

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K102286

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 dipstick sandwich lateral flow immunochromatographic assay

E. Applicant:

Immuno-Mycologies, Inc.

F. Proprietary and Established Names:

CrAg Lateral Flow Assay

G. Regulatory Information:

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

H. Intended Use:

1. Intended use:

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum.

2. Indication for use:

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum.

The CrAg Lateral Flow Assay is a prescription use laboratory assay which can aid in the diagnosis of cryptococcosis

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Not applicable

I. Device Description:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay which detects cryptococcal antigen in serum. The assay consists of CrAg Lateral Flow test strips which have a gold-conjugated antibody and a gold-conjugated, anti-cryptococcal antibody deposited onto a sample membrane and anti-Crypto antibody and control-line capture antibody striped onto a membrane. Also in the kit is a specimen diluent.

J. Substantial Equivalence Information:

1. Predicate Device names

Latex-*Cryptococcus* Antigen Detection System

2. Predicate K number:

K791382

Comparison with predicate:

Table 1: Comparison Between New Device and Predicate Device

SIMILARITIES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex-Cryptococcus Antigen Detection System (K791382)
Intended Use		
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum and CSF
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
Device Description		
Sample Matrix	Serum	Serum and CSF
Instruments	None	None
Detection Antibody	Anti-cryptococcal monoclonal antibody	Anti-cryptococcal antibody
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex-Cryptococcus Antigen Detection System (K791382)
Intended Use		
Intended Use	No difference	No difference
Indication For Use	No difference	No differences
Device Description		
Assay Principle	Lateral flow assay	Latex agglutination
Assay Components	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies	Positive control, negative control, latex cards, latex conjugated anti-cryptococcal polyclonal antibodies
Serum Pre-Treatment	Serum dilution	Pronase with pronase inhibitor
CSF Pre-Treatment	none	Boil
Detection Antibodies	Gold-conjugated anti-cryptococcal monoclonal antibodies	Latex-conjugated anti-cryptococcal polyclonal antibodies
Storage Requirements	20-25°C	4°C ± 2°

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

Not Applicable

L. Test Principle:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum. For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal monoclonal antibodies and gold-conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti-cryptococcal antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated control goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Serum:

Repeatability and reproducibility with serum specimens were determined by spiking a serum specimen pool that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System with cryptococcal antigen at four concentrations: Negative, high negative (C_5), low positive (near C_{95}), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5-A2. One site was internal (Site 1) and two were a South African reference laboratory (Site 2) and a US hospital laboratory (Site 3). For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent

negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

SERUM PANEL	Site 1 % Pos	Site 2 % Pos	Site 3 % Pos	Overall % Pos
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	7% (2/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

As expected, the high negative tested positive nearly 5% of the time and 3% of the time in serum. All other samples performed 100% as expected across all sites, all runs, and all operators.

b. Linearity/assay reportable range:

Not Applicable

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

The CrAg Lateral Flow Assay has a three-month expiration date when stored at room temperature. Real-time stability testing is on-going. Initial accelerated stability was determined by testing three lots of kits stored at 25°C and 37°C for three months. Buffer was spiked with cryptococcal antigen at four concentrations: 1.5, 3.0, 6.0 and 9.0ng/ml. Non-spiked buffer was used as a negative control. Each sample was tested in triplicate by one operator. Each spiked sample replicate tested positive and each negative control tested negative on all lots of the CrAg Lateral Flow Assay, stored at both 25°C and 37°C. This initial data indicate that the CrAg Lateral Flow Assay is stable at room temperature for three months. Stability studies are on-going to demonstrate a one-year expiration dating.

d. Detection limit:

Detection Limit/Analytical Cut-off

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running varying concentrations of cryptococcal antigen diluted in LF Specimen Diluent. Test results determined that the analytical cut-off is 1.25ng/ml (Table 3).

Table 3. Analytical Cut-Off

Sample Concentration (ng/ml)	No. Positive	No. Tested	% Positive
0.50	0	24	0%
0.75	0	24	0%
1.00	4	24	17%
1.25	12	24	50%
1.50	21	24	88%
1.75	24	24	100%
2.00	24	24	100%
2.50	24	24	100%
3.00	24	24	100%
3.50	24	24	100%
4.00	24	24	100%

Analytical specificity:**Table 4. Analytical Specificity**

Pathology	No. of Specimens	No. of Replicates Per Specimen	Total Tests	Total Number of Positives	% Positive (No Pos/Total)
Non-Fungal Pathologies					
HAMA (Human anti-Mouse antibody) Positive	5	3	15	0	0%
Syphilis	10	3	30	0	0%
Rubella	5	3	15	0	0%
Mycoplasma	10	3	30	0	0%
Toxoplasmosis	7	3	21	0	0%
CMV Infection	10	3	30	0	0%
Rheumatoid factor*	10	3	30	0	0%
Total	57	3	171	0	0%
Fungal Pathologies					
Penicilliosis	5	3	15	0	0%
Sporotrichosis	6	3	18	0	0%

Blastomycosis	10	3	30	0	0%
Coccidioidomycosis	10	3	30	0	0%
Histoplasmosis	10	3	30	0	0%
Candidiasis	10	3	30	0	0%
Aspergillosis **	10	3	30	3	10%
<i>Aspergillus terreus</i> Culture Filtrate	3	3	9	0	0%
<i>Aspergillus niger</i> Culture Filtrate	3	3	9	0	0%
<i>Aspergillus flavus</i> Culture Filtrate	3	3	9	0	0%
<i>Aspergillus fumigatus</i> Culture Filtrate	3	3	9	0	0%
<i>Paracoccidioides brasiliensis</i> Culture Filtrate	3	3	9	6	67%
Total	76	3	228	9	3.9%

* Rheumatoid factor concentrations tested ranged from 112IU/ml to 6479IU/mls.

** The three positives were the result of three replicates of the same specimen, which was positive.

In addition to the cross-reactivity study, interference testing was also performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C95 concentration. All specimens were then tested at IMMY, on one lot of CrAg Lateral Flow assay in triplicate: spiked and unspiked. Percent positivity was determined for each condition. All of the unspiked specimens had negative results on the CrAg Lateral Flow Assay. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the CrAg Lateral Flow Assay. However, it is possible that hemolyzed samples could lead to false negatives due to the high background color on the strip. As such, a statement is included in the Limitations of the Procedure section of the package insert:

“Hemolyzed serum samples could lead to false negative results due to the high background color on the strip.”

Due to specimen availability, the following conditions were not tested in the CrAg Lateral Flow Assay: *Candida dubliniensis*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, *Cladosporium trichoides*, *Neisseria meningitidis*, *Salmonella typhi*, *Pneumocystis carinii*, *Trichosporon beigeli*,

Zygomycetes, ANA+, HAV, HCV, *Staph*, and *Strep*.

f. Assay cut-off:

High dose hook effect concentrations with serum specimens were determined by spiking negative serum that were negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

2. Comparison studies:

a. Method comparison with predicate device:

The CrAg Lateral Flow Assay was compared to the gold standard diagnoses of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay. These studies contained a mix of both prospective and retrospective specimens. A summary table of the data collected is included below.

Serum	Culture/India Ink	
	Positive	Negative
CrAg	91	0
LFA Assay	0	123

Serum	Calculated	95% CI
Sensitivity	100%	96.0% - 100%
Specificity	100%	97.0% - 100%

b. Matrix comparison:

Serum Specimens

A panel of 197 serum specimens that were submitted to a US reference laboratory for cryptococcal antigen testing, using a Cryptococcal Antigen EIA, were collected and stored frozen until method comparison studies were performed. Of the 197 specimens, 96 were positive and 101 were negative by the EIA. All specimens were analyzed, according to EP12-A2, concurrently in the CrAg Lateral Flow Assay and in the IMMY Latex-*Cryptococcus* Antigen Detection System to ensure the data was not affected by the freeze-

thaw cycle. Each specimen was tested at IMMY in duplicate in both tests according to each test's package insert. The data is presented in a 2x2 contingency table (Table 8). The percent agreement positive, percent agreement negative, and 95% confidence interval are also presented (Table 9).

Table 8. Serum 2x2 Contingency Table

	EIA (+)	EIA (-)
CrAg Lat Flow (+)	96	7
CrAg Lat Flow (-)	0	94

Table 9. Serum Statistical Analysis

		95% CI
% Agreement Positive	100%	96-100%
% Agreement Negative	93%	86-97%

Semi-Quantitative Method Comparison

In addition, 62 of these specimens were tested using the semi-quantitative titration procedure in both the CrAg Lateral Flow Assay and the IMMY Late Cryptococcal Antigen Detection System (REF CR1003). Linear regression analysis of the data yielded an R² value of 0.890

3. Clinical studies:

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

Not Applicable

N. Proposed Labeling:

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

O. Conclusion:

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