Fortschritt in der Cryptococcose-Diagnostik

CrAg Lateral Flow Assay

Der neue CrAg Lateral Flow Assay der Firma Immuno-Mycologics Inc. (IMMY) aus den USA ist ein sensitiver und spezifischer Streifenschnelltest zum Nachweis von Cryptococcus Antigen aus Serum, Plasma, Urin und Liquor.

Die neue Testmethode benötigt keine Probenvorbereitung und nur eine einzige Probenverdünnung, so dass sie dem Anwender eine große Arbeitserleichterung verschafft. Durch den Einsatz einer ausgewählten Mischung spezifischer monoklonaler Antikörper werden alle bekannten Serotypen erfasst.

- Einfachste Handhabung - Serum-, Plasma-, Urin- bzw. Liquor probe verdünnen
  - Teststreifen hineingeben
  - Nach 10 Minuten Ergebnis ablesen

- Testdauer insgesamt nur 15 Minuten
- Erfasst alle bekannte Serotypen
- Höchste Sensitivität - Nachweigrenze nur 0,25 ng/ml
- Höchste Spezifität durch den Einsatz von monoklonalen Ak.
**CrAg Lateral Flow Assay**

*For the Detection of Cryptococcal Antigen – REF CR2003*

**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.

**EXPLANATION**

Cryptococcosis is caused by both species of the Cryptococcus species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) (4). Individuals with impaired cell-mediated immunity are at greatest risk of infection (6). Cryptococcosis is one of the most common opportunistic infections in AIDS patients (5). Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity (5). Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity (5). Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity (5).

**MATERIALS PROVIDED**

A. LF Specimen Diluent (2.5 ml, REF GLF025): Glycine buffered saline containing blocking agents and a preservative.

B. CrAg LF Test Strips (50 strips in desiccant vial, REF LFCR50).

C. CrAg Positive Control (1 ml, REF CB1020): Glycine buffered saline spiked with Cryptococcal Antigen

D. Package Insert

**PROCEDURE**

**Qualitative Procedure**

1. Add 1 drop of LF Specimen Diluent (REF GLF025) to an appropriate container (micro-centrifuge tube, micro-titer plate, test tube, etc.).

2. Add 40 µl of specimen to the container.

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 immediately (See READING THE TEST).

**Quantitative Titration Procedure**

1. Place 10 micro-centrifuge or test tubes in an appropriate rack and label them 1-10 (1.5 though 1:2560). Additional dilutions may be necessary if the specimen is positive at 1:2560.

2. Add 4 drops of LF Specimen Diluent (REF GLF025) 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.

6. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen in each one of the 10 tubes.

7. Wait 10 minutes.

8. Read and record the results immediately (See READING THE TEST).

**STABILITY AND STORAGE**

All reagents included in this kit should be stored at room temperature (22-25°C) until the expiration date listed on the reagent labels.

**SPECIMEN COLLECTION & PREPARATION**

For optimal results, sterile non-hemolyzed serum or CSF should be used. 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.

**PRECAUTIONS**

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. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimen. Always wear gloves when handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimen. Always wear gloves when handling patient

**READING THE TEST**

Read the reactions immediately. The presence of two lines (Test and Control), regardless of the intensity of the test line, 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 positive result. If the control line does not appear, the results are invalid and the test should be repeated.
This assay was not evaluated for cross-reactivity against the following organisms or pathologies:

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida dubliniensis</td>
<td>Pneumocystis carinii</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>Trichosporon beigelii</td>
</tr>
<tr>
<td>Candida parapsidis</td>
<td>Zygomycetes</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>Antinuclear antibody +</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Hepatitis A Virus</td>
</tr>
<tr>
<td>Cladosporium trichoides</td>
<td>Hepatitis C Virus</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>Streptococcus pneumonia</td>
</tr>
</tbody>
</table>

**High Dose Hook Effect (Prozone)**

Although rare, extremely high concentrations (>0.140 mg/mL) of Cryptococcal Antigen can result in weak test lines and, in extreme instances; yield a negative test result.

If prozone 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 AND SPECIFIC PERFORMANCE CHARACTERISTICS**

The CrAg Lateral Flow Assay was evaluated using 239 patient specimens (42 CSF, 197 Serum) that were submitted to a US reference laboratory for Cryptococcal Antigen Testing. These specimens were tested using the CrAg Lateral Flow Assay, the Immy Latex-Cryptococcal Antigen Detection System (REF CR1003), and the Meridian Premier™ Cryptococcal Antigen EIA. The results of these comparisons are shown in the tables below.

**Serum Agglutination Method Comparison**

<table>
<thead>
<tr>
<th>Pathology</th>
<th>% Positive</th>
<th>% Agreement</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicilliosis</td>
<td>5</td>
<td>0% (0/5)</td>
<td></td>
</tr>
<tr>
<td>Sporotrichosis</td>
<td>6</td>
<td>0% (0/6)</td>
<td></td>
</tr>
<tr>
<td>HAMA</td>
<td>5</td>
<td>0% (0/5)</td>
<td></td>
</tr>
<tr>
<td>Syphilis</td>
<td>10</td>
<td>0% (0/10)</td>
<td></td>
</tr>
<tr>
<td>Rubella</td>
<td>5</td>
<td>0% (0/5)</td>
<td></td>
</tr>
<tr>
<td>Mycoplasmosis</td>
<td>10</td>
<td>0% (0/10)</td>
<td></td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>7</td>
<td>0% (0/7)</td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>10</td>
<td>0% (0/10)</td>
<td></td>
</tr>
<tr>
<td>Blastomycosis</td>
<td>10</td>
<td>0% (0/10)</td>
<td></td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>10</td>
<td>0% (0/10)</td>
<td></td>
</tr>
<tr>
<td>Candidias</td>
<td>10</td>
<td>0% (0/10)</td>
<td></td>
</tr>
<tr>
<td>Aspergillus GM</td>
<td>10</td>
<td>0% (0/10)</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>10</td>
<td>0% (0/10)</td>
<td></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 (>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 fumigatus
- Aspergillus niger
- Aspergillus flavus

**REFERENCES**


**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 (C5) sample, a low positive sample and a moderate positive sample. This panel was tested twice per day at three sites with a total of 5 operators over a 5-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.