Dynamiker Biotechnology (Tianjin) Co., Ltd.

Dynamiker Cryptococcal Antigen Lateral Flow Assay (LFA)

Catalogue No.: DNK-1411-1

User Manual / 40 tests

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1. INTENDED USE

Rapid immunochromatographic test for the detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans and Cryptococcus gattii*) in human serum and cerebral spinal fluid (CSF).

2. INTRODUCTION

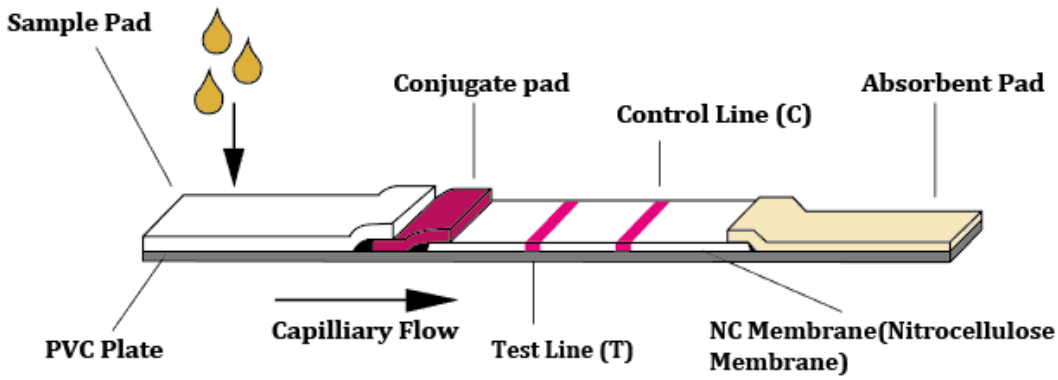
Cryptococcus spp. are important opportunistic pathogens that often related to meninges, lung and skin infections. *Cryptococcus Neoformans* meningitis (CNM) is the most common occurred fungal infection in central nervous system[1]. In recent years, CNM has increased significantly in morbidity and mortality. According to the statistics, the prevalence of *Cryptococcus* meningitis in the United States is about 5% to 10% in patients with AIDS, where a higher incidence rate can be found in certain developing countries[2]. Meanwhile, *Cryptococcus Neoformans* infect respiratory system and cause a yearly increasing of pulmonary cryptococcosis.

The Cryptococcal Antigen Lateral Flow Assay (LFA) is a prescription-use laboratory assay that provides aid to the diagnosis of cryptococcosis.

3. PRINCIPLE

The product uses lateral flow technique and double antibody sandwich format. *Cryptococcus* antibodies conjugated with gold particles are coated on fiberglass. Test line (T) and control line (C) on nitrocellulose membrane are coated with *Cryptococcus* antibodies and goat anti-mouse antibodies, respectively. Cryptococcal antigens in samples form antigen-antibody complexes with gold-conjugated antibodies and migrate further along on nitrocellulose membrane. *Cryptococcus* antibodies immobilized on Test line (T) interact with the above complexes and causing a visible red line. The wicking of samples cause free gold-conjugated antibodies to reach control line (C) and form red color. The absence of cryptococcal antigens in negative samples only cause color change in control line (C). Therefore, control line is included as the quality control standard to ensure the test is visually validated to be functioning correctly.

Samples are added into sample pad and test can be achieved within 15-20 minutes.



4. COMPONENTS

	Components
Test kit	40 packs of tests, Instruction for use
Pack	Test, desiccant, aluminum foil bag
Test	Test line (T): coated with cryptococcal antibodies with proper concentration; Control line (C): coated with goat anti-mouse antibodies with proper concentration; Conjugate pad: coated with gold-conjugated cryptococcal antibodies with proper concentration

5. STORAGE and EXPIRATION DATE

1. Test should be stored at 2-30ºC in dark and dry place for 18 months. DO NOT freeze the test.
2. Test is recommended to be used within 0.5 hour after opening.

6. SAMPLE COLLECTION and PREPARATION

1. Collect samples according to standard laboratory procedures. Avoid cross-contamination among samples. Sample labelling should be clear and correct without mistake.
2. Samples with severe hemolysis and high viscosity are not applicable.
3. Sample stability and storage

3.1 Sample transportation

Samples should be frozen during transportation. Sample transportation should comply with biosafety requirements.

3.2 Sample storage

Samples can be stored at 2-8ºC for up to 24 hours. For longer storage, store the samples at -20ºC. Avoid repeated freezing and thawing.

7. PROCEDURE

QUALITATIVE TEST：

1) Carefully refer to the instruction for use prior to performing the test.
2) Before test, ensure that tests and samples are at room temperature.
3) Place tests on flat and clean bench; dispense 80μL of samples and slowly add into sample pad.
4) Read and record the results after 15 minutes (No longer than 20 minutes). Abnormal results may occur after 20 minutes.

SEMI-QUANTITATIVE DETECTION:

1) Before test, ensure that tests and samples are at room temperature.
2) Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560.
3) Place 10 centrifuge 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.
4) Add 400μL sample diluent(Normal Saline) to each tube #1-10.
5) Add 100μL of sample to tube #1 and mix well.

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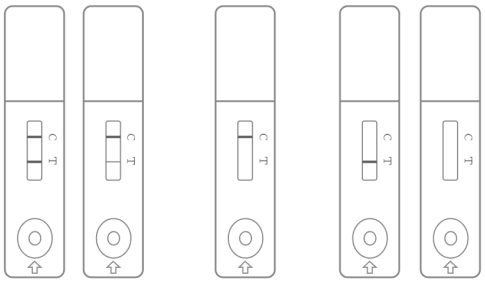
6) Transfer 400μL of specimen from tube #1 to tube #2 and mix well. Continue this dilution procedure through tube #10.
7) Open the test pack, place test strips on flat and clean bench, dispense 80μL of diluent samples (tube #1-10) and slowly add into sample pad.
8) Read and record the results after 15 minutes (No longer than 20 minutes). Abnormal results may occur after 20 minutes.
9) The patient's titer should be reported as the highest dilution that yields a positive result.

Note: Centrifuge the sample at 3000rpm with 30 seconds before test, if the samples are frozen or stored at 2-8 °C longer than 24 hours.

8. INTERPRETATION of RESULTS



Positive **Negative** **Invalid**

Positive (+): Presence of two red lines, test line (T) and control line (C), indicates cryptococcal antigens present in samples.

Negative (-): Appearance of single control line (C), no red test line (T), indicates the absence of cryptococcal antigens or limited amount (lower than assay limit of detection) of cryptococcal antigens in samples.

Invalid: No red control line (C) appears. Invalid results may due to incorrect operation or loss of efficacy in tests. Repeat test first, if problem remains, stop using productsin same lot member and contact with local distributor for support.

Note:

1. The intensity of red color in test line (T) is in proportional to the titer of cryptococcal antigens in samples. However, test results cannot be used as reference to indicate the titer level of cryptococcal antigen in samples.
2. Further repeated test and confirmatory test should be conducted on positive samples.

QUALITY CONTROL

1. Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered as an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.

2. External controls may be provided(by request only) in the kits to ensure that the tests are functioning properly. Also, the Controls may be used to demonstrate proper performance by the

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test operator. To perform a positive or negative control test, complete the steps in the Qualitative Procedure section with positive control and titration buffer as negative control.

9. LIMITATIONS of METHODOLOGY

1. The assay has not been established for matrices other than human CSF and serum.
2. Since the assay is developed for qualitative detection, quantitation of cryptococcal antigens in samples is not available.
3. Positive results indicate the presence of cryptococcal antigens in samples, rather than a diagnostic standard of *Cryptococcus* infection. Repeated test and confirmatory test is necessary to achieve diagnosis.
4. The assay has a limit of detection (LOD). Negative results cannot be used to rule out *Cryptococcus* infection.

10. PRECAUTIONS

1. The product is only for in vitro diagnosis.
2. Inspection of product packing and sealing as well as expiration date is necessary prior to performing the test.
3. Please re-collect samples for test if samples are in severe hemolysis.
4. Tests can be stored at room temperature. Ensure that tests are kept from moisture. Tests stored at low temperature (DO NOT FREEZE) should bring to room temperature before testing.
5. Test should be performed as quickly as possible. Long-time exposure of test to air and moisture will cause invalid results.
6. Overload of samples may result in unexpected results, such as false positives.
7. Accuracy of test can be affected by environment temperature (<10ºC or >40ºC) and relative humidity (>80%).

11. PERFORMANCE CHARACTERISTICS

LIMIT OF DETECTION

In order to establish the limit of detection, a C_5- C_{95} experiment was conducted by diluting purified cryptococcal antigen by titration buffer and testing 40 replicates per concentration using the DNK CrAg Lateral Flow Assay. The results of this testing are shown in the following table:

Concentration	Positive number	Positive rate %
0.075ng/ml	0	0%（0/40）
0.10ng/ml	0	0%（0/40）
0.25ng/ml	0	0%（0/40）
0.50ng/ml	15	37.5%（27/40）
0.75ng/ml	27	67.5%（32/40）
1.00ng/ml	38	95%（38/40）
1.25ng/ml	40	100%（40/40）
1.50ng/ml	40	100%（40/40）
1.75ng/ml	40	100%（40/40）

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2.00ng/ml	40	100%（40/40）
2.25ng/ml	40	100%（40/40）

C_5-C_{95} Interval	0.5-1.0 ng/mL

ACCURACY

The DNK CrAg LFA 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 datacollected is included below.

CSF	Culture/ India Ink		
DNK CrAg LFA		Positive	Negative
	Positive	121	0
	Negative	0	229
Sensitivity:100% (97.91%-100%)* Specificity:100% (96.16%-100%)* Agreement:100% (96.72%-100%)* *95% CI			

Serum	Culture/ India Ink		
DNK CrAg LFA		Positive	Negative
	Positive	79	2
	Negative	0	198
Sensitivity: 100% (97.63%-100%)* Specificity: 99% (90.54%-99.57%)* Agreement: 99.28% (97.28%-99.97%)* *95% CI			

ELISA METHOD COMPARISON

The DNK CrAg LFA was evaluated using Dynamiker *Cryptococcus neoformans* Antigen Assay (ELISA) with 300 serum samples. The results are shown below:

Serum	Dynamiker *Cryptococcus neoformans* Antigen Assay (ELISA)		
DNK CrAg LFA		Positive	Negative
	Positive	105	4
	Negative	0	191
Positive agreement: 100% (97.54%-100%)* Negative agreement: 97.94% (90.32%-98.82%)* *95% CI			

INTERFERENCE

The DNK CrAg LFA was evaluated for cross-reactivity against a panel of patients' serum specimens across a variety of different pathologies. The results are shown below.

Candidiasis	*Aspergillosis*
Histoplasmosis	*Coccidiodomycosis*
Blastomycosis	*Penicilliosis*
Mycoplasmosis	*Sporothrichosis*
Rheumatoid Factor	*Syphilis*
HAMA	*Rubella*
CMV	*Toxoplasmosis*

No cross-reactivity were found for above pathologies.

Antigens from the following pathologies were tested and exhibited no cross-reactivity.

Candida albicans	*Candida glabrata*
Candida parapsillosis	*Candida tropicalis*
Candida krusei	*Candida dubliniensis*
Aspergillus fumigatus	*Aspergillus flavus*
Aspergillus terreus	*Aspergillus niger*
Staphylococcus pneumoniae	*Hepatitis A virus*
Hepatitis C virus	*Cladosporium trichoides*
Salmonella typhi	*Staphylococcus aureus*
Neisseria meningitidis	*Staphylococcus aureus*

HIGH DOSE HOOK EFFECT (PROZONING)

Although rare, extremely high concentrations (>0.15 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.

12. REFERENCE

[1] The capsular dynamics of Cryptococcus neoformans. TRENDS in Microbiology, Vol. 14. No.11.

[2] The capsule of the fungal pathogen Cryptococcus neoformans. Adv Appl Microbiol, 2009, 68: 133–216.

13. MANUFACTURER

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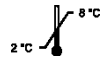
[SYMBOLS USED]

Symbol	Description
	Use By
LOT	Batch Code
	Manufacturer
	Keep Away from Sunlight
	Temperature Limitation
IVD	In Vitro Diagnostic Medical Device
EC REP	Authorized Representative in the European Community
CE	CE Mark

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