CRYPTOCOCCAL ANTIGEN

Latex particles coated with anti-cryptococcal globulin (ACGR) reacts with cryptococcal polysaccharide antigen (in CSF or serum) causing a visible agglutination.

I. Specimen Collection and Processing

5 mL of blood is collected in a serum separator tube and separated by centrifugation. The serum is removed to a vial and refrigerated until testing. Specimens are stored a -70^{o}C after testing.

Spinal fluid is collected in clean, sterile, centrifuge tubes. Specimens are stored refrigerated after testing.
Note: Fungus culture should also be set up.

II. Procedure

Reagents

Meridian CALAS (Cryptococcal Antigen Latex Agglutination System)

1. GBDA - Glycine buffered diluent with albumin.
2. ACGR - Anti-cryptococcal globulin reagent.
3. NGR - Normal globulin reagent.
4. AGC - Antiglobulin control. Rehydrate with 1.5 mL dH_2O.
5. NC - Negative control. Rehydrate with 2.5 mL dH_2O and **inactivate the negative at 56^{o}C for 30 minutes**.
6. CAC - Cryptococcal antigen control (Positive control).
7. Pronase - Rehydrate with 2.5 mL dH_2O.

Note: Ensure that all reconstituted vials are thoroughly dissolved before use

All reagents are stored refrigerated. Do not interchange reagents with a kit having a different lot number. Allow reagents to warm to room temperature before use. Mix gently before use.

Other Materials
Boiling water bath
56°C heating block
1.0 x 0.1 mL pipettes
Rotator
Small serologic test tubes
Test tube rack
Marking pen
Applicator sticks

The following are provided by Meridian:
Capillary pipettes
Rubber bulb
Ring slide

Method

Specimen preparation:

1. Store refrigerated if testing is not done immediately.

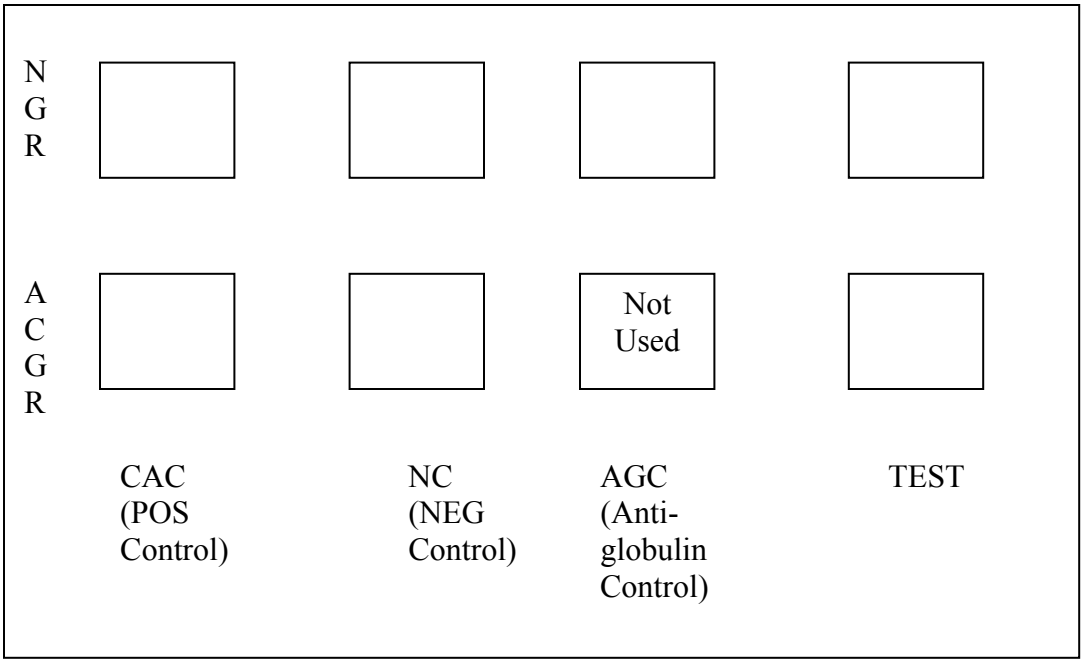
    (a) Inactivate serum by mixing 500 µL of serum and 500 µL of pronase solution in a 12 x 75 mm tube and incubate at 56°C for 15 minutes. Further inactivate in a boiling water bath for 5 minutes. **This constitutes a 1:2 dilution.**

    (b) Centrifuge CSF at 3500 rpm for 15 mins. Inactivate the supernatant in a boiling water bath for 5 minutes.

Performing the tests:

Note: Controls must be run each time a patient specimen is tested.

1. Set up and label the slide as follows:



2. Gently resuspend the latex particles in the ACGR and NGR reagents. Rock each reagent just prior to use.
   Place one drop of ACGR or NGR into the designated rings.

3. Place 25 µL (one drop) of the cryptococcal antigen control (CAC) into the designated rings. Repeat with the negative control (NC) and anti-globulin control (AGC)

4. Place 25 µL of specimen in the designated rings.

5. Using a separate applicator stick, mix the contents of each ring thoroughly, spreading the contents over the entire surface area.

6. Place the slide on the rotator and rotate at 125 rpm for 5 minutes.

7. Read the reactions immediately.

8. Rate the agglutination as follows:
   Positive = any evidence of agglutination (granulation or clumping)
   Negative = a homogenous suspension of particles with no visible clumping.

9. Patient specimens showing any agglutination in ACGR should be titrated against both ACGR and NGR reagents.

   (a) Prepare two-fold serial dilutions of the specimen using 200 µL of GBDA in each of 8 test tubes labelled as follows:

Tube 1 2 3 4 5 6 7 8

Serum 1:4 1:8 1:16 1:32 1:64 1:128 1:256 1:512

CSF 1:2 1:4 1:8 1:16 1:32 1:64 1:128 1:256

   (b) Transfer one drop of each dilution into 2 rings.
   (c) Add one drop of ACGR to one ring of each dilution.
   (d) Add one drop of NGR to each of the other rings.
   (e) Mix using separate applicator sticks.
   (f) Place the slide on the rotator and rotate at 125 rpm for 5 minutes.
   (g) Read the results as follows:

      1+ = fine granulation against a milky background
      2+ = small but definite clumps against a slightly cloudy background
      3+ = large and small clumps against a clear background
      4+ = large clumps against a clear background

   (h) If tube #8 gives an agglutination of 2+ or greater, the specimen must be further serially diluted until a titre may be obtained.

Interpretation of results

Negative: Negative result in initial screening tests against ACGR.

Positive: The titre is reported as the highest dilution showing a 2+ or greater reaction with ACGR and negative with NGR.

Nonspecific Interference: The titre with ACGR is at least 4-fold higher than the titre with NGR.

Uninterpretable test: The titre with ACGR is less than 4-fold greater than the titre with NGR.

III. Reporting

Telephone all positive reports.

Negative Report: "Cryptococcal antigen not detected by latex agglutination."

Positive Report: "Cryptococcal antigen detected at a titre of _____ by latex agglutination."

Non-specific or Uninterpretable Report:
"Cryptococcal antigen uninterpretable by latex agglutination."

IV. Precautions

The ring slide must be thoroughly cleaned after each use as follows:

(a) Soak in hypochlorite overnight.
(b) Scrub using detergent.
(c) Rinse well with tap water.
(d) Rinse 3 times with distilled water.
(e) Dry thoroughly using paper towels.
(f) Wipe clean with lint-free tissue.

V. Quality Control

The pattern of reactions for the controls must be as follows.

NGR	-	-	+
ACGR	+	-	
	CAC	NC	AGC

Failure to obtain this pattern indicates that the test must be repeated and the patient test results cannot be reported.

VI. References

Product Insert, 1986. Meridian Diagnostics Inc., 3471 River Hills Dr., Cincinnati, Ohio 45244. (513)-271-3700.