KCMC Biotechnology Laboratory, Microbiology	STANDARD OPERATING PROCEDURE	Effective Date 27 August 2006	SOP-Number MIC.036
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Title: CRYPTOCOCCAL ANTIGEN TEST

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Annual Review	
By	**Date**

Title: CRYPTOCOCCAL ANTIGEN TEST

Document History:

Version Number	Reason for Changes	Date

Copies distributed to:

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Title: CRYPTOCOCCAL ANTIGEN TEST

PURPOSE

For the qualitative and semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus neoformans* in cerebrospinal fluid (CSF) and serum.

PRINCIPLE:

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. Previously, the detection of this antigen in serum was hampered by the presence of rheumatoid factor. Pretreatment of serum specimens with Pronase reduces this nonspecific interference and enhances the detection of capsular polysaccharide antigens of *C. neoformans*.

CLINICAL APPLICATION

The LATEX-CRYPTOCOCCUS ANTIGEN TEST test has value as both a diagnostic and prognostic tool for management of patients with cryptococcal infection. With a sensitivity and specificity of 93-100% a positive reaction in serum or CSF from an untreated patient at titers of 1:4 or less is highly suggestive of infection. Titers of 1:8 or greater usually indicate active cryptococcosis. Patients with extrameningeal cryptococcosis have positive tests approximately 97% of the time.

SCOPE

This Standard Operating Procedure applies to all testing of serum and CSF for the presence of cryptococcal antigen by technical staff of the microbiology laboratory who have been trained in and are competent in performing this test.

STANDARD PRECAUTIONS

Observe standard precautions. Wear gloves when collecting and handling patient specimens. Dispose of all contaminated items in infectious waste container.

SPECIMENS: Serum and cerebrospinal fluid (CSF).

Patient Preparation: No special preparation.

Collection Containers/Minimal Volume

CSF – sterile tube/1 ml
Blood – Plain or serum separator vacutainer tube (no anticoagulant)/2 ml

Specimen collection

Cerebrospinal fluid (CSF)

1. Specimen will be collected by ward medical staff according to standard procedures for lumbar puncture.

Blood

Refer to MIC.012 SPECIMEN COLLECTION FOR ISAAC STUDIES for blood collection procedures.

EQUIPMENT AND MATERIALS

ImmunoMycologics Latex Cryptococcal Antigen Test kit:

- Specimen Diluent (10 ml, REF GB0020): Concentrated (10X) glycine Buffered saline (pH 8.6) containing albumin and a preservative.
- Cryptococcal Latex: (1.5 ml, REF CG0020 or 3.5 ml, REF CG0010): standardized latex particles sensitized with rabbit anti-cryptococcal globulin in glycine buffered saline containing a preservative. DO NOT FREEZE.
- Cryptococcal Antigen Positive Control (1 ml, REF CB0010): Purified capsular polysaccharide antigens containing a preservative.
- Negative Control (1 ml, REF N80110): Normal goat serum containing a preservative. Must be heat inactivated prior to use.

  Heat inactivation:
  1. Inactivate at 56^{o} C for 30 minutes.
  2. Mark on tube the date of heat inactivation.
- Pronase (1.75 ml, REF DE0010): Lyophilized Pronase containing a preservative. Reconstitute prior to use.

  Reconstitution of Pronase:
  1. Add 1.75 ml if distilled water to the lyophilized reagent.
  2. Aliquot 0.05 ml (50 ul) into sterile tubes and freeze at -20^{o} C or colder.
  3. Label tube “Pronase” add lot# and expiration date.
- Pronase Control (2 ml, REF R20000): Goat anti-rabbit globulin containing a preservative.
- Pronase Inhibitor (6 ml, REF EI0010): Contains an inhibitor for the Pronase.
- Cardboard Ring Slides (REF SC0020)

Materials not provided in test kit

Sterile 12x75 mm test tubes
Sterile distilled water
1 ml serological pipet
Test tube rack

Heat block (for 100 and 56°C)
Wooden applicator sticks
Timer
Pipettor (25 µl and 100 µl)
Pipette tips (2-200 ul)

SPECIMEN PREPARATION

Cerebrospinal Fluid (CSF)

1. Centrifuge at 1500xg/3500 rpm for 15 minutes to ensure the removal of all white cells and particulate matter.
2. Carefully aspirate the CSF into a sterile tube and seal.
3. Specimen may be processed immediately, refrigerated or frozen at –20°C.
4. Prior to testing incubate CSF specimen at 100°C for 5 minutes.

Blood

1. Permit blood to clot for 10 minutes or more at room temperature in collection tube.
2. Centrifuge 1500xg/3500 rpm for 15 minutes
3. Carefully aspirate the serum into a sterile tube and seal.
4. Prior to testing add 300 µl of serum to 50 µl aliquot of Pronase (REF DE0010) and seal tube with parafilm.
5. Incubate serum/Pronase solution at 56°C for 30 minutes.
6. Add 1 drop of Pronase Inhibitor (REF EI0010) and mix to terminate enzymatic digestion.
7. Specimen is ready for testing.

Specimen Storage: Specimens to be tested within 72 hours of collection may be stored at 2-8°C. For longer periods, store at -20°C.

CALIBRATIONS – NA

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UALITY CONTROL:

LATEX POSITIVE CONTROL SENSITIVITY CHECK:

Frequency: Each shipment of each lot and monthly thereafter.

Procedure:
Titer the Cryptococcal Antigen Antigen Positive Control to 4 x 2-fold dilutions (see Titration(Quantitative) Procedure).

Acceptable Results: The Cryptococcal Antigen Positive Control should titer 1:4 ± 1 dilution if the sensitivity of the Cryptococcal Latex reagent is satisfactory.

Corrective Actions for Unacceptable Results:
1. Restest a new Positive Latex control and repeat test on the reagent in use.
2. Do not use Control latex that has reduced sensitivity for patient testing.
3. Arrange for replacement of unsatisfactory reagent.

Documentation:
1. Record results on CRYPTOCOCCAL ANTIGEN TEST QC FORM
2. Complete a QC DEVIATION FORM on all unsatisfactory results.
3. Submit to supervisor for review.
4. Supervisor will review QC monthly.

PRONASE CONTROL PROTEOLYTIC REACTIVITY CHECK:

Frequency: Each shipment of each lot and monthly thereafter.

Procedure:
1. Substitute 300 ul of Pronase Control for specimen in steps 6-9 of the serum preparation procedure (Treated Pronase Control).
2. Test both the Pronase treated sample of Pronase Control and an untreated sample of Pronase Control using the Screening Procedure.

Acceptable Results:
1. The untreated Pronase Control reaction must be 2+ or greater.

2. The Pronase-treated Pronase Control reaction must be less than 1+. If >1+ this indicates reduced proteolytic activity.

Corrective Actions for Unacceptable Results:

1. Do not use Pronase Control with reduced activity for patient testing.
2. Arrange for replacement of unsatisfactory reagent.

Documentation:

1. Record Results on the CRYPTOCOCCAL ANTIGEN QC form.
2. Complete a QC DEVIATION FORM on all unsatisfactory results.
3. Submit to supervisor for review.
4. Review QC monthly.

DAILY QC – Positive and Negative Controls

Frequency: Each run of patient samples.

Controls/Acceptable Results:
Cryptococcal Antigen Positive Control/ $\geq$ 2+ agglutination
Cryptococcal Antigen Negative Control/<1+ agglutination

Corrective Actions for Unacceptable Results:

1. If results of one or both reagents are unacceptable do not interpret or report patient test results.
2. A positive reaction with the Negative control may indicate contamination or freezing of the latex particles causing false positive results.

Documentation:

1. Record results on the CRYPTOCOCCAL ANTIGEN QC form
2. Complete a QC DEVIATION FORM on all unacceptable results.
3. Submit to supervisor for review.
4. Supervisor will review QC monthly.

SCREENING (QUALITATIVE) PROCEDURE

1. Add 25 µl of Cryptococcus Antigen Positive Control, Negative Control 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 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 over a dark background (do not magnify).

INTERPRETATION OF SCREENING RESULTS

The graduations of the reaction strengths are described below. 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 on lid of box).

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.

REPORTING OF SCREENING RESULTS

Reference Range: Normal is negative.

Negative/No Reaction:
Report: NEGATIVE

Positive (2+ or greater) reaction:
Report : POSITIVE
If a 2+ or greater reaction is observed in the Screening Procedure, then the specimen must be titrated and the titer reported as the highest dilution showing a 2+ or greater reaction (e.g. Titer 1:256).

1+ Reaction:
Weak, 1+ reactions should be reported negative but first must rule out a prozone effect so the sample should be titrated. (Consult Supervisor)

Report: NEGATIVE if there is no prozone effect.

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TITRATION (QUANTITATIVE) PROCEDURE

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

1. Add 100 µl of Specimen Diluents (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, Negative Control 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.
9. Discard slide in infectious waste container.

INTERPRETATION OF TITRATION REACTIONS

1. The titer is the highest dilution showing a 2+ reaction.

REPORTING RESULTS

1. Enter test results on a Microbiology Laboratory Report form.
2. Contact Clerical Assistant to pick up report form and take to patient's medical ward.
3. Give report to a member of the medical ward team (preferably a physician) and have them sign the log book acknowledging receipt of the report.

LIMITATIONS OF THE PROCEDURE:

1. False-negative reactions may be caused by low titers, early infection, presence of immune complexes , prozone effect of high titers or poorly encapsulated strains with low production of polysaccharide .

2. False-positive reactions can occur due to the presence of rheumatoid factor, agar syneresis fluid, *Capnocytophaga animorsus*, *Trichosporon beigelii*, hydroxyethyl starch, sera with >200 mg Fe3+/dL, improper cleaning of the ring slide, and non-specific reactivity in HIV-infected patients.
3. Pronase treatment has been shown to reduce false positives, increase titers, and increase sensitivity in serum specimens.

SPECIFIC PERFORMANCE CHARACTERISTICS AND EXPECTED VALUES:

1. The latex agglutination test for *C. neoformans* antigens has both diagnostic and prognostic value.
2. The sensitivity and specificity for the LATEX-CRYPTOCOCCUS ANTIGEN TEST has been reported to be 93-100% and 93-100%, respectively.
3. A positive reaction in serum or CSF of an untreated patient at titers of 1:4 or less is highly suggestive of cryptococcal infection. Titers of 1:8 or greater usually indicate active cryptococcosis.
4. The antigen titer is usually proportional to the extent of infection, with increasing titers reflecting progressive infection and a poor prognosis. Declining titers indicate a positive response to therapy in the treated patient Failure of titers to decline indicates inadequate therapy.
5. Occasionally, however, low titers may persist for an indefinite period in the presence of nonviable fungus and clinical improvement. 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.

REFERENCES:

Merz, WG, Roberts GD. 2003. Algorithms for Detection and Indentification of Fungi. In: Manual of Clinical Microbiology. 8th Ed. ASM Press, Washington, DC.

Tanner, D. C., M. P. Weinstein, B. Fedorciw, K. L. Joho, J. J. Thorpe, and L. Reller. 1994. Comparison of commercial kits for detection of cryptococcal antigen. J.Clin.Microbiol. 32:1680-1684.

Package Insert, June 2005, LATEX-CRYPTOCOCCUS ANTIGEN DETECTION SYSTEM. Immuno-Mycologics, Inc. Norman, OK.

APPENDIX A – CRYPTOCOCCAL ANTIGEN QC form