**INTENDED USE**

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

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

**SUMMARY and EXPLANATION of the Test**

Cryptococcosis is caused by at least three species of Cryptococcus neoformans complex (C. neoformans and C. gattii) individuals with impaired cell-mediated immunity are at greatest risk of infection. 7 Cryptococcosis is one of the most common opportunistic infections in AIDS patients. 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. 8-11 The CrAg LFA utilizes highly sensitive and specific anti-cryptococcal mouse monoclonal antibodies. These antibodies are highly sensitive to glucuronosylmannan, (GMM) the primary antigen shed by the organism. The CrAg LFA shows increased sensitivity across all serotypes of the organism, especially serotype C (C. gattii). 5 Detection of CrAg with the CrAg lateral flow assay has been widely employed when cryptococcal disease is suspected. 12 Preclinical studies report that trained healthcare workers and laboratory personnel can use the assay as a point-of-care assay outside the laboratory. 13

**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 capturing to 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. 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.

**REAGENT STABILITY AND STORAGE**

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 come to room temperature before opening.

**REAGENT PREPARATIONS**

- Add 1 drop or pipette 40 µL of specimen to a tube. Insert a test strip into the tube and close the lid. Firmly cap all reagent bottles when not in use.
- Add 1 drop of LF Specimen Diluent (REF GLF065) to an appropriate labeled reservoir (disposable micro-centrifuge tube or test tubes, or micro-titer plate, etc.). It is also good practice to label the strip, prior to inserting into sample.
- Submerge the white end of a CrAg LF Test Strip into the specimen in each test tube.
- Add 5 µL of specimen to the reservoir and mix.
- Subtract the white end of a CrAg LF Test Strip (REF LFRC05) to the specimen. Note: Return unused strips to the desiccant vial and firmly close the lid. Firmly cap all reagent bottles when not in use.
- Obtain a test strip and insert it into the tube.
- Read and record the results (See READING THE TEST).

**REAGENT PREPARATIONS**

- Add 1 drop or pipette 40µL of LF Specimen Diluent (REF GLF065) to an appropriate labeled reservoir (disposable micro-centrifuge tube or test tubes, or micro-titer plate, etc.). It is also good practice to label the strip, prior to inserting into sample.
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**READ 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 titre should be reported as the highest dilution that yields a positive result. Note: titers obtained by IMMY’s CrAg LFA are not equivalent 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 3-4 titer levels. 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) has not been validated.

**QUALITY CONTROL**

A negative control can be evaluated by adding 1 drop of LF Specimen Diluent (REF GLF065) followed by 1 drop of CrAg Positive Control (CBT020) 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.

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Interpretations based upon the semi-quantitative methodology can be indicative of prognosis and response to treatment. Cryptococcal antigen titers greater than 1:640 are associated with meningitis development. 14 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 serum or CSF; or
- Titters obtained by the LFA are not equivalent to titers obtained by other cryptococcal antigen tests.
- 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

**WARRANTS and PRECAUTIONS**

- For In Vitro Use Only.

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expected values

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.
- 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, acetic acid, inositol, 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 samples across a variety of different pathogens.

The results of this testing are shown in the table below.

<table>
<thead>
<tr>
<th>Pathology</th>
<th># of Samples</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paniculosis</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>Sarcoptes scabiei</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>HAMA</td>
<td>5</td>
<td>0%</td>
</tr>
<tr>
<td>Syphilis</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>Rubella</td>
<td>5</td>
<td>0%</td>
</tr>
<tr>
<td>Mucoplasma</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>Tonsillitis</td>
<td>7</td>
<td>0%</td>
</tr>
<tr>
<td>Blastomycosis</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>Coccidioidomyces</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>Candidias</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>Aspergillus OM</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>Rhematoid Factor</td>
<td>10</td>
<td>0%</td>
</tr>
</tbody>
</table>

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 (10.1 mg/mL) antigens from Paracoccidioides brasilensis exhibited some cross-reactivity.

Antigens from the following organisms were tested and exhibited no cross-reactivity:
- Aspergillus terreus
- Aspergillus fumigatus
- Aspergillus niger
- Aspergillus flavus

This assay was not evaluated for cross-reactivity against the following organisms or pathogens:
- Candida dubliniensis
- Candida tropicalis
- Candida parapsidosis
- Candida auris
- Candida glabrata
- Candida lusitaniae
- Cryptococcus neoformans
- Salmonella typhi

High dose hook effect (prozoning)

Although rare, extremely high concentrations (>10.140 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.

-reduction and precision by spiking serum with cryptococcal antigen to produce a panel consisting of a negative sample, a high-negative (C) 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.

<table>
<thead>
<tr>
<th>Panel</th>
<th>Site 1 % Pos</th>
<th>Site 2 % Pos</th>
<th>Site 3 % Pos</th>
<th>Overall % Pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Positive</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Semi-quantitative</td>
<td>0%</td>
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<td>0%</td>
<td>0%</td>
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Cryptococcal antigenemia is caused by the fungus Cryptococcus neoformans, which can be isolated from numerous environmental sources. The fungus grows in soil, decaying vegetation, and animal droppings. Infection occurs when the fungus is inhaled or ingested, typically through the respiratory system. The disease can manifest in various forms, including meningitis, pulmonary infections, and disseminated infections in immunocompromised individuals.

The CrAg Lateral Flow Assay was validated for reproducibility and precision by spiking serum with cryptococcal antigen to produce a panel consisting of a negative sample, a high-negative (C) 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.

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</table>

BIBLIOGRAPHY


International Symbol Usage

Sufficient for ‘Y’ Tests

Reference Number

Lot Number

Manufactured by

Expiration Date

Single Use Only

Conforms to European Union Requirements

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