LIMING BIO

Cryptococcal Antigen Rapid Test Device

REF	502080	**Specimen:** Whole Blood /Plasma/ Serum/ Cerebral Spinal Fluid
Language: English		**Version:** 01
Effective Date: 2014-11		

For professional *in vitro* diagnostic use only.

INTENDED USE

The StrongStep® Cryptococcal Antigen Rapid Test Device is a rapid immune-chromatographic assay for the detection of the capsular polysaccharide antigens of Cryptococcus species complex (Cryptococcus neoformans and Cryptococcus gattii) in serum, plasma, whole blood and cerebral spinal fluid (CSF). The assay is a prescription-use laboratory assay which can aid in the diagnosis of cryptococcosis.

INTRODUCTION

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 in serum and CSF has been extensively utilized with very high sensitivity and specificity.

PRINCIPLE

The StrongStep® Cryptococcal Antigen Rapid Test Device has been designed to detect Cryptococcus species complex through visual interpretation of color development in the internal strip. The membrane was immobilized with anti Cryptococcal monoclonal antibody on the test region. During the test, the specimen is allowed to react with monoclonal anti-Cryptococcal antibody colored particals conjugates, which were precoated on the conjugate pad of the test. The mixture then moves on the membrane by capillary action, and interact with reagents on the membrane. If there were enough Cryptococcal antigens in specimens, a colored band will form at the test region of the membrane. Presence of this colored band indicates a positive result, while its absence indicates a negative result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

KIT COMPONENTS

20 Individually packed test devices	Each device contains a strip with colored conjugates and reactive reagents pre-spreaded at the corresponding regions.
3ml Titration Buffer	Glycine-buffered saline containing a preservative
20 Disposable pipettes	For adding specimens
1 Package insert	For operation instruction.
1 Positive control (on request only)	Contain inactived Cryptococcal Antigen and sodium azide. For External control.
1 Negative control (on request only)	Not contain Cryptococcal Antigen. For external control.

MATERIALS REQUIRED BUT NOT PROVIDED

Timer	For timing use.
Specimen collection container	For specimen collection.
Centrifuge	For preparing serum/plasma specimens
Pipettor	(40-μ L and 80-μ L)
Micro-titer plate	For specimen Semi-Quantitative Titration

PRECAUTIONS

- This kit is for IN VITRO diagnostic use only.
- This kit is for PROFESSIONAL use only.
- Read the instructions carefully before performing the test.
- This product does not contain any human source materials.
- Do not use kit contents after the expiration date.
- Handle all specimens as potentially infectious.
- Follow standard Lab procedure and biosafety guidelines for handling and disposal of potentially infective material. When the assay procedure is complete, dispose specimens after autoclaving them at 121℃ for at least 20 min. Alternatively, they can be treated with 0.5% Sodium Hypochlorite for hours before disposal.
- Do not pipette reagent by mouth and no smoking or eating while performing assays.
- Wear gloves during the whole procedure.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Cares should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- The Cryptococcal Antigen Rapid Test Device (Whole Blood /Plasma/ Serum/ Cerebral Spinal Fluid) is intended for use with human whole blood, plasma, serum, or Cerebral Spinal Fluid specimens only.
- Only clear, non-hemolyzed specimens are recommended for use with this test. Serum or plasma should be separated as soon as possible to avoid hemolysis.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Serum, plasma and Cerebral Spinal Fluid specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens.
- Containers containing anticoagulants such as EDTA, citrate, or heparin should be used for whole blood storage.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.
- Icteric, lipemic, hemolysed, heat treated and contaminated specimens may cause erroneous results.

Qualitative PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. For best results, the assay should be performed within one hour.
2. Transfer 2 drops of Plasma/ Serum/ Cerebral Spinal Fluid specimen (approximately 80μL) or 3 drops of whole blood specimen (approximately 120μL) to the specimen well (S) of the device with the provided disposable pipette, then start the timer.
   Avoid trapping air bubbles in the specimen well (S), and do not add any solution to the result area.
   As the test begins to work, color will migrate across the membrane.
3. Wait for the colored band(s) to appear. The result should be read at 10 minutes.

Semi-Quantitative Titration Procedure

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

1. Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560.
2. Place 10 micro-titer plate 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 or 160μ L of titration buffer to plate #1.
4. Add 2 drops or 80μ L of titration buffer to each of the plate labeled 2-10.

5. Add 40μ L of specimen to plate #1 and mix well.
6. Transfer 80μ L of specimen from plate #1 to plate #2 and mix well.
7. Continue this dilution procedure through plate #10.
8. Transfer 80μ L of diluted specimen from each of the 10 tubes to the specimen well (S) of the device, then start the timer.
9. Wait for the colored band(s) to appear. The result should be read at 10 minutes.

INTERPRETATION OF RESULTS

POSITIVE RESULT: 	Two colored bands appear within 10 minutes. One blue band appears in the Control Zone (C) and another red band appears in the Test Zone (T). The test result is positive and valid. For the semi-quantitative titration procedure, the patient's titer should be reported as the highest dilution that yields a positive result.
NEGATIVE RESULT: 	One blue bands appears in the Control Zone (C) within 10 minutes. No red band appears in the Test Zone (T). The test result is negative and valid.
INVALID RESULT: 	No blue band appears in the Control Zone (C) within 10 minutes. The test result is invalid. Repeat the test with a new test device.

NOTE:
1. The intensity of the color in test region (T) may vary depending on the concentration of aimed substances present in the specimen.
2. Insufficient specimen volume, incorrect operation procedure, or performing expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- 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.
- External procedural controls may provided(on 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 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.

LIMITATIONS OF THE TEST

- 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.
- As in case of all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test but should rather be made after all the clinical findings have been evaluated.

PERFORMANCE CHARACTERISTICS

- **Accuracy**

The Cryptococcal Antigen Rapid Test Device 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.

Whole blood		Culture/ India Ink	
StrongStep®		Positive	Negative
	Positive	76	2
Cryptococcosis Test	Negative	1	213
Relative Sensitivity: 98.70% (92.98%-99.97%)*			
Relative Specificity: 99.07% (96.68%-99.89%)*			
Overall Agreement: 98.97% (97.03%-99.79%)*			
***95% Confidence Interval**			

Serum		Culture/ India Ink	
StrongStep®		Positive	Negative
	Positive	65	1
Cryptococcosis Test	Negative	0	189
Relative Sensitivity: 100% (94.49%-100%)*			
Relative Specificity: 99.47% (97.11%-99.99%)*			
Overall Agreement: 99.61% (97.84%-99.99%)*			
***95% Confidence Interval**			

Plasma		Culture/ India Ink	
StrongStep®		Positive	Negative
	Positive	87	3
Cryptococcosis Test	Negative	1	254
Relative Sensitivity: 98.86% (93.82%-99.97%)*			
Relative Specificity: 98.83% (96.63%-99.76%)*			
Overall Agreement: 98.84% (97.06%-99.68%)*			
***95% Confidence Interval**			

CSF		Culture/ India Ink	
StrongStep®		Positive	Negative
	Positive	138	3
Cryptococcosis Test	Negative	0	322
Relative Sensitivity: 100% (97.36%-100%)*			
Relative Specificity: 99.08 % (97.32%-99.81%)*			
Overall Agreement: 99.35% (98.12%-99.87%)*			
***95% Confidence Interval**			

- **Interference**

The Cryptococcal Antigen Rapid Test Device 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.

Penicilliosis	***Sporothrichosis***
HAMA	***Syphilis***
Rubella	***Mycoplasmosis***
Toxoplasmosis	***CMV***
Blastomycosis	***Coccidiodomycosis***
Histoplasmosis	***Candidiasis***
Aspergillus GM+	***Rheumatoid Factor***

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the Cryptococcal Antigen Rapid Test Device. 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***
Candida dubliniensis	***Candida tropicalis***
Candida parapsilosis	***Candida krusei***
Candida glabrata	***Trichosporon beigelii***
Hepatitis A virus	***Cladosporium trichoides***
Hepatitis C virus	***Salmonella typhi***

Neisseria meningitidis	*Staphylococcus aureus*
Chlamydia pneumoniae	*Staphylococcus pneumoniae*

- **High dose hook effect (Prozoning)**
  Although rate, 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, specimens should be diluted to rule out false negative results.

LITERATURE REFERENCES

1. Doering, T.L. Annu. Rev. Microbiol. 2009; 63:223-247.
2. Goodman, J.S., L. Kaufman, and M. G. Koenig. N. Engl. J. Med. 1971; 285: 434-436.
3. Kozel, T. R. Trends Microbiol. 1995; 3:295-299.
4. Lin, X. and J. Heitman. T. Annu. Rev. Microbiol. 2006; 60: 69-105.
5. Park, B.J., K.A. Wannemuehler, B.J. Marston, N. Govender, P.G. Pappas, and T.M. Chiller. AIDS. 2009; 23: 525-530.
6. Zhou, Q. and W. J. Murphy. Immunol. Res. 2006; 35: 191-208.

GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer	Σ	Contains sufficient for <n> tests
	Do not reuse	EC REP	Authorized representative in the European Community

Liming Bio-Products Co., Ltd,
No. 12 Huayuan Road,Nanjing, Jiangsu, 210042
P.R. China.
Tel: (0086)25 85476723 Fax: (0086)25 85476387
E-mail: sales@limingbio.com
Website: www.limingbio.com
www.stddiagnostics.com
www.stidiagnostics.com