be used as an aid in the diagnosis of cryptococcosis. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.

2. Indication for use:

   The ALPHA Cryptococcal Antigen enzyme immunoassay (CrAg EIA) is a qualitative or semi-quantitative (titration) test system for the detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebrospinal fluid (CSF). The ALPHA Cryptococcal Antigen Enzyme Immunoassay is an assay which can be used as an aid in the diagnosis of cryptococcosis. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Spectrophotometer microplate reader equipped with 450 nm and/or 450/630 nm filters

I. Device Description:

The ALPHA Cryptococcal Antigen Enzyme Immunoassay (EIA) is a direct immunoenzymatic sandwich microplate assay which detects Cryptococcus antigens in serum and CSF. Anti- Cryptococcus antibodies bound to microwell plates are used as capture antibodies, and horseradish peroxidase (HRP)-conjugated anti-Cryptococcus antibodies are used as detection antibodies. The positive control and standard curve material are composed of cryptococcal capsular polysaccharide antigen in a buffered protein solution with a preservative.

Either serum or CSF is added to the microwells coated with the capture antibodies and incubated. If the patient specimen contains cryptococcal antigens that are recognized by the capture antibodies, those antigens will become bound to the microwells. The microwells are washed to remove unbound patient material, and HRP-conjugated detect antibody is added to the wells. If Cryptococcus antigens are bound to the microwells by the capture antibodies, the detect antibody will also become bound to the microwells. The wells are then washed to remove any unbound detect antibody. Next, tetramethylbenzidine (TMB) substrate is added to the microwells, and in the presence of HRP, a blue color will develop. The reaction is stopped by the addition of a stop solution. The optical density (OD) is determined with a microplate reader at 450 nm with reference at 630 nm (reference is optional).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Premier™ Cryptococcal Antigen EIA

2. Predicate 510(k) number(s):
K904393

3. Comparison with predicate:

SIMILARITIES		
Feature	**Device: ALPHA Cryptococcal Antigen EIA**	**Predicate: Premier Cryptococcal Antigen EIA**
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus*	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus*
Indication For Use	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis
Assay Principle	Enzyme immunoassay	Enzyme immunoassay
Sample Matrix	Serum and CSF	Serum and CSF
Assay Components	Antibody coated 96-well microplate, wash buffer, positive control, enzyme conjugate, TMB substrate, stop solution, sample diluent	Antibody coated 96-well microplate, wash buffer, positive control, enzyme conjugate, TMB substrate, stop solution, sample diluent
Detection Chemistry	HRP + TMB	HRP + TMB
Instruments	Optical Density Microplate Reader	Optical Density Microplate Reader
Detection Antibody	Anti-cryptococcal monoclonal antibody	Anti-cryptococcal antibody
Specimen Pre-treatment	None	None

DIFFERENCES		
Feature	**Device: ALPHA Cryptococcal Antigen EIA**	**Predicate: Premier Cryptococcal Antigen EIA**
Output	Positive or Negative Only	Positive, Negative, or Indeterminate
Reagent Application	Pipette or equivalent	Reagent dropper
Optional Visual Read	No	Yes

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

CLSI EP17-A2 Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.

L. Test Principle:

In the qualitative procedure, specimens are analyzed undiluted. In the semi-

quantitative titration procedure, specimens are analyzed after serial dilution in specimen diluent. Either serum or CSF is added to the microwells coated with the capture antibodies and incubated. If the patient specimen contains cryptococcal antigens that are recognized by the capture antibodies, those antigens will become bound to the microwells. The microwells are washed to remove unbound patient material, and HRP-conjugated detect antibody is added to the wells. If Cryptococcus antigens are bound to the microwells by the capture antibodies, the detect antibody will also become bound to the microwells. The wells are then washed to remove any unbound detect antibody. Next, tetramethylbenzidine (TMB) substrate is added to the microwells, and in the presence of HRP, a blue color will develop. The reaction is stopped by the addition of a stop solution. The optical density (OD) is determined with a microplate reader at 450 nm with reference at 630 nm (reference is optional).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The ALPHA Cryptococcal Antigen EIA was evaluated for reproducibility and precision by spiking serum and mock CSF with cryptococcal antigen to produce a panel consisting of a negative sample, a high negative (C5) sample, a low positive sample and a moderate positive sample. This panel was tested twice per day at three sites (Immuno-Mycologics, Inc., a national reference lab, and a clinical lab) with a total of five operators over a five-day period in order to determine both the inter-lab and the intra-lab reproducibility and precision of the assay.

The percent coefficient of variation (CV) for the positive CSF specimens ranged from 6.0% to 9.4% at the individual sites. The percent CV of the CSF results averaged over all sites ranged from 21.1% to 23.3%. The percent coefficient of variation for the positive serum specimens ranged from 7.7% to 13.6% at the individual sites. The percent CV of the serum results averaged over all sites ranged from 13.05% to 14.16%.

The study results are listed below.

		IMMY			National Reference Lab			Clinical Lab			Combined Data (3 Sites)		
Description	**Type**	Ave O.D.	Std Dev	% CV	Ave O.D.	Std Dev	% CV	Ave O.D.	Std Dev	% CV	Ave O.D.	Std. Dev.	% CV
Blank	Control	0.066	0.012	17.8	0.000	0.0027	3686.2	0.075	0.005	7.0	0.046	0.034	74.67
CRYPC1	Control	1.814	0.078	4.3	1.973	0.1083	5.5	2.473	0.158	6.4	2.059	0.295	14.34
Negative	Serum	0.022	0.011	48.7	0.012	0.0058	48.8	0.019	0.005	25.4	0.018	0.009	50.25
High Negative	Serum	0.040	0.008	19.2	0.028	0.0077	27.7	0.035	0.007	20.0	0.034	0.009	26.51
Low Positive	Serum	0.420	0.041	9.7	0.338	0.0436	12.9	0.372	0.038	10.3	0.377	0.053	14.16
Moderate Positive	Serum	1.683	0.229	13.6	1.666	0.1281	7.7	1.959	0.205	10.5	1.756	0.229	13.05
Negative	CSF	0.065	0.018	27.8	0.058	0.0087	15.1	0.099	0.010	10.0	0.072	0.022	29.85
High Negative	CSF	0.179	0.020	11.1	0.174	0.0190	10.9	0.298	0.027	9.2	0.211	0.059	28.09

Low Positive	CSF	0.346	0.028	8.0	0.339	0.0319	9.4	0.533	0.039	7.3	0.397	0.092	23.27
Moderate Positive	CSF	0.629	0.039	6.2	0.606	0.0443	7.3	0.929	0.056	6.0	0.707	0.149	21.13

The overall percent positive and percent negative result reproducibility was calculated by combining the data from all three sites. The reproducibility of results from low positive, moderate positive and negative samples was 100%.

Serum	Site 1 % Positive	Site 2 % Positive	Site 3 % Positive	Overall % Positive
Negative	0%	0%	0%	0%
High Negative	0%	0%	0%	0%
Low Positive	100%	100%	100%	100%
Moderate Positive	100%	100%	100%	100%

CSF	Site 1 % Positive	Site 2 % Positive	Site 3 % Positive	Overall % Positive
Negative	0%	0%	0%	0%
High Negative	0%	0%	83%	24%
Low Positive	100%	100%	100%	100%
Moderate Positive	100%	100%	100%	100%

b. *Linearity/assay reportable range:* Not applicable

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

Controls:

The Positive Control and Negative Control should 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.

Reagent	**Acceptable Blanked OD Parameters**
Positive Control	> 0.265
Negative Control	< 0.090

If the blanked OD of the Positive Control is not within these parameters, patient test results should be considered invalid and the assay should be repeated.

High Dose Hook Effect:

The effects of high doses were determined by spiking a serum specimen pool that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and ALPHA Cryptococcal Antigen EIA, with cryptococcal antigen at 1 mg/ml and testing it in triplicate at IMMY on one lot of ALPHA Cryptococcal Antigen EIA, according to the package insert.

It was observed that at extremely high concentrations, the signal can be

reduced due to the high dose hook effect. Although the signal is reduced leading to lower than expected EIA values, the result is still positive. The semi-quantitative titration procedure can differentiate between a low EIA score due to the high dose hook effect and a low EIA score due to low concentrations of Cryptococcal Antigen.

Sample stability:

Three CrAg-Negative human CSF specimens and four CrAg-Negative human serum specimens were spiked with concentrations of cryptococcal antigen near and slightly above the cutoff of the assay. The specimens were tested on the ALPHA CrAg EIA immediately after spiking and then they were frozen at < -20 °C for the Day 0 time point. The specimens were then divided into three aliquots and tested as follows:

- Aliquot 1 – Tested daily in triplicate, subjected to a freeze-thaw cycle each day
- Aliquot 2 – Frozen for one week, thawed and tested in triplicate
- Aliquot 3 – Frozen for two weeks, thawed and tested in triplicate

A reduction in OD values was observed after multiple freeze-thaw cycles and after storage at < -20 °C for one week. The OD value reductions were large enough to cause some very low positive samples (near 0.300 Blanked OD) to become negative when stored frozen for one week or subjected to more than two freeze-thaw cycles. Specimens should be tested as soon as possible after collection and multiple freeze-thaw cycles should be avoided.

d. Detection limit/ Analytical cut-off:

The limit of detection (LoD) was estimated at IMMY according to Clinical and Laboratory Standards Institute (CLSI) EP-17A. The limit of the blank (LoB) was estimated by running 80 replicates of normal human serum and 80 replicates of artificial CSF. The initial LoD was estimated by running 20 replicates of 4 concentrations near the LoB in both serum and CSF.

The LoD was established by testing 20 replicates of both serum and CSF spiked with Cryptococcal antigen at a range of concentrations near the initial LoD. Results were considered positive if they yielded a blanked OD that was greater than the cut-off.

Serum data:

Concentration	% Positive
4.9 ng/mL	0% (0/24)
5.0 ng/mL	8% (2/24)
5.1 ng/mL	54% (13/24)
5.2 ng/mL	71% (17/24)
5.3 ng/mL	100% (24/24)

CSF data:

Concentration	% Positive
1.3 ng/mL	0% (0/24)
1.4 ng/mL	0% (0/24)
1.5 ng/mL	4% (1/24)
1.6 ng/mL	83% (20/24)
1.7 ng/mL	100% (24/24)

Analysis shows that the LoD for serum is 5.3 ng/ml and the LoD for CSF is 1.7 ng/ml.

e. Analytical specificity:

Cross reactivity:

Analytical specificity for the ALPHA Cryptococcal Antigen EIA was determined by running specimens from potentially cross-reacting medical conditions unrelated to cryptococcosis. The following specimens were run on one lot of the ALPHA Cryptococcal Antigen EIA. A total of 118 serum specimen and 15 fungal culture filtrates were tested. Culture filtrates were tested at three different dilutions: undiluted, 1:10, and 1:100. Dilutions were made in 1X Specimen Diluent. Percent positive was determined for each condition.

Pathology	**Number of Specimens**	**Number of Positives**	**% Positive**
HAMA	5	0	0
Syphilis	10	0	0
Rubella	5	0	0
Mycoplasma	10	0	0
Toxoplasmosis	7	0	0
CMV Infection	10	0	0
Rheumatoid factor	10	0	0
Penicilliosis	5	0	0
Sporotrichosis	6	0	0
Blastomycosis	10	0	0
Coccidioidomycosis	10	0	0
Histoplasmosis	10	0	0
Candidiasis	10	0	0
Aspergillus GM+	10	1	10

Culture filtrate results:

Organism	Number of Specimens	Number of Positives	% Positive
Aspergillus terreus	3	0	0
Aspergillus niger	3	0	0
Aspergillus flavus	3	0	0
Aspergillus fumigatus	3	0	0
Paracoccidioides brasiliensis	3	2	67

As expected, some fungal cross-reactivity was observed. One *Aspergillus* galactomannan positive specimen (positive by Bio-Rad's Platelia™ Aspergillus EIA) was positive in the ALPHA Cryptococcal Antigen EIA and *Paracoccidioides brasiliensis* culture filtrates undiluted and 1:10 were positive as well. The total percent positive for fungal pathologies was 3.9%, well below the acceptance criteria of 10%. However, *Paracoccidioides brasiliensis* culture filtrate had a total percent positive of 67%.

Rheumatoid factor is known to cause false-positive results in the Cryptococcal latex agglutination method. Rheumatoid factor did not cause false positives in the ALPHA Cryptococcal Antigen EIA between the range of 112 IU/ml and 6479 IU/ml. The normal reference range for rheumatoid factor is 0-14 IU/ml.

Interfering substances:

Interference testing was performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C_{95} concentration. All specimens were then tested at IMMY on one lot of the ALPHA Cryptococcal Antigen EIA in triplicate: spiked and unspiked. Percent positivity was determined for each condition. All of the unspiked specimens had negative results on the ALPHA Cryptococcal Antigen EIA. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the ALPHA Cryptococcal Antigen EIA.

f. Assay cut-off:

The assay cutoff was determined through Receiver Operator Curve (ROC) analysis using the IMMY CrAg Lateral Flow Assay (LFA) as the means of classifying a specimen as a true positive or negative. ROC Analysis was performed on a dataset consisting of 995 combined serum and CSF specimens

ROC Analysis suggested a blanked OD cutoff value of greater than 0.265 which yielded a positive percent agreement of 97.4% and a negative percent agreement of 99.9% when compared to the IMMY CrAg LFA. This cutoff

was confirmed through the method comparison comparing the results from the IMMY Cryptococcal Antigen EIA to the predicate device.

Assay cut-offs

$x \leq 0.265$	Negative
$x > 0.265$	Positive

2. Comparison studies:

a. Method comparison with predicate device:

A three-site (IMMY and two national reference laboratories) split specimen comparison study was performed on both serum and CSF specimens that had been submitted for cryptococcal antigen EIA testing. A total of 1782 specimens (CSF n=426; serum n=1356) were tested in both the ALPHA Cryptococcal Antigen EIA and a commercial Cryptococcal antigen EIA according to their respective package inserts. The resulting data from the split sample comparison is shown in the tables below.

Serum 2x2 Contingency Table

		Other Manufacturer's EIA	
		Pos	Neg
IMMY ALPHA CrAg EIA	Pos	131	28
	Neg	2	1195

Serum Data Analysis

	Calculated	95% CI
% Positive Agreement	98.5% (131/133)	94.7 - 99.6%
% Negative Agreement	97.7% (1195/1223)	96.7 - 98.4%

CSF 2x2 Contingency Table

		Other Manufacturer's EIA	
		Pos	Neg
IMMY ALPHA CrAg EIA	Pos	29	3
	Neg	2	392

CSF Data Analysis

	Calculated	95% CI
% Positive Agreement	93.5% (29/31)	79.3 - 98.2%
% Negative Agreement	99.2% (392/395)	97.8 - 99.7%

Supplemental studies:

A multi-site split-specimen comparison study was performed on both serum and CSF specimens that had been submitted for cryptococcal antigen EIA testing. A total of 1782 specimens (CSF n=426; serum n=1356) were tested in both the ALPHA Cryptococcal Antigen EIA and the IMMY Cryptococcal Antigen Lateral Flow Assay (LFA) according to their respective package inserts. Analysis of this data yielded percent positive agreements of 96.9% and 93.8% in serum and CSF, respectively. Analysis indicated percent negative agreements of 99.8% and 99.5% in serum and CSF, respectively.

b. Matrix comparison:

See sections M1a and M1d for comparisons of assay Precision, Reproducibility, and detection limits in serum and CSF matrices.

3. Clinical studies:
   a. Clinical sensitivity: Not applicable

   b. Clinical specificity: Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable): See M2a method comparison with predicate device

4. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

   The frequency of cryptococcosis is dependent on several factors including: patient population, type of institution, and epidemiology. In these studies, the IMMY Alpha Cryptococcal Antigen EIA exhibited 97.6% agreement positive and 98.1% agreement negative with another manufacturer's EIA (serum and CSF combined).

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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