IMMY

Latex-Cryptococcus Antigen Detection System

REF #: CR1004 and Individual Reagents

IVD

INTENDED USE

The LATEX-CRYPTOCOCCUS ANTIGEN TEST is a simple and rapid latex agglutination test for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of *Cryptococcus neoformans* in serum and cerebrospinal fluid (CSF) as an aid in the diagnosis of Cryptococcosis.

SUMMARY AND EXPLANATION OF THE TEST

Cryptococcosis is caused by the encapsulated yeast *Cryptococcus neoformans*. Individuals with impaired cell-mediated immune (CMI) function due to acquired immunodeficiency syndrome (AIDS) (39), lymphoproliferative disorders (38), steroid therapy (13), and organ transplantation (12) are at increased risk of Cryptococcosis. AIDS accounts for 80-90% of Cryptococcal infections (24) where Cryptococcosis occurs in 5-10% of AIDS patients in the United States (24). The incidence of Cryptococcosis in other parts of the world, such as Africa, is as high as 30% (8). Cryptococcosis is the fourth most common opportunistic, life-threatening infection among AIDS patients (23).

The LATEX-CRYPTOCOCCUS ANTIGEN TEST is a simple, sensitive test capable of detecting *C. neoformans* polysaccharide antigens in serum and CSF (1), and is superior in sensitivity to the India ink mount (4,6). Clinical studies established the prognostic value of the test (9,16,21,22) and showed it to be a valuable aid in establishing a diagnosis when the culture was negative (14). *C. neoformans* antigens were present in both serum and CSF in 86% of 330 confirmed cases of Cryptococcal meningitis (22). Antigen was detected in CSF specimens in 99% of these 330 cases but in only 87% of serum samples alone (22). Paired serum and CSF specimens allowed detection of the antigen in each confirmed case (22).

BIOLOGICAL PRINCIPLES

The LATEX-CRYPTOCOCCUS ANTIGEN TEST is based upon the principle that anti-Cryptococcal antibody-coated latex particles will agglutinate with specimens containing Cryptococcal capsular polysaccharide antigens (4,6). Previously, the detection of this antigen in serum was hampered by the presence of rheumatoid factor (3,15). Pretreatment of serum specimens with Pronase (REF DE0010) reduces nonspecific interference and enhances the detection of capsular polysaccharide antigens of *C. neoformans* (17) due to rheumatoid factor (34) and immune complexes (33).

REAGENTS PROVIDED

A. **Specimen Diluent** (10 ml, REF GB0020): Concentrated (10X) glycine buffered saline (pH 8.6) containing albumin and a preservative.
B. **Cryptococcal Latex:** (3.5 ml, REF CG0010): Standardized latex particles sensitized with rabbit anti-cryptococcal globulin in glycine buffered saline containing a preservative. DO NOT FREEZE.
C. **Cryptococcal Antigen Positive Control** (1 ml, REF CB0010): Purified capsular polysaccharide antigens containing a preservative.
D. **Negative Control** (1 ml, REF N80110): Normal goat serum containing a preservative.
E. **Pronase** (1.75 ml, REF DE0010): Lyophilized Pronase containing a preservative.
F. **Pronase Control** (2 ml, REF R20000): Goat anti-rabbit globulin containing a preservative.
G. **Pronase Inhibitor** (6 ml, REF EI0010): Contains an inhibitor for the Pronase.
H. **Disposable Ring Slides** (REF SC0020)
I. **Package Insert**

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipette(s) capable of measuring and delivering 25 - 100 µL and associated disposable tips
- 1-mL serological pipettes
- Distilled or DI water
- Disposable borosilicate glass test tubes (non-siliconized), 10 or 12 X 75 mm, for specimen dilutions & pronase aliquots
- Test tube rack
- Waterbath or heat block (56 °C)
- Wooden applicator sticks
- Rotator set to 100 rpm
- Timer
- Biohazard waste receptacle

REAGENT STABILITY AND STORAGE

All reagents (except Pronase after rehydration, which must be frozen at -20°C or colder) should be stored at 2-8°C. Prolonged periods at room temperature should be avoided. Avoid FREEZING latex suspensions as this causes granularity, which might be interpreted as a false positive reaction. The frozen aliquots of Pronase may continue to be used as long as they continue to destroy the activity of the Pronase Control in monthly tests and are within the expiration dating.

REAGENT PRECAUTIONS

1. Do not use reagents containing foreign matter, particulates or aggregates, which indicate contamination or improper storage or handling. Discard if these conditions are found.
2. 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.
3. Do not use kit or any kit reagents after the stated expiration date.
4. IMMY cannot guarantee the performance of its products when used with materials purchased from other manufacturers. The use of other products with this test has not been evaluated and may result in erroneous results.
5. NEVER heat-inactivate Pronase Control as this could cause aberrant control reactions.
6. Do not store rehydrated Pronase in a frost-free type freezer.
7. **R**eagents are preserved with sodium azide [0.095% (w/w)], which is a skin irritant. Avoid skin contact with the kit components. Do not mix reagents with acid as this may result in the formation of hydrazoic acid, an extremely toxic gas. Additionally, disposal of reagents containing sodium azide into lead or copper plumbing can result in the formation of explosive metal azides. It is therefore recommended that excess reagents simply be discarded in an appropriate waste receptacle.
8. N80110 is labeled as:

Danger

H334	May cause an allergy or asthma symptoms or breathing difficulties if inhaled.
P261	Avoid breathing mist/vapors/spray.
P284	[In case of inadequate ventilation] wear respiratory protection.
P304 + P341	If inhaled: If breathing is difficult, remove person to fresh air and keep comfortable for breathing.
P342 + P311	If experiencing respiratory symptoms: Call a poison center or doctor.
P501	Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

9. DE0110 is labeled as:

Danger

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H334	May cause an allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P261	Avoid breathing mist/vapors/spray.
P264	Wash hands, forearms and face thoroughly after handling.
P271	Use only outdoors or in a well-ventilated area.
P280	Wear protective gloves, protective clothing, chemical goggles, & face protection.
P284	[In case of inadequate ventilation] wear respiratory protection.
P302 + P352	If on skin: Wash with plenty of water.
P304 + P340	If inhaled: Remove person to fresh air and keep comfortable for breathing.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P312	Call a poison center or doctor if you feel unwell.
P332 + P313	If skin irritation occurs: Get medical advice/attention.
P337 + P313	If eye irritation persists: Get medical advice/attention.
P342 + P311	If experiencing respiratory symptoms: Call a poison center or doctor.
P362 + P364	Take off contaminated clothing and wash it before reuse.
P403 + P233	Store in a well-ventilated place. Keep container tightly closed.
P405	Store locked up.
P501	Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

WARNINGS AND PRECAUTIONS FOR USERS

1. For In Vitro Diagnostic use only.
2. Wear protective clothing, including lab coat, eye/face protection, and disposable gloves, and handle the kit reagents and patient samples with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
3. Specimens must not contain bacteria, visible lipids, or other obvious signs of contamination.
4. Care should be taken not to introduce syneresis fluid, which is present in various types of agar, into any specimens prior to testing as this may cause spurious results.
5. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimen.

REAGENT PREPARATION

Reconstitute or dilute the following reagents with the indicated volume of distilled or DI water:

1. **Pronase** (REF #: DE0010) 1.75 ml; Reconstituted Pronase should be aliquoted into test tubes in 0.05 ml (50 µl) amounts, sealed, and frozen immediately at –20°C or colder. Do NOT use siliconized tubes.
2. **Specimen Diluent** (REF #: GB0020) Dilute 1:10
3. Latex solutions must appear as homogeneous suspensions.
4. When using the negative control (REF #: N80110) for the first time, heat inactivate at 56°C for 30 minutes.
5. The ring slides (REF #: SC0020) should be discarded after each use.

SPECIMEN PREPARATION

CEREBROSPINAL FLUID (CSF)

1. Collect specimen aseptically following accepted procedures.
2. Centrifuge at 1000 x g for 15 minutes to ensure the removal of all white cells and particulate matter.
3. Carefully aspirate the CSF into a sterile container and seal.
4. Specimen may be processed immediately, refrigerated, preserved by freezing at –20°C or by adding thimerosal to provide a final concentration of 0.01%.
5. Incubate CSF specimen at 100°C for 5 minutes.
6. Specimen is ready for testing (see **PROCEDURE).**

SERUM

1. Collect whole blood aseptically following accepted procedures. The specimen must not contain anticoagulants as this will invalidate the test.
2. Permit blood to clot for 10 minutes or more at room temperature in a collection tube.
3. Centrifuge 1000 x g for 15 minutes
4. Carefully aspirate the serum into a sterile container and seal.
5. Specimen may be processed immediately, refrigerated, preserved by freezing at -20°C or by adding thimerosal to provide a final concentration of 0.01%.
6. Add 300 µl of serum to 50 µl aliquot of Pronase (REF DE0010) and seal tube with parafilm.
7. Incubate serum/Pronase solution at 56°C for 30 minutes.
8. Add 1 drop of Pronase Inhibitor (REF EI0010) and mix to terminate enzymatic digestion.
9. Specimen is ready for testing (see **PROCEDURE).**

PROCEDURE

SCREENING PROCEDURE

1. Add 25 µl of Cryptococcus Antigen Positive Control (REF CB0010), Negative Control (REF N80110) and each heat-treated CSF and/or Pronase-treated serum specimen onto

separate rings of the ring slide. Use a new pipette tip for each reagent and specimen.
2. Add 25 µl of Cryptococcal Latex (REF CG0020 or CG0010) to each ring.
3. Using separate applicator sticks, thoroughly mix the contents of each ring.
4. Rotate by hand or place the ring slide on a rotator set to 100 rpm (+/- 25) for 5 minutes at room temperature.
5. Read the reactions immediately (see **Reading the Test).**

TITRATION PROCEDURE

Patient specimens showing a 1 + or greater reaction should be titrated.

1. Add 100 µl of Specimen Diluent (REF GB0020) to each of 10 tubes labeled 1-10 and place in a rack (1:2 through 1:1024). Additional dilutions may be necessary if the specimen is positive at 1:1024.
2. Add 100 µl of patient specimen to tube #1 and mix well.
3. Transfer 100 µl from tube #1 to tube #2 and mix well. Continue this dilution procedure through tube #10.
4. Add 25 µl of Cryptococcus Antigen Positive Control (REF CB0010), Negative Control (REF N80110) and each specimen dilution onto separate rings of the ring slide.
5. Add 25 µl of Cryptococcal Latex (REF CG0020 or CG0010) to each ring.
6. Using separate applicator sticks, thoroughly mix the contents of each ring.
7. Rotate by hand or place the ring slide on a rotator set to 100 rpm (+/- 25) for 5 minutes at room temperature.
8. Read the reactions immediately (see **Reading the Test).**

READING THE TEST PROCEDURE

Read the reactions immediately over a dark background and rate them on a scale from negative to 4+. Do Not Magnify. For comparison, the Cryptococcus Antigen Positive Control should give a 2+ or greater reaction and the Negative Control should be less than 1+ (see Reference Reaction Pictures). The graduations of the reaction strengths are as follows:
Negative (-): a homogeneous suspension of particles with no visible clumping.
One Plus (1+): fine granulation against a milky background.
Two Plus (2+): small but definite clumps against a slightly cloudy background.
Three Plus (3+): large and small clumps against a clear background.
Four Plus (4+): large clumps against a very clear background.

QUALITY CONTROL

LATEX CONTROL:

Periodically, the sensitivity of the Cryptococcal Latex reagent (REF CG0020 or CG0010) may be tested by titering the Cryptococcal Antigen Positive Control (REF CB0010). The Cryptococcal Antigen Positive Control should titer 1:4 ± 1 dilution if the sensitivity of the Cryptococcal Latex reagent is satisfactory.

PRONASE CONTROL:

At least once monthly, a frozen aliquote of Pronase (REF DE0010) should be tested for proteolytic activity by substituting a 300 µl aliquote of Pronase Control (REF R20000) for specimen in steps 6-9 in serum specimen preparation. Both the Pronase-treated sample of Pronase Control and an untreated sample of Pronase Control should be tested simultaneously using the **Screening Procedure** above. The untreated Pronase Control must be 2+ or greater and the Pronase-treated Pronase Control must be less than 1+ **(NOTE:** The Pronase-treated Pronase Control reactions may be slightly rough, but should be less than a 1+ reaction). If the Pronase-treated Pronase Control is greater than 1+, then the proteolytic activity of Pronase has diminished and a new vial of Pronase should be rehydrated, aliquoted, frozen, and tested.

RESULTS

CONTROL REACTIONS:

The Cryptococcal Antigen Positive Control must be 2+ or greater, and the Negative Control must be less than 1+ with the Cryptococcal Latex. If either control is incorrect, one or both of the reagents is unsatisfactory (or the tests were performed improperly) and any patient tests with the reagents are invalid. A positive reaction with the Negative Control may indicate possible contamination or freezing of the Cryptococcal Latex, which could produce false positive results in patient specimens. The Pronase Control detects the presence of rabbit globulin on the latex particles. Failure of the Pronase Control to give a positive reaction indicates that one of the reagents is unsatisfactory.

PATIENT SPECIMENS:

1. **Negative:** If the screening test performed on the undiluted patient specimen was negative or a 1 + reaction, then the test should be reported as negative. However, 1 + reactions may be suggestive of Cryptococcosis (22). If the clinical symptoms of the patient are suggestive of Cryptococcosis, subsequent specimens and culture are strongly recommended. Weakly reactive specimens (e.g. 1+) should be checked for prozone effect of high titers by testing using the **Titration Procedure** (29,32). If prozoning is suspected, repeat the test with both a 1:10 and a 1:100 dilution of the specimen.
2. **Positive:** If a 2+ or greater reaction is observed in the **Screening Procedure,** then the specimen is titrated using the **Titration Procedure.** The titer is reported as the highest dilution showing a 2+ or greater reaction.

LIMITATIONS OF THE PROCEDURE

A negative test does not exclude the possibility of cryptococcal infection, particularly when a single patient specimen has been tested and the patient has symptoms consistent with Cryptococcosis (1). False-negative reactions may be caused by low titers, early infection, presence of immune complexes (30), prozone effect of high titers (32), or poorly encapsulated strains with low production of polysaccharide (28). False-positive reactions can occur due to the presence of rheumatoid factor (3,19), agar syneresis fluid (7,11,20), *Capnocytophaga animorsus (36), Trichosporon beigelii (25),* hydroxyethyl starch (26), sera with >200 mg Fe^{3+}/dL (10), improper cleaning of the ring slide (5), and non-specific reactivity in HIV-infected patients (37). Pronase treatment has been shown to reduce false positives (17), increase titers (17,18), and increase sensitivity (35) in serum specimens.

EXPECTED VALUES AND SPECIFIC PERFORMANCE CHARACTERISTICS

The latex agglutination test for *C. neoformans* antigens has both diagnostic and prognostic value (29). A positive reaction in serum or CSF of an untreated patient at titers of 1:4 or less is highly suggestive of Cryptococcal infection (29). Titers of 1:8 or greater usually indicate active Cryptococcosis (29). The antigen titer is usually proportional to the extent of infection, with increasing titers reflecting progressive infection and a poor prognosis (29). Declining titers indicates a positive response to therapy in the treated patient (1,29). Failure of titers to decline indicates inadequate therapy (29). Occasionally, however, low titers may persist for an indefinite period in the presence of nonviable fungus and clinical improvement (1,29). When antigen titration is being used to monitor therapy, all titrations should be performed with the same manufacturer's kit. It is also good practice to titer serial specimens simultaneously to minimize laboratory variation.

The sensitivity and specificity for the LATEX CRYPTOCOCCUS test has been reported to be 93-100% and 93-100%, respectively (31,35). The lower detection limits for polysaccharide antigens from different serotypes of C. neoformans were determined by spiking serum with known concentrations of purified antigens (27). Serotype A antigen was detected at a level of 0.5 ng/ml; serotype B antigen was detected at 0.5 ng/ml; serotype C antigen was detected at 25 ng/ml; and serotype D antigen was detected at 0.5 ng/ml (2).

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INTERNATIONAL SYMBOL USAGE

	Storage 2-8 °C	LOT	Lot Number
	Manufactured by	REF	Reference Number
	Expiration Date	IVD	In Vitro Diagnostic
	Sufficient for "#" Tests		Consult Instructions for Use

IMMY, Inc.
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