PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Cryptococcus Antigen Procedure (IMMY CrAg Lateral Flow Assay)

Department of Microbiology

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1.0 Purpose & Principle

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). 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 gold-conjugated, anti-CrAg antibodies. The gold-labeled 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 gold-labeled 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 gold-conjugated control antibody to move to the control line. Immobilized antibodies at the control line will bind to the gold-conjugated control antibody and form a visible control line. 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. Cryptococcal antigen titers can be useful for monitoring therapeutic efficacy. Semi-quantitative testing can be performed with the lateral flow assay by performing serial dilutions of the specimen as described in the test procedure below.

2.0 Clinical Significance

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*). Individuals with impaired cell-mediated immunity are at greatest risk of infection. Cryptococcosis is one of the most common opportunistic infections in AIDS patients. Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity and specificity.

3.0 Scope

This procedure is classified under CLIA as Moderately Complex. It should be carried out by technical personnel familiarized and trained to perform the test. Testing includes but is not limited to: test performance and interpretation, QC checks, and documentation.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne and airborne pathogens

To perform this procedure, you must use:

- Gloves – must be worn when handling specimens.
- Laboratory Coat – must be worn when handling specimens and reagents.
- Biological Safety Cabinet – must be used when performing the test.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- MSDS and chemical hygiene plan

5.0 Specimen Collection, Handling and Storage

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

6.0 Materials

6.1 General

- Pipette capable of delivering 40 to 80 µL and pipette tips
- Timer
- Test tubes

6.2 Reagents

CrAg Lateral Flow Assay kit (REF CR2003), 50 Reactions. Store at 22-25°C.

- LF Specimen Diluent (2.5 mL): Glycine-buffered saline containing blocking agents and a preservative
- CrAg LF Test Strips (50 strips in desiccant vial). Unused test strips should be stored in the LF test strip vial with the desiccant cap firmly attached.
- CrAg Positive Control (1 mL): Glycine-buffered saline spiked with cryptococcal antigen

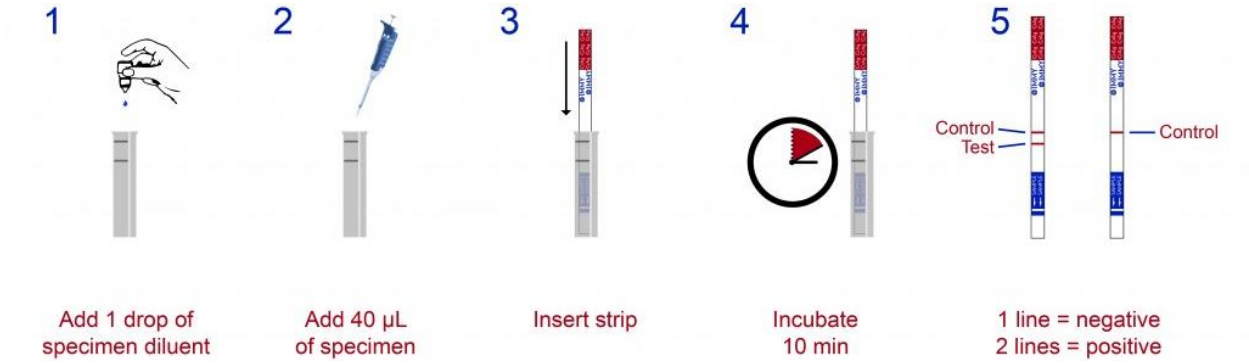
7.0 Interfering Substances

This assay was evaluated by the manufacturer for the potential of interference due to serum conditions including icteric, hemolyzed, and lipemic samples. These samples exhibited no interference in the assay. Hemolyzed samples, however, could lead to false negatives due to the high background color on the strip.

8.0 Procedure

8.1 Qualitative Procedure

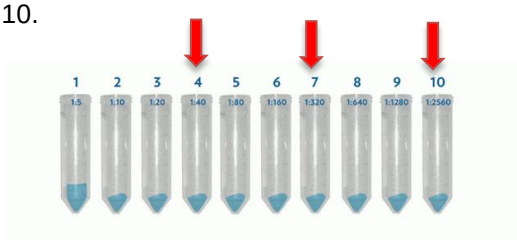
1. Add 1 drop (~ 40 µL) of LF Specimen Diluent to a test tubes.
2. Add 40 µL of specimen to the container and mix.
3. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen.
4. Wait 10 minutes.
5. Read and record the results.



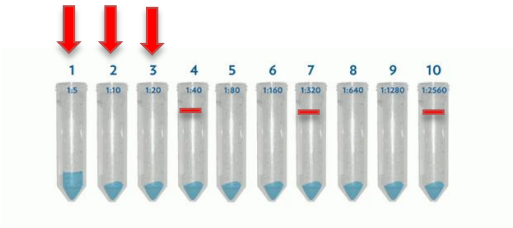
8.2 Semi-Quantitative Titration Procedure

Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560.

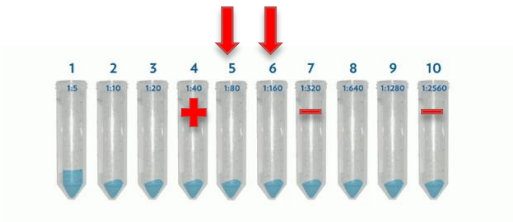
1. Place 10 micro-centrifuge or test tubes in an appropriate rack and label them 1-10 (1:5 through 1:2560).
2. Add 4 drops of LF Specimen Diluent to tube #1.
3. Add 2 drops of LF Specimen Diluent to each of the tubes labeled 2-10.
4. Add 40 µL of specimen to tube #1, and mix well.
5. Transfer 80 µL of specimen from tube #1 to tube #2 and mix well. Continue this dilution procedure through tube #10. Discard 80 µL from tube 10 for a final tube volume of 80 µL.
6. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen in tube # 4, 7, and 10.



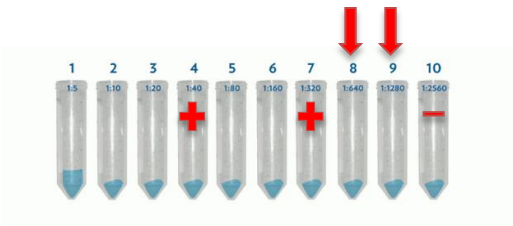
7. Wait 10 minutes.
8. Read the results.
9. If tubes # 4, 7, and 10 are negative, test tubes 1, 2, and 3. If tubes 1, 2, and 3 are negative, repeat the original qualitative assay to verify the positive result.



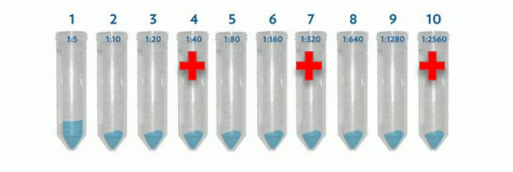
10. If tube # 4 is positive and 7 is negative, test tubes 5 and 6.



11. If tubes # 4 and 7 are positive and tube 10 is negative, test tubes 8 and 9.



12. If tube # 10 is positive, no additional testing is necessary.



9.0 Interpretation & Reporting of Results

Read the reactions. The presence of two lines (test and control), regardless of the intensity of the test line, indicates a positive result.

9.1 Positive Results

For the semi-quantitative titration procedure, the patient's titer should be reported as the highest dilution that yields a positive result. Specimens that are positive in tube # 10 can be reported as ≥ 1:2560. If only the original qualitative assay is positive, but none of the titration samples are positive, the titer can be reported as 1:2 after the original qualitative result has been verified by repeat testing. If the repeat is negative, report the result as negative.

1	2	3	4	5	6	7	8	9	10
1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560

Document the result on the Cryptococcus Antigen Test Log and enter the positive result with the titer in LIS. The following comment should be added to the report for positive CSF specimens:

A fungus culture should be ordered to determine the species of Cryptococcus and to recover the organism for potential susceptibility testing. [CRYPTC]

9.2 Negative Results

A single control line indicates a negative result. Report the test as negative. If the control line does not appear, the results are invalid and the test should be repeated.

10.0 Quality Control & Quality Assurance

10.1 Internal controls

An internal control is contained within each test strip and, therefore, evaluated with each test. A pink-red band appearing at the Control line serves as a procedural control and indicates the test has been performed correctly, that proper flow occurred and that the test reagents were active at the time of use. The Control line must be present in order to report the test results. Document the internal control result on the Test Log.

10.2 External controls

New lots and/or shipments should be checked using the same lot of control material that was used to check the old lot. This is accomplished by saving a specific lot of control materials from a shipment of kits and then using that lot of controls for testing subsequent lots/shipments that are received. The control materials may be used until the manufacturer's expiration date printed on the bottle. Document the control material lot number used for QC on the Package Insert Verification log.

External Quality Control Procedure

A positive control (CrAg Positive Control) can be evaluated by adding 1 drop of LF Specimen Diluent followed by 1 drop of CrAg Positive Control to a tube. A negative control can be evaluated by adding 2 drops of LF Specimen Diluent to a tube. Insert a test strip into the tubes, and read after 10 min. Two lines (test and control) indicate a positive result, and one line (control) indicates a negative result. If external controls fail to produce the expected results, notify the supervisor and/or technical specialist. Lots and/or shipments that do not perform as expected cannot be used for patient testing. All results from external controls should be documented in LIS.

10.3 Quality Assurance

All test results should be documented on the Cryptococcus Antigen Test Log and resulted in LIS. Results entered into LIS should be reviewed by a second technologist on the same shift or the beginning of the next shift. Document review on the Cryptococcus Antigen Test Log.

11.0 Limitations

1. The assay performance characteristics have not been established for matrices other than serum and CSF.
2. 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 result depends on the pretest likelihood of cryptococcal disease being present. Testing should only be done when clinical evidence suggests the diagnosis of cryptococcal disease.
3. Testing hemolyzed serum samples could lead to false negatives due to the high background color on the strip.
4. 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 a prozone effect is suspected in weakly positive or negative test results, the semi-quantitative titration procedure should be followed to rule out false negative results.

12.0 Verification Information

The CrAg Lateral Flow Assay has been approved by the FDA for clinical diagnostic testing. No modifications have been made to the FDA-cleared assay. The manufacturer's published findings for analytical sensitivity and specificity can be found in the package insert. In this evaluation, clinical specimens were used to verify the accuracy and precision of the assay.

A total of 81 clinical specimens, including 48 serum samples and 33 CSF samples, were tested with the lateral flow assay. These samples had been previously tested with the Cryptococcal Antigen Latex Agglutination System (CALAS) by Meridian Bioscience, Inc. Specimens were stored at -70°C prior to verification testing with the CrAg Lateral Flow Assay. Testing was performed by 11 different users. There was 100% correlation between the lateral flow and the latex assays. The table below summarizes the results.

Performance of the Lateral Flow Assay Compared to Latex Agglutination

	Serum			CSF			Combined Total
	LTX POS	LTX NEG	Total	LTX POS	LTX NEG	Total	
LF POS	23	0	23	8	0	8	31
LF NEG	0	25	25	0	25	25	50
Total	23	25	48	8	25	33	81

Three samples were selected for titration for comparison to the latex assay. This included 2 CSF samples and 1 serum sample. The dilution scheme used by the two assays is slightly different, so an exact comparison was not possible. However, the titrations were generally within one two-fold dilution of each other.

Performance of Lateral Flow Titration Compared to Latex Agglutination

	Lateral Flow	Latex
Serum	1:320	1:512
CSF #1	1:2560	> 1:1024
CSF #2	1:640	1:256

13.0 References

1. IMMY CrAg Lateral Flow Assay package insert, Rev. 3-27-2012.
2. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

14.0 Document Control History

Reviewed by director (AR): 04/29/2014

Reviewed by supervisor (JC): 04/29/2014

Changes and updates: 06/16/2014 Removed, “Additional dilutions may be necessary if the specimen is positive at 1:2560” from titration procedure section. 01/23/2015 Added instructions for handling specimens that do not titer beyond primary test.