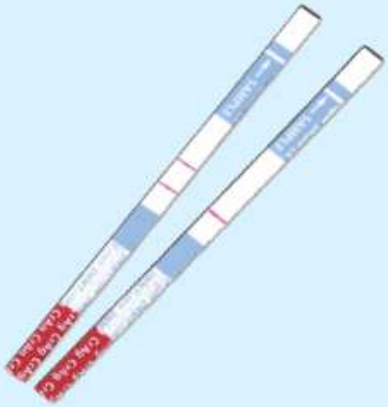
Fortschritt in der Cryptococcose-Diagnostik

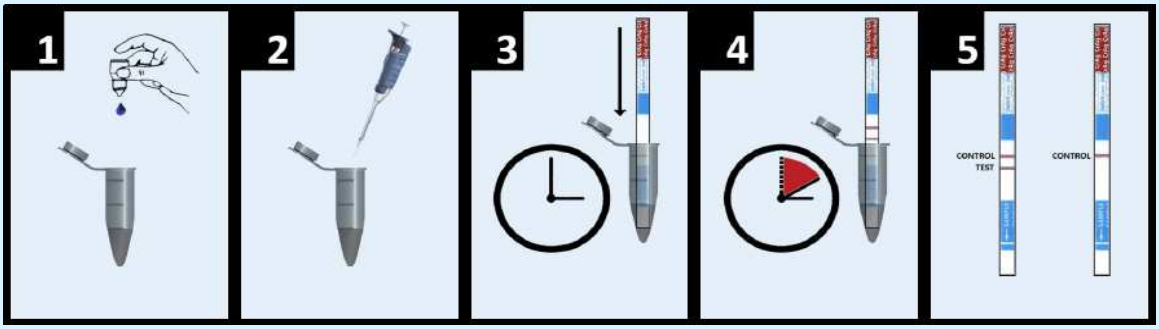
CrAg Lateral Flow Assay

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

Die neue Testmethode benötigt keine Proben-vorbereitung und nur eine einzige Probenverdünnung, so dass sie dem Anwender eine große Arbeit-serleichterung verschafft. Durch den Einsatz einer aus-gewählten Mischung spezifischer monoklonaler An-tikörper 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 - Nachweisgrenze nur 0,25 ng/ml**
- **Höchste Spezifität durch den Einsatz von monoklonalen Ak.**

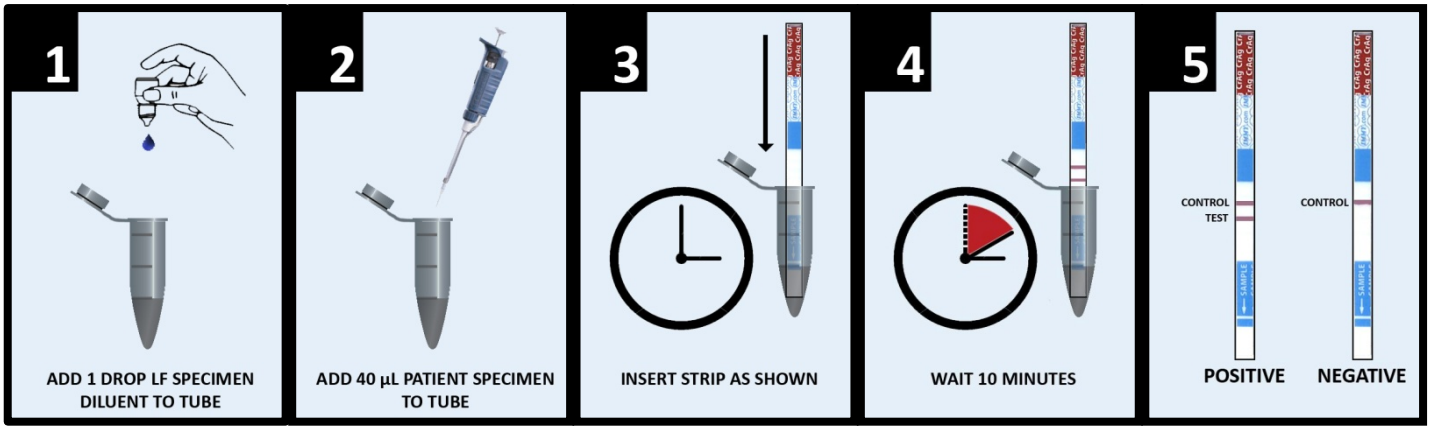


CHECK DIAGNOSTICS
Gesellschaft für Point-of-Care Testing mbH

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 and specificity (1-3).

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 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 Test 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.

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

MATERIALS NOT PROVIDED

A. Pipettor (40 µL and 80 µL)
B. Timer
C. Disposable micro-centrifuge tubes, test tubes, or a micro-titer plate

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 reagents in this kit as some reagents are preserved with 0.095% (w/w) sodium azide. Sodium azide should never be flushed down the drain since this chemical may react with lead or copper plumbing to form potentially explosive metal azides. Excess reagents should be discarded in an appropriate waste receptacle.

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.

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).

Semi-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).

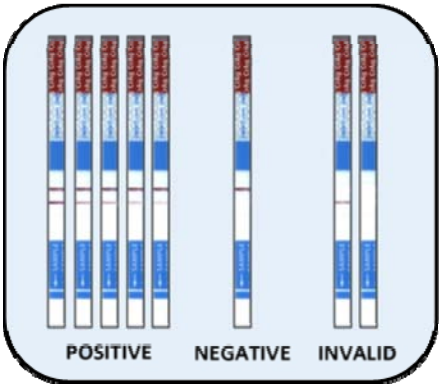
READING THE TEST

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 negative result. If the control line does not appear, the results are invalid and the test should be repeated.

READING THE TEST (cont.)



QUALITY CONTROL

A positive control (CrAg Positive Control REF CB0020) can be evaluated by adding 1 drop of LF Specimen Diluent (REF GLF025) 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 (REF GLF025) 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.

INTERPRETATION OF RESULTS

The Control Line must be present for a valid test. The presence of two bands (a control band and a band in the test zone) indicates a positive result.

LIMITATIONS OF THE PROCEDURE

Testing hemolyzed serum samples could lead to false negatives due to the high background color on the strip.

Cross-Reactivity Analysis

The CrAg Lateral Flow Assay was evaluated for cross-reactivity against a panel of patients' specimens across a variety of different pathologies. The results of this testing are shown in the table below.

Pathology	Number of Specimens	% Positive
Penicilliosis	5	0 % (0/5)
Sporothrichosis	6	0 % (0/6)
HAMA	5	0 % (0/5)
Syphilis	10	0 % (0/10)
Rubella	5	0 % (0/5)
Mycoplasmosis	10	0 % (0/10)
Toxoplasmosis	7	0 % (0/7)
CMV	10	0 % (0/10)
Blastomycosis	10	0 % (0/10)
Coccidiomycosis	10	0 % (0/10)
Histoplasmosis	10	0 % (0/10)
Candidiasis	10	0 % (0/10)
Aspergillus GM+	10	10 % (1/10)
Rheumatoid Factor	10	0 % (0/10)

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 *Paracoccidiodes 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

This assay was not evaluated for cross-reactivity against the following organisms or pathologies:

Candida dubliniensis
Candida tropicalis
Candida parapsidosis
Candida krusei
Candida glabrata
Cladosporium trichoides
Neisseria meningitidis
Salmonella typhi
Pneumocystis carinii
Trichosporon beigelii
Zygomycetes
Antinuclear antibody +
Hepatitis A Virus
Hepatitis C Virus
Staphylococcus aureus
Streptococcus pneumoniae

High Dose Hook Effect (Prozoning)

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 prozoning 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.

Latex Agglutination Method Comparison

Serum & CSF		Immy LA	
		Pos	Neg
CrAg LFA Assay	Pos	121	2
	Neg	0	116

Serum & CSF	Calculated	95% CI
% Positive Agreement	100% (121/121)	97% - 100 %
% Negative Agreement	98% (116/118)	94% - 99.5%
Total % Agreement	99.2% (237/239)	97% - 99.8%

Meridian EIA Method Comparison

Serum & CSF		Meridian EIA	
		Pos	Neg
CrAg LFA Assay	Pos	116	7
	Neg	0	116

Serum & CSF	Calculated	95% CI
% Positive Agreement	100% (116/116)	97% - 100 %
% Negative Agreement	94% (116/123)	89% - 97%
Total % Agreement	97% (232/239)	94% - 99%

Semi-Quantitative Method Comparison

In addition, 79 of these specimens (17 CSF, 62 Serum) were tested using the semi-quantitative titration 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^2 value of 0.890.

Limit of Detection

In order to establish the limit of detection, a C_5- C_{95} experiment was conducted by diluting purified Cryptococcal Antigen in LF Specimen Diluent (REF GLF025) and testing 24 replicates per concentration using the CrAg Lateral Flow Assay. The results of this testing are shown in the following table:

Concentration	# Positive	% Positive
0.50 ng/mL	0	0% (0/24)
0.75 ng/mL	0	0% (0/24)
1.00 ng/mL	4	17% (4/24)
1.25 ng/mL	12	50% (12/24)
1.50 ng/mL	21	88% (21/24)
1.75 ng/mL	24	100% (24/24)
2.00 ng/mL	24	100% (24/24)
2.50 ng/mL	24	100% (24/24)
3.00 ng/mL	24	100% (24/24)

$C_5 – C_{95}$ Interval	1.0 – 1.5 ng/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_5) 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.

SERUM PANEL	Site 1 % Pos	Site 2 % Pos	Site 3 % Pos	Overall % Pos
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	7% (2/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

CSF PANEL	Site 1 % Pos	Site 2 % Pos	Site 3 % Pos	Overall % Pos
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	10% (3/30)	0% (0/30)	0% (0/15)	4% (3/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

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