



Cryptococcal Antigen Lateral Flow Assay Performance Summary

Intended Use

The cryptococcal antigen lateral flow assay is an immunochromatographic test system for the qualitative or semiquantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebrospinal fluid (CSF). The CrAg lateral flow assay is a prescription use laboratory assay, which can aid in the diagnosis of cryptococcosis.

Device Description

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) (11). Individuals with impaired cell-mediated immunity are at greatest risk of cryptococcal infection (22). Cryptococcosis is one of the most common opportunistic infections in AIDS patients (14). Globally, cryptococcosis causes approximately one million cases every year and in sub-Saharan Africa cryptococcosis may be more common than tuberculosis (14). Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity and specificity (5,7,9).

The CrAg Lateral Flow Assay (LFA) is a dipstick sandwich immunochromatographic assay. For the qualitative procedure, specimens are diluted 1:2 in Specimen Diluent and analyzed (Figure 1). For the semi-quantitative procedure, specimens are diluted 1:5 in Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure (Figure 1). 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.

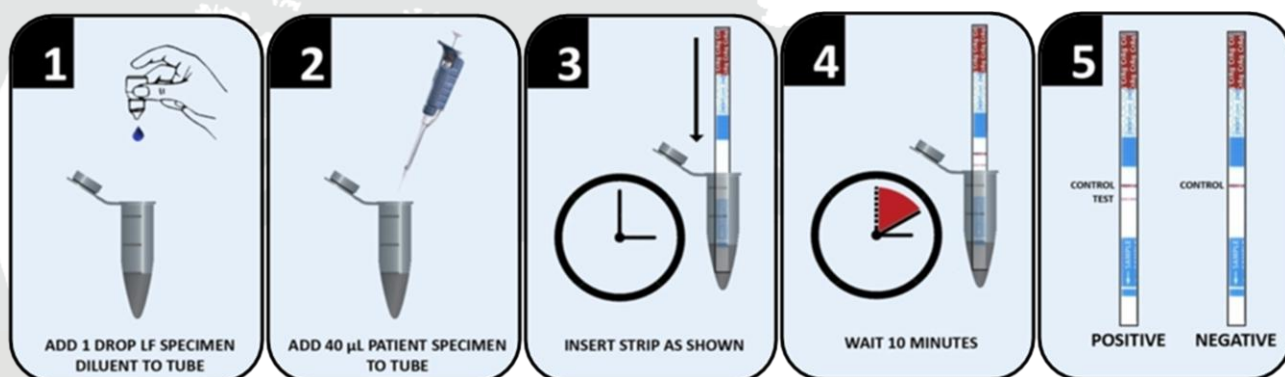


Figure 1. CrAg Lateral Flow Assay Qualitative Procedure



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-conjugated antibody-antigen complex will continue to wick up the membrane where it will interact with the test line. The test line is immobilized, anti-cryptococcal, monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-conjugated antibodies and the immobilized antibodies, causing a visible line to develop at the test line site. 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 antibody to move to the control line, where the gold-conjugated control antibody will cause a visible line to develop. A positive test result will create two lines, while a negative test result will create one line. If the control line fails to develop a line, then the test is not valid.

Performance Summary

Precision Studies (Repeatability & 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 CLSI EP5-A2. One site was internal (Site 1) and the remaining two were a US 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 1). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 1).

CSF:

Repeatability and reproducibility with CSF specimens were determined by spiking a mock CSF 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 CLSI EP5-A2. One site was internal (Site 1) and the remaining two were a US 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 1). For reproducibility, overall



percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 1).

Table 1. CrAg LFA Serum and CSF Precision

Sample	Serum								CSF							
	1		2		3		4		5		6		7		8	
Neg/Pos	Med. Pos.	Low Pos.	High Neg	Neg.	Med. Pos.	Low Pos.	High Neg.	Neg.	Med. Pos.	Low Pos.	High Neg.	Neg.	Med. Pos.	Low Pos.	High Neg.	Neg.
	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Site 1	0	30	0	30	28	2	30	0	0	30	0	30	27	3	30	0
%	0	100	0	100	93	7	100	0	0	100	0	100	90	10	100	0
Site 2	0	30	0	30	30	0	30	0	0	30	0	30	30	0	30	0
%	0	100	0	100	100	0	100	0	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0	0	15	0	15	15	0	15	0
%	0	100	0	100	100	0	100	0	0	100	0	100	100	0	100	0
Total	0	75	0	75	73	2	75	0	0	75	0	75	72	3	75	0
%	0	100	0	100	97	3	100	0	0	100	0	100	96	4	100	0

Analytical Sensitivity (Lower Limits of the Assay/Analytical Cutoff)

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running varying concentrations of cryptococcal antigen diluted in Lateral Flow (LF) Specimen Diluent, according to CLSI EP12-A2. 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 1.25 ng/ml.

Analytical Specificity (Cross-Reactivity)

Analytical specificity for the CrAg Lateral Flow Assay was determined by evaluating specimens from potentially cross-reactive medical conditions unrelated to cryptococcosis. The following specimens were run in triplicate on one lot of the CrAg Lateral Flow Assay. A total of 118 serum specimens and 15 fungal culture filtrates was tested. Culture filtrates were tested at three different dilutions: Undiluted, 1:10, and 1:100. Dilutions were made in LF Specimen Diluent. Percent positive was determined for each condition (Table 2).





Table 2. CrAg LFA Analytical Specificity

Pathology	# Specimens	% Positive Results
HAMA	5	0%
Syphilis	10	0%
Rubella	5	0%
Mycoplasma	10	0%
Toxoplasmosis	7	0%
CMV Infection	10	0%
Rheumatoid factor*	10	0%
Penicilliosis	5	0%
Sporotrichosis	6	0%
Blastomycosis	10	0%
Coccidioidomycosis	10	0%
Histoplasmosis	10	0%
Candidiasis	10	0%
Aspergillosis**	10	10%
<i>Aspergillus terreus</i> culture filtrate	3	0%
<i>Aspergillus niger</i> culture filtrate	3	0%
<i>Aspergillus flavus</i> culture filtrate	3	0%
<i>Aspergillus fumigatus</i> culture filtrate	3	0%
<i>Paracoccidioides brasiliensis</i> culture filtrate	3	67%
Total	133	2.3%

* Rheumatoid factor concentrations tested ranged from 112 IU/ml to 6,479 IU/ml.

** The three positives were the results of three replicates of the same specimen.

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 strips.



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 beigelii*, *Zygomycetes*, ANA+, HAV, HCV, *Staphylococcus*, and *Streptococcus*.

High Dose Hook Effect

High dose hook effect concentrations with serum and CSF specimens were determined by spiking negative serum and a mock CSF (LF Specimen Diluent) 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 500 ug/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 cryptococcal antigen concentrations higher than 200 ug/ml can produce high dose hook effects and therefore may produce false negative results. CSF specimens with cryptococcal antigen concentrations higher than 140 ug/ml can produce high dose hook effects and therefore may produce false negative results.

Method Comparisons

Comparison to Latex-Cryptococcal Antigen Detection System

Cryptococcal antigen detection is one of the gold standards to aid in the diagnosis of cryptococcosis (17). Furthermore, antigen detection has been shown to be a useful tool to monitor therapy efficacy (2,4,15,16). The IMMY Latex-*Cryptococcus* Antigen Detection System is one of several tests currently used to detect cryptococcal antigen (1,3,6,8,10,13,18-21). Therefore, the IMMY Latex-*Cryptococcus* Antigen Detection System served as a reference method to which the CrAg Lateral Flow Assay was compared.

Serum Specimens:

A panel of 197 serum specimens submitted to a US reference laboratory for cryptococcal antigen testing using Meridian's Premier™ Cryptococcal Antigen EIA was collected and stored frozen until method comparison studies were performed. Of the 197 specimens, 96 were positive and 101 were negative using the Meridian EIA. All specimens were analyzed, according to CLSI EP12-A2, concurrently in the CrAg Lateral Flow Assay and in the IMMY Latex-*Cryptococcus* Antigen Detection System to ensure



the specimens were 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 3). The percent agreement positive, percent agreement negative, percent overall agreement and 95% confidence interval are also presented (Table 4).

Table 3. CrAg LFA and Latex Serum Contingency Table

	CrAg Latex (+)	CrAg Latex (-)
CrAg LFA (+)	101	2
CrAg LFA (-)	0	94

Table 4. CrAg LFA and Latex Serum Statistical Analysis

		95% CI
% Agreement Positive	100%	96-100%
% Agreement Negative	97.9%	93-99%
% Overall Agreement	99.0%	96-100%

CSF Specimens:

A panel of 42 CSF specimens submitted to a US reference laboratory for cryptococcal antigen testing using Meridian's Premier™ Cryptococcal Antigen EIA was collected and stored frozen until method comparison studies were performed. Of the 42 specimens, 20 were positive and 22 were negative using the Meridian EIA. All specimens were analyzed, according to CLSI EP12-A2, concurrently in the CrAg Lateral Flow Assay and in the IMMY Latex-*Cryptococcus* Antigen Detection System to ensure the specimens were 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 5). The percent agreement positive, percent agreement negative, percent overall agreement and 95% confidence interval are also presented (Table 6).

Table 5. CrAg LFA and Latex CSF Contingency Table

	CrAg Latex (+)	CrAg Latex (-)
CrAg LFA (+)	20	0
CrAg LFA (-)	0	22

Table 6. CrAg LFA and Latex CSF Statistical Analysis

		95% CI
% Agreement Positive	100%	84-100%
% Agreement Negative	100%	85-100%
% Overall Agreement	100%	92-100%

Semi-Quantitative Serum and CSF Analysis:

Sixty-two serum and 17 CSF specimens that tested positive by the IMMY Latex-*Cryptococcus* Antigen Detection System during the qualitative method comparison were stored frozen (-80° C), then analyzed in the CrAg Lateral Flow Assay (LFA) to determine the specimens' titers (semiquantitative analysis). Concurrently, the specimens were tested in the IMMY Latex-*Cryptococcus* Antigen Detection System (LA) to determine the latex titer for each specimen. The entire panel was tested at IMMY according to





each test's package insert. Cryptococcus Antigen Latex titers versus CrAg Lateral Flow Test titers were plotted and regression analysis performed (Figure 2). The data show a strong correlation between the two tests ($R^2 = 0.885$).

Comparison to Meridian Premier™ Cryptococcal Antigen EIA

A panel of 197 serum specimens submitted to a US reference laboratory for cryptococcal antigen testing using Meridian's Premier™ Cryptococcal Antigen EIA was collected and stored frozen until method comparison studies were performed. Of the 197 serum specimens, 96 were positive and 101 were negative using the Meridian EIA. A panel of 42 CSF specimens submitted to a US reference laboratory for cryptococcal antigen testing using Meridian's Premier™ Cryptococcal Antigen EIA was collected and stored frozen until method comparison studies were performed. Of the 42 CSF specimens, 20 were positive and 22 were negative using the Meridian EIA.

Serum Specimens:

The original EIA results for all the serum specimens were used to compare the CrAg Lateral Flow Assay to Meridian's Premier™ Cryptococcal Antigen EIA. The data is presented in a 2x2 contingency table (Table 7). The percent agreement positive, percent agreement negative, percent overall agreement and 95% confidence interval are also presented (Table 8). Five of seven LFA positive, EIA negative specimens were CrAg Latex positive.

Table 7. CrAg LFA and EIA Serum Contingency Table

	CrAg EIA (+)	CrAg EIA (-)
CrAg LFA (+)	96	7
CrAg LFA (-)	0	94

Table 8. CrAg LFA and EIA Serum Statistical Analysis

		95% CI
% Agreement Positive	100%	96-100%
% Agreement Negative	93.1%	86-97%
% Overall Agreement	96.4%	93-98%

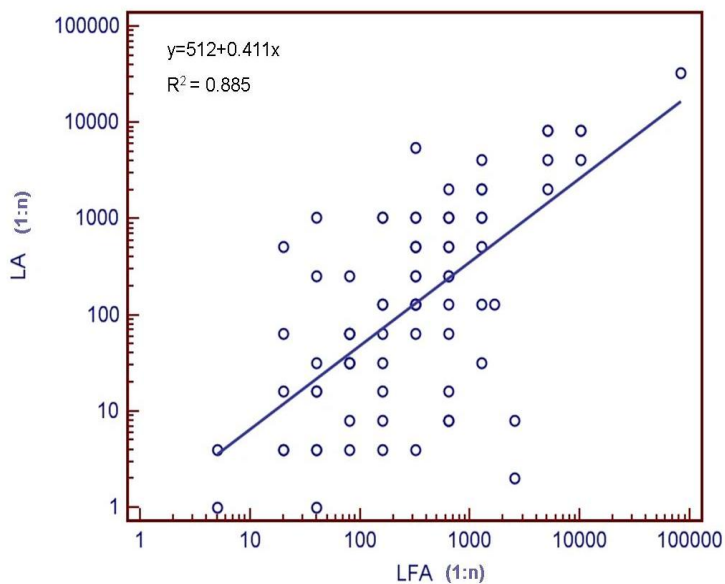


Figure 2. Regression Analysis comparing CrAg Lateral Flow Titers to CrAg Latex Titers





CSF Specimens

The original EIA results for all the CSF specimens were used to compare the CrAg Lateral Flow Assay to Meridian's Premier™ Cryptococcal Antigen EIA. The data is presented in a 2x2 contingency table (Table 9). The percent agreement positive, percent agreement negative, percent overall agreement and 95% confidence interval are also presented (Table 10).

Table 9. CrAg LFA and EIA CSF Contingency Table

	CrAg EIA (+)	CrAg EIA (-)
CrAg LFA (+)	20	0
CrAg LFA (-)	0	22

Table 10. CrAg LFA and EIA CSF Statistical Analysis

		95% CI
% Agreement Positive	100%	84-100%
% Agreement Negative	100%	85-100%
% Overall Agreement	100%	92-100%

Comparison to Meridian Premier™ Cryptococcal Antigen EIA – Thailand Study

The Centers for Disease Control and Prevention (CDC) compared the CrAg Lateral Flow Assay to the Meridian Premier™ Cryptococcal Antigen EIA using stored sera from HIV-infected patients hospitalized with acute respiratory illness in Thailand (12). The CrAg Lateral Flow Assay results were read at 15 minutes. The data is presented in a 2x2 contingency table (Table 11). The percent agreement positive, percent agreement negative, percent overall agreement and 95% confidence interval are also presented (Table 12). The CrAg Lateral Flow Assay was compared to cryptococcal blood culture results (Table 13) and the sensitivity and specificity were calculated (Table 14). When compared to culture, the Meridian Premier™ Cryptococcal Antigen EIA had a sensitivity and specificity of 94.1% (73-99% 95% CI) and 87.3% (82-91% 95% CI), respectively.

Table 11. CrAg LFA and EIA Contingency Table

	CrAg EIA (+)	CrAg EIA (-)
CrAg LFA (+)	87	2
CrAg LFA (-)	4	371

Table 12. CrAg LFA and EIA Statistical Analysis

		95% CI
% Agreement Positive	95.6%	89-98%
% Agreement Negative	99.5%	98-100%
% Overall Agreement	98.7%	97-99%

Table 13. CrAg LFA and Culture Contingency Table

	Culture (+)	Culture (-)
CrAg LFA (+)	17	22
CrAg LFA (-)	0	175

Table 14. CrAg LFA and Culture Statistical Analysis

		95% CI
Sensitivity	100%	82-100%
Specificity	88.8%	84-93%





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