510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K112422

B. Purpose for Submission:

To obtain substantial equivalence for a modification to add CSF to the original 510(k) 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-Mycologics, 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 the capsular polysaccharide antigens of *Cryptococcus* species complex *(Cryptococcus neoformans and Cryptococcus gattii)* in serum and cerebral spinal fluid (CSF).

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, and cerebral spinal fluid (CSF).

   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 cerebral spinal fluid (CSF). 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. The kit also includes a specimen diluent.

J. Substantial Equivalence Information:

1. Predicate Device name

   Immuno-Mycologics' CrAg Lateral Flow Assay

2. Predicate K number:

   K102286

Comparison with predicate:

Table 1: Comparison Between New Device and Predicate Device

SIMILARITIES		
Feature	**CrAg LFA (New Device – K112422)**	**CrAg LFA (Serum Only) (K102286)**
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		
Technology	Lateral Flow Assay	Lateral Flow Assay
Instruments	None	None
Assay Components	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies
Specimen Pre-Treatment	Dilution	Dilution
Detection Antibody	Anti-cryptococcal monoclonal antibody	Anti-cryptococcal monoclonal antibody
Storage Requirements	20-25°C	20-25°C
DIFFERENCES		
Feature	**Cryptococcal Antigen Lateral Flow: New Device – K112422**	**Cryptococcal Antigen Lateral Flow: Serum Only – K102286**
Intended Use		
Intended Use	Immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus species complex (Cryptococcus neoformans and Cryptococcus gatti)* in serum and CSF	Immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus species complex (Cryptococcus neoformans and Cryptococcus gatti)* in serum
Sample Matrix	CSF	Serum

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 CSF. 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.

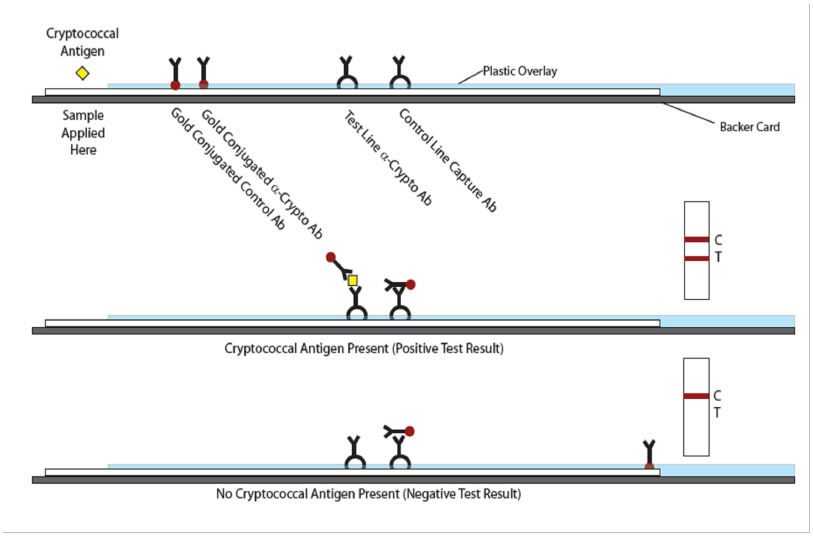

Figure1. CrAg Lateral Flow Assay Schematic

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The CrAg Lateral Flow Assay was evaluated for reproducibility and precision by spiking mock CSF samples with cryptococcal antigen to produce a panel consisting of a negative sample, a high-negative (C_5) sample, a low-positive sample and a moderate-positive sample. This panel was tested twice per day at three sites 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 results of this study are shown in the tables below.

Table 2. Repeatability at 3 Different Sites

CSF 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	10% (3/30)	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)

b. Linearity/assay reportable range:

Not Applicable

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

Real time stability testing is on-going. Initial accelerated stability was determined by testing three lots of kits stored at 25°C (real-time) for 18 months and 37°C (accelerated) for 12 months. LF specimen Diluent was spiked with cryptococcal antigen at four concentrations: 1.5, 3.0, 6.0 and 9.0ng/ml. Non-spiked diluent 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 kits, stored at both 25°C and 37°C after storage for six months. At 12 months, all samples passed on all kits stored at 25°C. Kits stored at 37°C were not tested. This initial data in addition to established stability of currently available lateral flow tests indicate that the CrAg Lateral Flow test kit is stable at room temperature for 18 months.

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. The concentration where 50% of the results were positive and 50% of the results were negative determined our analytical cut-off. The analytical cut-off is 1.25ng/ml.

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%

Analytical specificity:

Analytical specificity for the CrAg Lateral Flow Assay was determined by evaluating potentially cross-reacting medical conditions unrelated to cryptococcosis. Specimens were tested in triplicate. Percent positive was determined for each condition (Table 3).

Table 3. Analytical Specificity

Pathology	# of Samples	% Positive
Penicilliosis	5	0 % (0/5)
Sporothrichosis	6	0 % (0/6)
HAMA	5	0 % (0/5)
Syphilis	10	0 % (0/10)
Rubella	5	0 % (0/5)
Mycoplasmosis	10	0 % (0/10)
Toxoplasmosis	7	0 % (0/7)
CMV	10	0 % (0/10)
Blastomycosis	10	0 % (0/10)
Coccidiomycosis	10	0 % (0/10)
Histoplasmosis	10	0 % (0/10)
Candidiasis	10	0 % (0/10)
Aspergillus GM+	10	10 % (1/10)
Rheumatoid Factor*	10	0 % (0/10)

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

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the CrAg Lateral Flow Assay at high concentrations (>0.1 mg/ml), antigens from *Paracoccidiodes brasiliensis* exhibitied some cross-reactivity. Antigens from *Aspergillus terreus, Aspergillus fumigatus, Aspergillus niger,* and *Aspergillus flavus* did not exhibit cross-reactivity.

The assay was not evaluated for cross-reactivity against the following organisms or pathologies:

Candida dubliniensis	*Pneumocystis carinii*
Candida tropicalis	*Trichosporon beigelii*
Candida parapsidosis	*Zygomycetes*
Candida krusei	Antinuclear antibody +
Candida glabrata	Hepatitis A Virus
Cladosporium trichoides	Hepatitis C Virus
Neisseria meningitidis	*Staphylococcus* spp.
Salmonella typhi	*Streptococcus pneumonia*
Mycobacterium tuberculosis	*Streptococcus* spp.
	Diphtheroid
Enterovirus	*H. influenzae* type B
Enterobacteriaceae	*Herpes simplex viruses*
Enterococcus spp.	*Listeria monocytogenes*
Epstein Barr	Syneresis fluid condensation
Trichosporon beigelii	*Staphylococcus aureus*

The assay was not evaluated for potential interference related to specimen pretreatment with 2-mercaptoethanol or with specimens including the following substances: bloody CSF, cloudy CSF, white blood cells, xanthochromic CSF, bilirubin, protein, systemic lupus erythmatosus (SLE), sarcoidosis, or *N.memingitides*.

The effect of pronase on the CrAg LFA was determined by pronase-treating five Cryptococcal EIA positive specimens and five Cryptococcal EIA negative specimens. The samples were analyzed both untreated and pronase-treated. All treated, positives samples remained positive and all treated, negative samples remained negative. Therefore, pronase does not affect the CrAg LFA.

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable

b. Matrix comparison:

A matrix comparison study was performed on 86 paired serum and CSF specimen

that were collected prospectively. Percent agreement positive is 100%. Percent agreement negative is 88.9%, and overall agreement is 96.5%. Of the 3 discrepant results, one patient was diagnosed with cryptococcal meningitis approximately 5-6 week after the LFA was performed.

3. Clinical studies:

The CrAg Lateral Flow Assay was compared to the standard reference method for diagnoses of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay. These studies contained prospective specimens. A summary of the data collected is included in the following table.

CSF		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	1
	Negative	0	77

CSF	Calculated	95% CI
Sensitivity	100%	94.4% - 100%
Specificity	98.7%	93.1% - 99.8%

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 this study, 100% (65/65) of true positives as determined by culture and/or India Ink were detected. The apparent false positive in the CrAg LFA, compared to culture/India ink, could be the result of CrAg detection having a higher sensitivity (100%) than culture (83%) and India ink (84%). Therefore, it is expected to have culture/India Ink negative/LFA positive results.

N. Proposed Labeling:

The labeling is sufficient and 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.