CrAg® LFA

CRYPTOCOCCAL ANTIGEN
LATERAL FLOW ASSAY

For the Detection of Cryptococcal Antigen – REF CR2003

℞ ONLY | IVD For In Vitro Use Only | 2°C – 30°C

IMMY

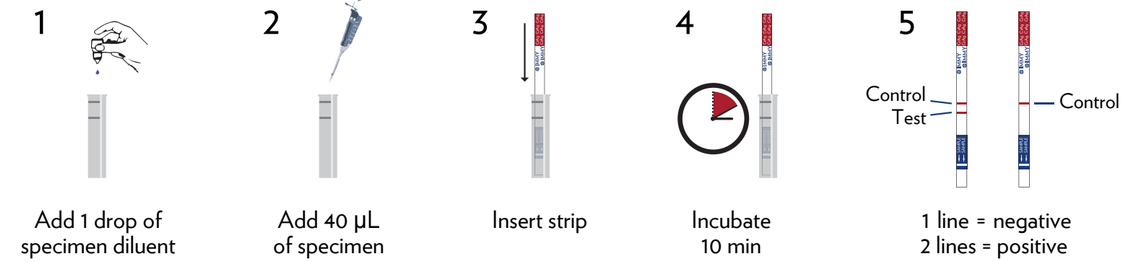
INTENDED USE
The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of Cryptococcus species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebral spinal fluid (CSF).

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

SUMMARY and EXPLANATION of the Test
Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*).[1] Individuals with impaired cell-mediated immunity are at greatest risk of infection.[2] Cryptococcosis is one of the most common opportunistic infections in AIDS patients.[3] Cryptococcosis is responsible for 15% of HIV deaths worldwide.4 Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity and specificity.[5-6] The CrAg LFA utilizes highly sensitive and specific anti-cryptococcal mouse monoclonal antibodies. These antibodies are highly sensitive to glucuronoxylomannan, (GXM) the primary antigen shed by the organism. The CrAg LFA shows increased sensitivity across all serotypes of the organism, especially serotype C (*C. gattii*).[7-9] Detection of CrAg with the CrAg lateral flow assay has been widely employed when cryptococcal disease is suspected.[10-13] Preliminary reports suggest that trained lay healthcare workers and laboratory personnel can use the assay as a point-of-care assay outside the laboratory.[14]

BIOLOGICAL PRINCIPLES
The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay. Specimens and specimen diluent are added into an appropriate reservoir, such as a test tube, and the lateral flow device is placed into the reservoir. The test uses specimen wicking to capture gold-conjugated, anti-CrAg monoclonal antibodies and gold-conjugated control antibodies deposited on the test membrane. If CrAg is present in the specimen, then it binds to the anti-CrAg antibodies. The antibody-antigen complex continues to wick up the membrane where it will interact with the test line, which has immobilized anti-CrAg monoclonal antibodies. The antibody-antigen complex forms a sandwich at the test line causing a visible line to form. With proper flow and reagent reactivity the wicking of any specimen, positive or negative, will cause the control antibody to move to the control line. Immobilized antibodies at the control line will bind to the control antibody and form a visible control line. Note: The control line is a migration control and not a specimen addition control. Positive test results create two lines (test and control). Negative test results form only one line (control). If a control line fails to develop then the test is not valid.

QUALITATIVE – BASIC PROCEDURE



Add 1 drop of specimen diluent | Add 40 µL of specimen | Insert strip | Incubate 10 min | 1 line = negative 2 lines = positive

WARNINGS and PRECAUTIONS
For In Vitro Diagnostic Use only.

WARNINGS FOR USERS
1. Use of this kit with samples other than human serum and CSF is not recommended.
2. Wear protective clothing, including lab coat, eye/face protection, and disposable gloves, and handle the kit reagents and patient samples with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
3. Avoid splashing samples or solutions.
4. Biological spills should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach, 70% ethanol, or 0.5% Wescodyne Plus™. Materials used to wipe up spills may require biohazardous waste disposal.
5. Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous wastes must be handled and discarded in accordance with all local, regional, and national regulations.
6. The CrAg Lateral Flow Assay Test Strips (REF LFCR50) may be biohazardous after running specimens. Handle and dispose of accordingly.
7. Refer to Hazards and Precautionary Information section for hazards associated with specific reagents. Safety Data Sheets are available upon request.

REAGENT PRECAUTIONS
1. Specific standardization is necessary to produce our high-quality reagents and materials. The user assumes full responsibility for any modification to the procedures published herein.
2. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimens.
3. Always wear gloves when handling reagents in this kit as some reagents are preserved with less than 0.1 % (w/w) sodium azide. Sodium azide should never be flushed down the drain as this chemical may react with lead or copper plumbing to form potentially explosive metal azides. Excess reagents should be discarded in an appropriate waste receptacle.
4. The following components are not test system lot dependent: LF Specimen Diluent (REF GLF065) and therefore can be used with any lot of CrAg LFA Strips (REF LFCR50), provided they have not expired.
5. The control line is a migration control and not intended as a specimen addition control.

REAGENTS
1. LF Specimen Diluent (6.5 mL, REF GLF065): Glycine-buffered saline containing blocking agents and a preservative
2. CrAg LF Test Strips (50 strips in desiccant vial, REF LFCR50). Strips are 0.4 cm wide by 7.6 cm tall
3. CrAg Positive Control (1 mL, REF CB1020): Glycine- buffered saline spiked with cryptococcal antigen (strain 184A – clinical isolate from Tulane University)[15]
4. Package insert

MATERIALS NOT PROVIDED
1. Pipettor (40-µL and 80-µL) or disposable 40 µL plastic Pastettes.
2. Timer
3. Disposable, flat-bottom micro-centrifuge tubes, test tubes, or a micro-titer plate that can hold the test strip

REAGENT PREPARATIONS
The entire kit should be at room temperature before and during use. If the kit is stored at or below 4°C, allow the kit to come to room temperature before opening.

REAGENT STABILITY AND STORAGE
All reagents included in this kit should be stored at the stated temperature (2-30°C) until the expiration dates listed on the reagent labels. Unused test strips should be returned immediately to the LF test strip vial with the cap firmly attached. All reagents should be tightly capped immediately after use.

SPECIMEN COLLECTION & PREPARATION
For optimal results, sterile non-hemolyzed specimens should be used. Collect CSF and serum specimens aseptically following accepted procedures. If a delay is encountered in specimen processing, storage at 2-8°C for up to 72 hours is permissible. CSF and serum may be stored for longer periods at <-20°C, provided they are not repeatedly thawed and refrozen. CSF and serum in transit should be maintained at 2-8°C not <-20°C.

PROCEDURE
REFER TO *REAGENTS* SECTION FOR A LIST OF MATERIALS PROVIDED.

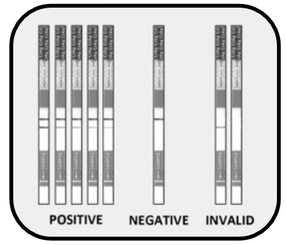
Qualitative Procedure
1. Add 1 drop or pipette 40uL of LF Specimen Diluent (REF GLF065) to an appropriate labeled reservoir (disposable micro-centrifuge tube, test tubes, or micro-titer plate, etc.). It is also good practice to label the strip, prior to inserting into sample.
2. Add 40 µL of specimen to the reservoir and mix.
3. Submerge the white end of a CrAg LF Test Strip (REF LFCR50) into the specimen. *Note*: Return all unused strips to the desiccant vial and firmly close the lid. Firmly cap all reagent bottles when not in use.
4. Wait 10 minutes. *Note: you can read the results 10 minutes to 2 hours after inserting the strips.*
5. Read and record the results (See READING THE TEST).

Semi-Quantitative Titration Procedure
1. Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560.
2. Place 10 micro-centrifuge or test tubes in an appropriate rack and label them 1-10 (1:5 through 1:2560). Additional dilutions may be necessary if the specimen is positive at 1:2560. For methods to conserve strips, request our Titration Algorithm Procedure.
3. Add 4 drops or pipette 160 µL of LF Specimen Diluent (REF GLF065) to tube #1.
4. Add 2 drops or pipette 80 µL of LF Specimen Diluent (REF GLF065) to each of the tubes labeled 2-10.
5. Add 40 µL of specimen to tube #1 and mix well.
6. Transfer 80 µL of specimen from tube #1 to tube #2 and mix well. Continue this dilution procedure through tube #10. Discard 80 µL from tube #10 and 40 µL from tube #1 for final tube volumes of 80 µL.
7. Submerge the white end of a CrAg LF Test Strip into the specimen in each of the 10 tubes.
8. Wait 10 minutes.
9. Read and record the results (See READING THE TEST).
Note: you can read the results 10 minutes to 2 hours after inserting the strips.

READING THE TEST
Read the reactions. The presence of two lines (test and control), regardless of the intensity of the test line, including faint lines, indicates a positive result. For the semi-quantitative titration procedure, the patient's titer should be reported as the highest dilution that yields a positive result. Note: titers obtained by IMMY's CrAg LFA are not equivocal to titers obtained from other cryptococcal antigen assays.

A single control line indicates a negative result. If the control line does not appear, the results are invalid and the test should be repeated. Faint line intensity could be indicative of a high titer specimen. The semi-quantitative procedure should be run to rule out high titer inhibition of test line. The control line is a migration control and not intended as a specimen addition control.

The stability of the control and test lines beyond the reading time (10 minutes – 2 hours) has not been validated.



Note: The test strip may be aligned with the strip image on the kit label on the kit bag to ensure correct interpretation of results.

QUALITY CONTROL
A positive control can be evaluated by adding 1 drop of LF Specimen Diluent (REF GLF065) followed by 1 drop of CrAg Positive Control (REF CB1020) to a tube. A negative control can be evaluated by adding 2 drops of LF Specimen Diluent (REF GLF065) to a tube. Insert a test strip into the tube and read after 10 minutes. Note: you can read the results 10 minutes to 2 hours after inserting the strips.
Two (2) lines (test and control) indicate a positive result and one line (control) indicates a negative result.
Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS
The control line must be present for a valid test. The presence of two lines (a control line and a line in the test zone) regardless of the intensity of the test line, including faint lines, indicates a positive result. The control line is a migration control and not intended as a specimen addition control.

Faint line intensity could be indicative of a high titer specimen. The semi-quantitative procedure should be run to rule out high titer inhibition of test line.

The stability of the control and test lines beyond the reading time (10 minutes to 2 hours) has not been validated.

Interpretations based upon the semi-quantitative methodology can be indicative of prognosis and response to treatment. Cryptococcal antigen titers greater than 1:160 are associated with meningitis development.[16-17]
Negative results do not rule out the diagnosis of disease. The specimen may be drawn before detectable antigen is present.

LIMITATIONS OF THE PROCEDURE
- The assay performance characteristics have not been established for samples other than CSF or serum.
- Titers obtained by the LFA are not equivalent to titers obtained by other cryptococcal antigen tests.[18]
- Depending on the disease and organism prevalence, testing should not be performed as a screening procedure for the general population. The predictive value of a positive or negative serologic result depends on the pretest likelihood of cryptococcal disease being present. This test can be

used in the context of prevention/screening and diagnosis of cryptococcal diseases.

- Testing hemolyzed serum samples could lead to false negatives due to the high background color on the strip.
- Weakly encapsulated strains can lead to false negative results.[19]
- According to published reports, T. beigelii can cause false positives.[20]
- Patients with high levels (>40 ug/ml) of Human anti-mouse antibodies (HAMA) may cause false positives.
- This assay was not evaluated for potential interference related to specimen pretreatment with 2-mercaptoethanol or with specimens including the following substances: Vaginal cream, caffeine, ascorbic acid, itraconazole, amphotericin B, acetaminophen, or acetylsalicylic acid.

CROSS-REACTIVITY ANALYSIS

The CrAg Lateral Flow Assay was evaluated for cross-reactivity against a panel of patients' serum specimens across a variety of different pathologies. The results of this testing are shown in the table below.

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)
Coccidiodomycosis	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)

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 Paracoccidioides brasiliensis exhibited some cross-reactivity.

Antigens from the following organisms were tested and exhibited no cross-reactivity:

Aspergillus terreus
Aspergillus niger
Aspergillus fumigatus
Aspergillus flavus

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

Candida dubliniensis
Candida tropicalis
Candida parapsidosis
Candida krusei
Candida glabrata
Cladosporium trichoides
Streptococcus pneumoniae
Salmonella typhi
Pneumocystis carinii
Zygomycetes
Antinuclear antibody +
Hepatitis A Virus
Hepatitis C Virus
Staphylococcus aureus
Neisseria meningitidis
Mycobacterium tuberculosis

HIGH DOSE HOOK EFFECT (PROZONING)

Although rare, extremely high concentrations (>0.140 mg/mL) of cryptococcal antigen can result in weak test lines and, in extreme instances, yield negative test results. If prozoning is suspected in weakly positive or negative test results, the semi-quantitative titration procedure should be followed to rule out false negative results.

EXPECTED VALUES

The frequency of cryptococcosis is dependent on several factors including patient population, type of institution, and epidemiology. In this study, 100% of true positives as determined by culture and/or India Ink were detected.

SPECIFIC PERFORMANCE CHARACTERISTICS

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 LFA	Positive	91	0
	Negative	0	123

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

CSF		Culture/India Ink	
		Positive	Negative
CrAg LFA	Positive	65	0
	Negative	0	99

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

EIA METHOD COMPARISON

The CrAg Lateral Flow Assay was evaluated using 197 serum specimens that were submitted to a US reference laboratory for cryptococcal antigen testing. These specimens were tested using the CrAg Lateral Flow Assay and a commercially available cryptococcal antigen EIA. The results of these comparisons are shown in the tables below.

SERUM		CrAg EIA	
		Positive	Negative
CrAg LFA	Positive	96	7
	Negative	0	94

Serum	Calculated	95% CI
Sensitivity	100% (96/96)	96%-100%
Specificity	100% (94/101)	86%-97%

IMMY LATEX AGGLUTINATION METHOD COMPARISON

The CrAg Lateral Flow Assay was evaluated using 197 serum specimens that were submitted to a US reference laboratory for cryptococcal antigen testing. These specimens were tested using the CrAg Lateral Flow Assay and the IMMY Cryptococcal Antigen Latex Agglutination Assay. This comparison yielded an overall percent agreement of 99%.

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 Latex Cryptococcal Antigen Detection System (REF CR1003). Linear regression analysis of the data yielded an R2 value of 0.905.

LIMIT OF DETECTION

In order to establish the limit of detection, a C_5- C_{95} experiment was conducted by diluting purified cryptococcal antigen in LF Specimen Diluent (REF GLF065) and testing 24 replicates per concentration using the CrAg Lateral Flow Assay. The results of this testing are shown in the following table:

Concentration	# Positive	% Positive
0.50 ng/mL	0	0% (0/24)
0.75 ng/mL	0	0% (0/24)
1.00 ng/mL	4	17% (4/24)
1.25 ng/mL	12	50% (12/24)
1.50 ng/mL	21	88% (21/24)
1.75 ng/mL	24	100% (24/24)
2.00 ng/mL	24	100% (24/24)
2.50 ng/mL	24	100% (24/24)
3.00 ng/mL	24	100% (24/24)

C_5-C_{95} Interval	1.0-1.5 ng/mL

REPRODUCIBILITY AND PRECISION

The CrAg Lateral Flow Assay was evaluated for reproducibility and precision by spiking serum 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 table below.

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)

HAZARDS & PRECUATIONARY INFORMATION

There are no hazards associated with the reagents in this kit.

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International Symbol Usage

Σ	Sufficient for "#" Tests	REF	Reference Number
	Protect from Humidity	LOT	Lot Number
	Manufactured by	IVD	In Vitro diagnostics
	Expiration Date		Single Use Only
2°C 30°C	Storage 2-30 °C		
CE	Conforms to European Union Requirements		

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