WARNINGS and PRECAUTIONS

1. Always wear gloves when handling reagents in this test kit as some reagents are preserved with 0.095% (w/w) sodium azide. Sodium azide should never be flush down the drain as this chemical may be toxic to the environment.
2. Sodium azide should never be flush down the drain as this chemical may be toxic to the environment.

SUMMARY and EXPLANATION of the Test

Cryptococcosis is caused by both species of the Cryptococcus species complex (Cryptococcus neoformans and Cryptococcus gattii) (6). Individuals with impaired cell-mediated immunity are at greatest risk of infection (8). Cryptococcosis is one of the most common opportunistic infections in AIDS patients (6). Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity and specificity (2-3).

BIOLICAL 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 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), regardless of the intensity of the test line, which 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. 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.

QUALITATIVE – BASIC PROCEDURE

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

MATERIALS PROVIDED.

1. Pipettor (40-µL and 80-µL)
2. Timer
3. Disposable micro-centrifuge tubes, test tubes, or a micro-titer plate

REAGENT PREPARATIONS

The entire kit should be at room temperature (20-25 °C) before and during use.

REAGENT STABILITY AND STORAGE

All reagents included in this kit should be stored at room temperature (20-25 °C) until the expiration dates listed on the reagent labels. Unused test strips should be stored in the LF test strip vial with the desiccant cap firmly attached.

PROCEDURE

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 hours 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.

QUALITY CONTROL

A positive control (CrAg Positive Control REF CB0020) can be evaluated by adding 1 drop of LF Specimen Diluent (REF GLF065) followed by 1 drop of CrAg Positive Control to a tube. A negative control can be evaluated by adding 2 drops of UF Specimen Diluent (REF GLF065) to a tube. Insert a test strip into the tubes, and read after 10 minutes. 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) indicates a positive result.

Reading the Test

Positive test results form only one line (control), if a control line fails to develop then the test is not valid.

Add 1 drop of specimen diluent Add 40 µL of specimen Insert strip Incubate 10 min

1 line = negative
2 lines = positive

Add 1 drop of pipette 40uL of LF Specimen Diluent (REF GLF065) to an appropriate reservoir (disposable micro-centrifuge tube, test tubes, or micro-titer plate, etc.). Add 40 µL of specimen to the container and mix. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip (REF LFCR50) into the specimen. Wait 10 minutes. Read and record the results (See READING THE TEST).

crAg LF Test Strips (50 strips in desiccant vial, REF LFCR50)

Package insert

CrAg Positive Control (1 mL, REF CB0020): Glycine-buffered saline spiked with cryptococcal antigen (strain S14A – clinical isolate from Tulane University (Infection & Immunity, June 1983, p. 1052-1059))

LFCR50

Materials Not Provided

1. Pipettor (40-µL and 80-µL)
2. Timer
3. Disposable micro-centrifuge tubes, test tubes, or a micro-titer plate

Reagent Preparations

The entire kit should be at room temperature (20-25 °C) before and during use.

Reagent Stability and Storage

All reagents included in this kit should be stored at room temperature (20-25 °C) until the expiration dates listed on the reagent labels.

Unused test strips should be stored in the LF test strip vial with the desiccant cap firmly attached.

Specimen Collection & Preparation

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 hours 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.

Procedure

1. Add 1 drop or pipette 40uL of LF Specimen Diluent (REF GLF065) to an appropriate reservoir (disposable micro-centrifuge tube, test tubes, or micro-titer plate, etc.).
2. Add 40 µL of specimen to the container and mix.
3. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip (REF LFCR50) into the specimen.
4. Wait 10 minutes.
5. Read and record the results (See READING THE TEST).

Semi-Quantitative Titration Procedure

Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560. Additional dilutions may be necessary if the specimen is positive at 1:2560. Add 4 drops or pipette 160uL of LF Specimen Diluent (REF GLF065) to tube #1.
6. Add 2 drops or pipette 80uL of LF Specimen Diluent to each of the tubes labeled 2-10. Add 20 µL of specimen to tube #1, and mix well.
7. 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.
8. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen in each of the 10 tubes.
9. Wait 10 minutes.
10. Read and record the results (See READING THE TEST).

Reading the Test

Read the reactions. The presence of two lines (test and control), regardless of the intensity of the test line, indicates a positive 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) indicates a positive result.
In order to establish the limit of detection, a Cr-Cr experiment was conducted by diluting purified cryptococcal antigen in LF Specimen Diluent (REF GF605) and testing 24 replicates per concentration using the CrAg Lateral Flow Assay. The results of this testing are shown in the following table:

### LIMIT OF DETECTION

<table>
<thead>
<tr>
<th>Concentration</th>
<th># Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 mg/mL</td>
<td>0</td>
<td>0% (0/24)</td>
</tr>
<tr>
<td>0.07 mg/mL</td>
<td>0</td>
<td>0% (0/24)</td>
</tr>
<tr>
<td>0.30 mg/mL</td>
<td>1</td>
<td>4% (1/24)</td>
</tr>
<tr>
<td>0.50 mg/mL</td>
<td>4</td>
<td>17% (4/24)</td>
</tr>
<tr>
<td>1.00 mg/mL</td>
<td>12</td>
<td>50% (12/24)</td>
</tr>
<tr>
<td>1.50 mg/mL</td>
<td>21</td>
<td>88% (21/24)</td>
</tr>
<tr>
<td>2.00 mg/mL</td>
<td>24</td>
<td>100% (24/24)</td>
</tr>
<tr>
<td>2.50 mg/mL</td>
<td>24</td>
<td>100% (24/24)</td>
</tr>
<tr>
<td>3.00 mg/mL</td>
<td>24</td>
<td>100% (24/24)</td>
</tr>
</tbody>
</table>

C<sub>0</sub> - C<sub>0.005</sub> = 1.0 – 1.5 mg/mL

### REPRODUCIBILITY AND PRECISION

The CrAg Lateral Flow Assay was evaluated for reproducibility and precision by spiking serum and mock CSF with cryptococcal antigen to produce a panel consisting of a negative sample, a high-negative (C<sub>0</sub>) 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- and intra-assay reproducibilities and precisions of the assay. The results of this study are shown in the tables below.

### 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.

### IMMUNOFLUORESCENT AGGLUTINATION METHOD

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 used in the semi-quantitative titer 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 R<sup>2</sup> value of 0.905.