**TITLE: IMMY CRYPTOCOCCAL ANTIGEN (CRAG) LATERAL FLOW**

**ASSAY FOR SERUM AND CSF**

**PRINCIPLE / PURPOSE:** The CrAg Lateral Flow Assay is an immunochromatographic

test system for the qualitative or semi-quantitative detection of the capsular

polysaccharide antigens of Cryptococcus neoformans and Cryptococcus gattii in serum

and CSF. It is a rapid aid in the diagnosis of pulmonary, meningeal and systemic

cryptococcosis. Titers can monitor the response to antifungal therapy.

The test is a dipstick sandwich immunochromatographic assay. Specimens and

specimen diluents are added to a test tube and the lateral flow device is placed into the

test tube. 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, 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. The

gold-monoclonal antibodies 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 a specimen, positive or negative,

will cause the gold-conjugated 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 line.

**COMPLEXITY LEVEL:** Moderate

**SAFETY:**

The required personal protective equipment for this procedure:

* Gloves
* Approved lab coats
* Shield
* Approved Protective eyewear
* Safety Cabinet Hood

**SPECIMEN:**

**Type:** CSF and sterile non-hemolyzed Serum

**\*Reject hemolyzed specimens**

**Stability and Storage**:

1. If specimens are not tested on the day of collection, store the specimens in a sealed, labeled tube at 2-8ºC. Stable for 72 hours.
2. If longer periods of storage are required, samples may be stored at -20°C; do not repeatedly thaw and refreeze.
3. Specimens in transit should be maintained at 2-8ºC.

**Specimen Potential Interferences:**

1. This assay was evaluated for the potential interference due to serum conditions including icterus, hemolysis and lipemia with no interference exhibited.
2. Hemolyzed specimens could lead to a false negative due to high background color in the strip.
3. Bloody, cloudy, elevated WBC counts, elevated protein and xanthochromic in CSF have not been evaluated for interference but such has not been reported.
4. Cross reactivity to a wide variety of fungi and viruses has not been shown.
5. Rheumatoid factor does not interfere.

**EQUIPMENT AND MATERIALS:**

**Supplied in the kit:**

1. LF Specimen diluent (Glycine-buffered saline containing blocking agents and a preservative)
2. CrAg LF test strips (50 strips in desiccant vial)
3. CrAg positive control – contains 1 ml glycine buffered saline spiked with
4. cryptococcal antigen
5. Package insert

**Other materials required:**

1. Pipettor (40 uL and 80 uL)
2. Disposable test tubes.
3. Timer

**Reagent Storage Requirements:**

1. Store all reagents at room temperature. Stable until expiration date.
2. Unused test strips should be stored in the LF test strip vial with the desiccant can firmly attached.

**Safety Precautions:**

1. Potential biohazardous materials – the human serum which is used in the manufacture of the positive control has been shown to be non-reactive for the presence of hepatitis B surface antibodies, HIV, and HCV using FDA licensed test methods. However, since no test method can assure the absence of all infectious agents, all human specimens must be considered potentially infectious and handled with care.
2. The reagents in the kit contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with copious amounts of water to prevent azide build-up.

**QUALITY CONTROL:**

Perform the External Positive and Negative Controls provided in the kit each day of

patient testing.

1. ***External Positive Control:*** Place 1 drop of LF diluent followed by 1 drop of CrAg positive control into a labeled test tube.
2. ***External Negative Control:*** Place 2 drops of LF Specimen Diluent into a labeled test tube.
3. Insert a test strip into each tube.
4. Wait 10 minutes.
5. Read results:
* Positive control strip should have two lines present, the internal control line and a line in the test zone.
* Negative control strip should have a single internal control line present. The absence of an internal control line means that the results are invalid and the test should be repeated.

 6. Record results on Micro Kit QC Log.

**If the expected results do not occur, do not use the kit or report patient results**.

**Parallel Testing must be performed with each new lot/shipment of kits.**

1. Upon receipt of a new lot number or shipment:
* Run the old kit Positive and Negative controls with the new kit test strips.
* Run the new kit Positive and Negative controls with the old kit test strips.

 2. Document these results on the Microbiology Parallel Test Log.

**If the expected results do not occur, do not use the kit or report patient results**.

**PROCEDURE:**

***Qualitative Test****:*

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1. Label a disposable micro-centrifuge/test tube with the patient full name and accession number.
2. Add 1 drop of LF specimen diluent to the appropriately labeled tube.
3. Add 40 uL of specimen to the tube and mix.
4. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen (tube).
5. Wait 10 minutes.
6. Read the reactions:

**Invalid:** If the control line does not appear, the results are invalid and the test should

be repeated.

**Negative:** The presence of a single internal control line is a negative result. Record

results on Cryptococcal Test Log. See Interpretation and Reporting Results Section for details on how to enter results in LIS.

**Positive:** The presence of two lines (test and internal control), regardless of

intensity of the test line, indicates a positive result. Record results on

Cryptococcal Test Log and proceed to *Semi-quantitative Titration* section

below.

**NOTE: once the positive is resulted in LIS, a reminder to make**

**sure a fungal culture with smear needs to be ordered will appear beside the result box (“Order FC”). Check in Inquiry under Patient ID to make sure a fungal culture with smear is ordered. If not, order a fungal culture with smear. This is for CSF specimens only.**

***Semi-quantitative Titration:***

1. Label 10 disposable test tubes with:

* accession number
* patient’s full name
* tube numbers 1 through 10
* the appropriate dilution factor (1:5 through 1:2560)

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

1:2560:

1. Add 4 drops of Specimen diluent to the 1:5 tube.
2. Add 2 drops of Specimen diluent to each of the tubes labeled 1:10 – 1:2560.
3. Add 40 uL of specimen to the 1:5 tube and mix well.
4. Transfer 80uL of specimen from the 1:5 tube to the 1:10 tube and mix well.
5. Continue the dilution procedure through the 1:2560 tube.
6. Discard 80uL from the1:2560 tube for a final tube volume of 80uL.

3. Submerge the white end of a Cryptococcal Antigen Lateral Flow Strip into tubes

labeled **1:40, 1:320,** and **1:2560** first to minimize the number of test strips used. (see chart below)

*4.* Wait 10 minutes.

*5.* Based on which tube yields a positive result, use the chart below to determine which

tubes to test next*.* NOTE: If the result is negative at the 1: 5 dilution stage, report

titer result as the reciprocal of 1:2.

6. Report the titer as the reciprocal of the last dilution that yields a positive result (If the

1:2560 tube is positive, report titer as >2560).

7. Record the result on Cryptococcal Test Log.

8. See Interpretation and Reporting Results Section for details on how to enter results in LIS.

**Note: A positive Cryptococcal test is a critical value and the result must be called**

**to a licensed caregiver (RN, physician).**

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**INTERPRETATION AND REPORTING RESULTS:**

**Reference Ranges:** Negative

**Procedures for Abnormal Results:** A positive test is considered a critical result and must be called to the RN or physician. Document the full name of the person the result was given to, date, time, and your initials.

**Reporting Format:**

**In Sunquest:**

1. Go to Result Entry
2. Resulting Mode: Manual
3. Worksheet: ARHEM
4. Click Binoculars and enter CID#
5. Enter test result CALAS:

**POS = POSITIVE FOR CRYPTOCOCCAL ANTIGEN**

**NEG = NEGATIVE FOR CRYPTOCOCCAL ANTIGEN (continue to #9)**

1. Hit TAB, then “RBV”. Enter name, date, time and your initials.
2. If positive, under CATTR, enter the Titer (e.g. “40”).
3. If positive and sample=CSF, a Fungal culture will reflex with a new Acc#. Look in Inquiry under Patient ID to confirm it has been ordered.
4. If negative, TAB to next line (CATTR) and type HIDE.
5. SAVE.

**LIMITATIONS OF PROCEDURE:**

1. The assay performance characteristics have not been established for specimens

other than serum and CSF.

2. Testing of hemolyzed serum samples may cause a false negative result due to

background staining of the strip.

3. The performance of this device has not been established with specific HIV

therapies. Patients on HIV therapy were included in the FDA submission and no

discrepancies were found in comparison to the gold standard.

4. This assay has been shown to be more sensitive than the previous Latex

Agglutination assay and therefore the sensitivity and specificity is virtually 100%.

5. As this assay is more sensitive, titers may be higher in patients who have had a prior

titer performed by the Latex Agglutination assay.

6. All positive results must have a titer performed, as a falling titer is an effective

monitor of response to anti-fungal therapy.

7. Negative results do not rule out the diagnosis of disease if specimens are drawn

before detectable antigen levels are present.

8. Extremely high concentrations of Cryptococcal antigen can results in weak test lines

or negative test results (prozone).

**REFERENCES:**

1. Hansen, J et al, Large-scale evaluation of the immune-mycologics lateral flow and

enzyme-linked immunoassays for detection of cryptococcal antigen in serum and

cerebrospinal fluid, Clin Vaccine Immunol, 20: 52-55, 2013.

2. Jarvis, JN et al, *Evaluation of a novel point-of-care cryptococcal Antigen Test on*

*Serum, Plasma and Urine from Patients with HIV-associated cryptococcal*

*meningitis*, Clin Inf Dis, 53:1019-23, 2011.

3. Binncker, MJ et al, *Comparison of four assays for the detection of cryptococcal*

*antigen*, Clin Vaccine Immunol, 1988, 2012.

4. Binnicker, MJ, Mayo Clinic Laboratories, Rochester, MN, personal communication,

March 2013.

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HISTORY PAGE

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SOP CHANGE CONTROL

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