CrAg Rapid Test

Cat. No.:DTS737
Pkg.Size:50 Tests

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

General Description

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

Principle Of The Test

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). Negative test results form only one line (control). If a control line fails to develop then the test is not valid.

Reagents And Materials Provided

1. LF Specimen Diluent (2.5 mL): Glycine-buffered saline containing blocking agents and a preservative
2. CrAg LF Test Strips (50 strips in desiccant vial)
3. CrAg Positive Control (1 mL): Glycine-buffered saline spiked with cryptococcal antigen (strain 184A - clinical isolate from Tulane University (Infection & Immunity, June 1983, p. 1052-1059))
4. Package insert

Materials Required But Not Supplied

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

Specimen Collection And Preparation

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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.

Reagent Preparation

The entire kit should be at room temperature (22-25 °C) before and during use.

Reagent Stability

All reagents included in this kit should be stored at room temperature (22-25 °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.

Assay Procedure

REFER TO REAGENTS SECTION FOR A LIST OF MATERIALS PROVIDED.

Qualitative Procedure

1. Add 1 drop of LF Specimen Diluent 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 into the specimen.
4. Wait 10 minutes.
5. Read and record the results (See Interpretation of Results).

Semi-Quantitative Titration Procedure

1. Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560.
2. Place 10 micro-centrifuge or test tubes in an appropriate rack and label them 1-10 (1:5 through 1:2560). Additional dilutions may be necessary if the specimen is positive at 1:2560.
3. Add 4 drops of LF Specimen Diluent to tube #1.
4. Add 2 drops of LF Specimen Diluent to each of the tubes labeled 2-10.
5. Add 40 µL of specimen to tube #1, and mix well.
6. 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 for a final tube volume of 80 µL.
7. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen in each of the 10 tubes.
8. Wait 10 minutes.
9. Read and record the results (See Interpretation of Results).

Quality Control

A positive control can be evaluated by adding 1 drop of LF Specimen Diluent 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 to a tube. Insert a test strip into the tubes, and read after 10 minutes. Two 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.

Reading The Test

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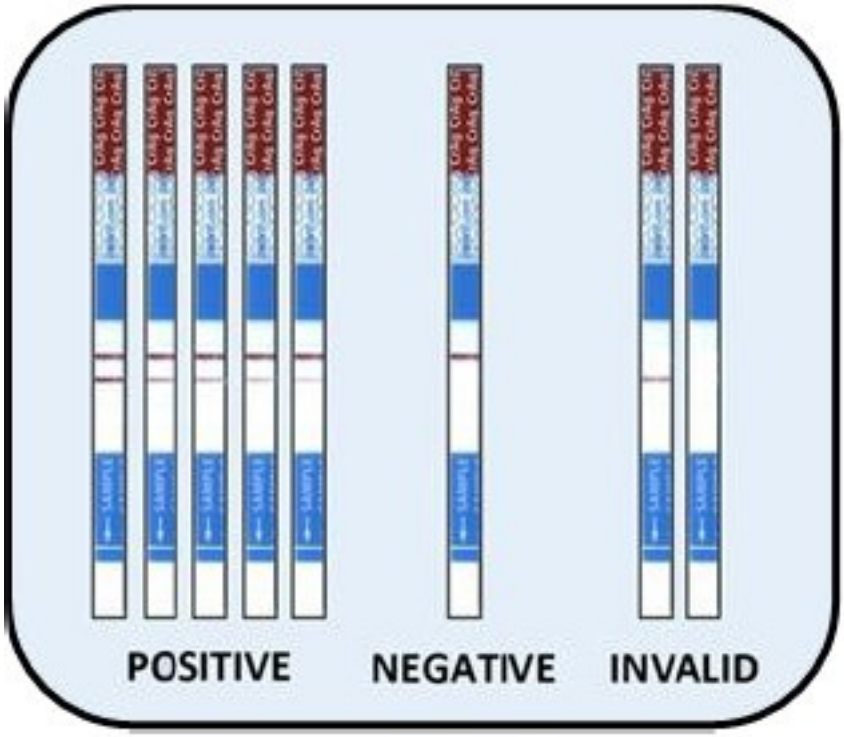
Read the reactions. The presence of two lines (test and control), regardless of the intensity of the test line, indicates a positive result.
For the semi-quantitative titration procedure, the patient's titer should be reported as the highest dilution that yields a positive result.
A single control line indicates a negative result. If the control line does not appear, the results are invalid and the test should be repeated.



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.
Negative results do not rule out the diagnosis of disease. The specimen may be drawn before detectable antigen is present.
The magnitude of the measured result, above the cutoff, is not indicative of the total amount of antigen present.

Expected Values

The frequency of cryptococcosis is dependent on several factors including: patient population, type of institution, and epidemiology. In this study, 100% (65/65) of true positives as determined by culture and/or India ink were detected. The apparent false positive in the CrAg LFA, compared to culture/India ink, could be the result of CrAg detection having a higher sensitivity (100%) than culture (83%) and India ink (84%). Therefore, it is expected to have culture-/India ink-negative/LFA-positive results. When compared to a commercially available EIA, 100% (96/96) of EIA positives were positive and 93% (94/101) EIA negatives were LFA-negative. Therefore, it is expected to have EIA-negative/LFA-positive results.

High Dose Hook Effect

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Although rare, extremely high concentrations (>0.140 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.

Specific Performance Characteristics

The CrAg Lateral Flow Assay was compared to the gold standard diagnoses of cryptococcosis (culture and/or India ink) to evaluate the sensitivity and specificity of the assay. These studies contained a mix of both prospective and retrospective specimens. A summary table of the data collected is included below.

Serum		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	91	0
	Negative	0	123

Serum	Calculated	95% CI
Sensitivity	100%	96.0% - 100%
Specificity	100%	97.0% - 100%

CSF		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	1
	Negative	0	77

CSF	Calculated	95% CI
Sensitivity	100%	94.4% - 100%
Specificity	98.7%	93.1% - 99.8%

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.

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Serum		CrAg EIA	
		Positive	Negative
CrAg LFA Assay	Positive	96	7
	Negative	0	94

Serum	Calculated	95% CI
% Positive Agreement	100% (96/96)	96% - 100 %
% Negative Agreement	93% (94/101)	86% - 97%

LATEX AGGLUTINATION 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 the 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 tested using the semi-quantitative titration procedure in both the CrAg Lateral Flow Assay and the Latex Cryptococcal Antigen Detection System. Linear regression analysis of the data yielded an R2 value of 0.905.

LIMIT OF DETECTION

In order to establish the limit of detection, a C5- C95 experiment was conducted by diluting purified cryptococcal antigen in LF Specimen Diluent and testing 24 replicates per concentration using the CrAg Lateral Flow Assay. The results of this testing are shown in the following table.

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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 (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 five operators over a five-day period in order to determine both the inter-lab and the intra-lab reproducibilities and precisions of the assay. The results of this study are shown in the tables 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)

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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)	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)

Cross-Reactivity

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	# of Samples	% 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)
Coccidioidomycosis	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.

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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, Pneumocystis, carinii, Candida tropicalis, Trichosporon beigelii, Candida, parapsidosis, Zygomycetes, Candida krusei, Antinuclear antibody +, Candida glabrata, Hepatitis A Virus, Cladosporium trichoides, Hepatitis C Virus, Neisseria meningitidis, Staphylococcus spp., Salmonella typhi, Mycobacterium tuberculosis, Enterovirus, Enterobacteriaceae, Enterococcus spp., Epstein Barr, Trichosporon beigelii, Streptococcus pneumonia, Streptococcus spp., Diphtheroid, H. influenzae type B, Herpes simplex viruses, Listeria monocytogenes, Syneresis fluid condensation, Staphylococcus aureus.

Interferences

This assay was not evaluated for potential interference related to specimen pretreatment with 2-mercaptoethanol or with specimens including the following substances: bloody CSF, cloudy CSF, white blood cells, xanthochromic CSF, bilirubin, protein, systemic lupus erythmatosus (SLE), sarcoidosis, or N. memingitides.
This assay was evaluated for the potential of interference due to serum conditions including icteric, hemolyzed, and lipemic samples. These samples exhibited no interference in the assay. Hemolyzed samples, however, could lead to false negatives due to the high background color on the strip.

Precautions

1. 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.
2. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimens.
3. 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 as 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.

Limitations

1. The assay performance characteristics have not been established for matrices other than serum and CSF.
2. 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. Testing should only be done when clinical evidence suggests the diagnosis of cryptococcal disease.
3. Testing hemolyzed serum samples could lead to false negatives due to the high background color on the strip.
4. The performance of this device has not been evaluated with specific HIV therapies. The patient samples included in the study were obtained from patients undergoing HIV therapy, but information on the therapy received was not available.

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