Cryptococcal Antigen Latex Agglutination System (CALAS®)

Latex Agglutination System for the detection of Cryptococcal Antigen in Serum and CSF

REF 140100, 140050 IVD **Rx Only**

INTENDED USE
The Cryptococcal Antigen Latex Agglutination System (CALAS) is a qualitative and semiquantitative test system for the detection of capsular polysaccharide antigens of *Cryptococcus neoformans* in serum and cerebrospinal fluid (CSF).[8]

SUMMARY AND EXPLANATION OF THE TEST
A simple, sensitive latex test capable of detecting the capsular polysaccharide of *C. neoformans* in CSF and serum was described and proven to be superior in sensitivity to the India Ink mount.[2, 3] Clinical studies established the prognostic value of the test[4, 6, 8, 9] and showed it to be a valuable aid in establishing a diagnosis when the culture was negative.[5]

Kaufman and Blumer[9] reported that *C. neoformans* antigen was present in both the serum and spinal fluid in 86% of 330 confirmed cases of cryptococcal meningitis. Antigen was detected in CSF specimens in 99% of these 330 cases but in only 87% of serum samples. Paired serum and CSF specimens allowed detection of the antigen in each confirmed case. Parallel serologic studies for both antigen and antibody are recommended to ensure detection of extrameningeal cryptococcosis.

Newly emerging disease states and therapies have been shown to increase the opportunity for nonspecific interference in some serum specimens. Pretreatment of serum specimens with pronase prior to utilization of the CALAS kit reduces nonspecific interference and enhances the detection of capsular polysaccharide antigens of *Cryptococcus neoformans*.

BIOLOGICAL PRINCIPLES
CALAS utilizes latex particles coated with anti-cryptococcal globulin (**Detection Latex**). The Detection Latex reacts with the cryptococcal polysaccharide antigen causing a visible agglutination. Latex particles coated with normal globulin (**Control Latex**) act as one of the control reagents. Nonspecific agglutination may occur due to the presence of certain macroglobulins (e.g. rheumatoid factors) in patient specimens. Treatment of serum specimens with pronase (Meridian Bioscience, Inc. Catalogue #140050) removes rheumatoid factor and other nonspecific interference.[12] These macroglobulins can be demonstrated in serum from patients with rheumatoid arthritis, sarcoidosis, cirrhosis, syphilis, scleroderma, psoriasis, gout, systemic lupus erythematosus and other conditions.[1, 11] Nonspecific interference is detected by the Control Latex reagent.

Agglutination of both the Control Latex and the Detection Latex requires two-fold serial titration of the specimen with both reagents. A four-fold higher titer with the Detection Latex than with the Control Latex is suggestive of cryptococcal disease but requires follow-up with specimens collected later in the course of the disease and culture results.[10] Titers with less than four-fold differences are considered equivocal test results and further follow-up is recommended (see INTERPRETATION OF RESULTS).

REAGENTS/MATERIALS PROVIDED
The maximum number of tests obtained from this test kit is listed on the outer box.

1. **Sample Diluent** - Glycine buffered saline (pH 8.4 ± 0.1) containing bovine serum albumin and 0.01% Thimerosal as a preservative.
2. **Detection Latex** - Standardized latex particles coated with an optimal dilution of rabbit anticryptococcal globulin, in glycine buffered saline (pH 8.4 ± 0.1) containing less than 0.01% Thimerosal as a preservative.
3. **Control Latex** - Standardized latex particles coated with an optimal dilution of normal rabbit globulin, in glycine buffered saline (pH 8.4 ± 0.1) containing less than 0.01% Thimerosal as a preservative.
4. **Antibody Control** - Lyophilized goat anti-rabbit serum containing 0.01% Thimerosal as a preservative.
5. **Negative Control** - Lyophilized normal human serum containing 0.10% Sodium Azide as a preservative. Each donor unit used in the preparation of this reagent has been found to be nonreactive for Hepatitis B surface antigen, anti-Hepatitis C and HIV-I / II antibodies by FDA approved test procedures.
6. **Positive Control** - Purified *Cryptococcus neoformans* polysaccharide antigen containing 0.01% Thimerosal as a preservative.
7. **Pronase** - Lyophilized containing 0.10% Sodium Azide as a preservative. Each vial contains enough enzyme to treat 10 serum specimens. Extra Pronase may be ordered (catalogue # 140050), that provides enough reagent to perform a minimum of 50 tests.
8. **Disposable Reaction Cards**
9. **Reaction Reference Photograph**
10. **Package Insert**

MATERIALS NOT PROVIDED

Purified water	Small serologic test tubes
1 x 0.01 mL pipettes	Rack
Rotator (optional)	Marking pen
Applicator sticks	25 µL, 100 µL, 200 µL (or equivalent) pipetter
Waterbath or heat block (56 C and 100 C)	

PRECAUTIONS
1. All reagents are for in vitro diagnostic use only.
2. Controls must be run each day prior to running patient specimens.
3. Reagents in each kit are matched and may give improper results if interchanged with a kit having a different lot number.
4. Do not use reagents containing foreign matter, particulates or aggregates which indicate contamination or improper storage or handling.
5. Specimens must not contain bacterial or other obvious signs of contamination.
6. **Heat inactivate the Negative Control each day the test is used.** Otherwise, reformation of certain globulins may occur and result in a false positive test.
7. **Never** heat inactivate the **Antibody Control** Reagent as this could cause aberrant control reactions.
8. Sodium azide is a skin irritant. Avoid skin contact with the kit components. Do not mix with acid as this may result in the formation of hydrazoic acid, an extremely toxic gas.
9. Do not store specimens in a frost free type freezer. Repeated freezing and thawing of the specimens can affect the test results.
10. 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.

WARNING
Because no test method can offer complete assurance that human T-lymphotrophic virus type I / II lymphadenopathy associated virus (HIV-I / II), hepatitis B virus, hepatitis C virus, or other infectious agents are absent, these controls should be handled by the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories". Some reagents in this kit contain sodium azide. Disposal of reagents containing sodium azide into lead or copper plumbing can result in the formation of explosive metal azides. This can be avoided by flushing with a large volume of water during such disposal.

HAZARD and PRECAUTIONARY STATEMENTS

 CALAS Pronase Reagent	**Signal Word** Danger **Hazard Statements** H302 - Harmful if swallowed H316 - Causes mild skin irritation H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled Contains Proteinase, streptomyces griseus **Precautionary Statements - EU (§28, 1272/2008)** P261 - Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray P304 + P341 - IF INHALED: If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing P308 + P313 - IF exposed or concerned: Get medical advice/ attention P342 + P311 - If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician
 CALAS Negative Control	**Signal Word** Danger **Hazard Statements** H300 - Fatal if swallowed **Precautionary Statements - EU (§28, 1272/2008)** P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician P321 - Specific treatment (see supplemental first aid instructions with this material)

SHELF LIFE AND STORAGE
Store the CALAS kit at 2-8 C. Reagents are preserved with 0.01% thimerosal or 0.10% sodium azide; however, prolonged periods at room temperature should be avoided. Latex suspensions must not be frozen as this causes irreversible clumping.

Pronase, once reconstituted, can be stored at 2-8 C for approximately one month. Discard the solution if it becomes cloudy or contaminated. Once reconstituted it is suggested that the pronase solution be aliquotted and frozen if it will not be used within one month. Frozen aliquots of pronase can be stored at -20 C until the expiration date stated on the pronase vial label. Repeat freezing and thawing should be avoided. Do not store in a frost free type freezer. The expiration date of each CALAS kit is indicated on the kit label. Discontinue use of the kit if the included controls do not provide the proper reactions. If this occurs and reagents are still within their labeled expiration dating, please contact Meridian Technical Support at 1-800-343-3858.

REAGENT PREPARATION

Reconstitute the following reagents with the indicated volume of deionized water:

a. **Antibody Control** 1.45 mL
b. **Negative Control** 2.40 mL
c. **Pronase** 2.50 mL

Reconstituted pronase should be aliquotted and frozen if it will not be used within one month.

Allow the reconstituted vials to stand at room temperature for 30 minutes before mixing them gently. Avoid foaming. Contents must be completely in solution prior to use. Heat inactivate the **Negative Control** each day the test is used.

Mix each kit vial by rocking gently prior to each use. Latex solutions must appear as homogeneous suspensions.

SPECIMEN COLLECTION AND PREPARATION

A. **Cerebrospinal Fluid** (Meridian ***does not*** recommend that CSF specimens be routinely pretreated with pronase. However, recent evidence[14] suggests that pronase treatment of CSF specimens may be useful with some specimens).
   1. Collect specimen aseptically following accepted procedures.
   2. Centrifuge at 1000 xg for 15 minutes to ensure the removal of all white cells and particulate matter.
   3. Carefully aspirate the spinal fluid 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. **We recommend that CSF be inactivated by placing in a boiling water bath for 5 minutes prior to each test.**[7] This tends to limit nonspecific interference.
   6. Allow to cool 3-4 minutes before testing.

B. **Serum** (It is recommended that all serum specimens be treated with pronase as described below).
   1. Collect whole blood aseptically according to in-house 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 at 1000 xg 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 200 µL of serum specimen to 200 µL of the pronase solution.
   7. Incubate serum/pronase solution at 56 C for 15 minutes.
   8. Immediately place the serum/pronase solution in a boiling water bath for **a full five minutes** to terminate enzymatic digestion.
   9. Allow solution to cool to room temperature.
   10. Specimen is ready for testing (see PROCEDURE).

   Note: For titering purposes, patient specimen has been diluted 1:2 with the pronase solution.

C. **Negative Control** – Heat inactivate the **Negative Control** at 56 C for 30 minutes. It must be heat inactivated each day of use.

TEST PROCEDURE

1. Remove enough cards to run controls once for the day and each patient specimen.
   NOTE: Controls do not need to be run on each card with each patient sample. See CALAS CONTROLS.
2. Consult the figure in the Procedure on the kit box label for a convenient system of setting up and labeling the controls and patient specimens on the Disposable Card(s).
3. Holding the **Positive Control** vial in a vertical position, squeeze one free-falling drop of reagent into each of the two designated rings.
4. Place 25 µL of the **Antibody Control** and **Negative Control** to the appropriate rings.
5. Place 25 µL of patient specimen in each of the two designated rings (see Figure).
6. Holding the **Detection Latex** in a vertical position, squeeze one free-falling drop of reagent into each of the designated rings.
7. In a similar fashion, add one drop of the **Control Latex** into each of the designated rings.
8. Using separate applicator sticks, mix the contents of the rings.
9. Rock the slide by hand or place it on a rotator and rotate at 125 ± 25 rpm for five minutes.
10. Read the results immediately and rate them on a scale ranging from negative to 4+. For comparison purposes, refer to the Reaction Card.
11. The gradations 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.

Titration:

Patient specimens showing a 2+ or greater reaction with either the **Detection Latex** or **Control Latex** should be titrated with both reagents.

Prepare two-fold serial dilutions of the specimens as follows:

1. Place 0.25 mL of **Sample Diluent** in each of 5 test tubes labeled 1-5 and place in a rack.
2. Using a clean pipette, place 0.25 mL of patient specimen in tube # 1 and mix well.
3. Transfer 0.25 mL from tube # 1 to tube # 2 and mix well. Continue this dilution procedure through tube # 5. Transfer 0.25 mL from the fifth tube into a "holding" tube since further dilutions may be necessary.

Tube	1	2	3	4	5
Pronase treated specimens	1:4	1:8	1:16	1:32	1:64
Nonpronase treated specimens	1:2	1:4	1:8	1:16	1:32

4. Label the Disposable Card(s) to accommodate both the **Detection Latex** and **Control Latex** titration series of tests.
5. Beginning with tube # 4, transfer 25 µL of this dilution to each of two marked rings.
6. Repeat step 5 for Tubes # 3 through # 1. This procedure allows the use of a single pipette tip to place the four dilutions on the Disposable Card(s).
7. Add one drop of gently mixed **Detection Latex** to each labeled ring in the **Detection Latex** series.
8. Add one drop of gently mixed **Control Latex** to each labeled ring in the **Control Latex** series.
9. Using a separate segment of an applicator stick, mix the contents of each ring thoroughly spreading to the edge of the ring.
10. Rock the slide by hand or place it on a rotator and rotate at 125 ± 25 rpm for 5 minutes.
11. Read the results immediately and rate them on a scale ranging from negative to 4+. For comparison refer to the supplied Reaction Reference Photograph.

INTERPRETATION OF RESULTS

Control Reactions:

The pattern of control reagent agglutination reactions must be identical to that illustrated in the diagram on the inside lid of the kit box. Failure to obtain this pattern indicates that either one or more of the reagents is unsatisfactory or the tests were performed improperly and must be repeated. In either case, patient test results cannot be reported in the absence of satisfactory control readings.

The **Positive Control** should give a positive reaction with the **Detection Latex** and a negative reaction with the **Control Latex.** This tests the **Detection Latex** for its sensitivity to cryptococcal antigen. A positive reaction between the **Control Latex** and the **Positive Control** may indicate contamination of one or both of the control vials.

The **Antibody Control** detects the presence of rabbit globulin on the latex particles. Failure of the **Antibody Control** to give a positive reaction with the **Control Latex** indicates that one of the reagents is unsatisfactory.

The **Negative Control** should give negative reactions with both the **Detection Latex** and **Control Latex.** A positive reaction with either reagent may indicate possible contamination or freezing which could produce false positive results with patient specimens. A positive reaction may also occur by neglecting to heat inactivate the **Negative Control.**

Patient Specimens:

A. **Negative:** If a negative or a 1+ reaction is observed in the initial screening test against the **Detection Latex,** the specimen is reported as negative. However, 1+ reactions may be suggestive of cryptococcus.[9] If the status of a patient suggests a cryptococcal infection, subsequent specimens and culture are strongly recommended. If prozoning is suspected, repeat the Patient Test procedure with both a 1:10 and a 1:100 dilution of the specimen in the supplied **Sample Diluent** buffer.

B. **Positive:** If a 2+ or greater reaction against **Detection Latex** is seen in the initial screening test, the specimen is titrated with the **Detection Latex** and **Control Latex** reagents. The titer is reported as the highest dilution showing a 2+ or greater reaction. While CSF titers of 1:4 or less are presumptive evidence of central nervous system infection by *C. neoformans*, additional follow-up and culture are strongly recommended. CSF titers of 1:8 or greater from patients with meningitis strongly suggest infection by *C. neoformans*. However, diagnosis should be confirmed by identification of the organism from culture or by microscopic examination of the specimen. The false positive rate associated with non-pronase treated serum with titers of less than 1:8 may be as high as 32%.[9] Appropriate follow-up is strongly recommended.

C. **Positive with Nonspecific Interference:** If the specimen titer with the **Detection Latex** is at least 4-fold higher than the non-specific interference (**Control Latex**) titer (e.g., **Detection Latex** titer 1:32, **Control Latex** titer 1:8 or lower), the test should be reported "Positive with nonspecific interference," and specimen titers against **Detection Latex** and **Control Latex** should be stated.[1, 11]

D. **Invalid test due to nonspecific interference:** If the specimen titer against the **Detection Latex** is not at least 4-fold higher than that with the **Control Latex,** the test should be reported "Invalid due to nonspecific interference"[1, 11] (see LIMITATIONS OF THE PROCEDURE).

The CALAS test appears to have both diagnostic and prognostic value since progressive disease is usually accompanied by increasing antigen titers. Declining titers are usually associated with clinical improvement (with or without therapy). Inadequate therapy is indicated by stationary or rising titers on subsequent sequential specimens.[10] Cryptococcal antigen in body fluids of the untreated patient indicates active infection. However, in some treated patients, CALAS titers remain positive at low levels for extended periods during which the organism can no longer be demonstrated.

QUALITY CONTROL

This test should be performed per applicable local, state, or federal regulations or accrediting agencies.

PRONASE ACTIVITY CONTROL

Each serum/pronase solution processed acts as a control to determine if the CALAS Pronase has lost activity. If gelatinization or coagulation of the serum/pronase solution occurs, the CALAS Pronase has lost significant activity and should not be used. Note that cloudiness is likely to occur upon boiling and is not indicative of a compromised Pronase Reagent.

CALAS CONTROLS

Reliable results are obtained only if a satisfactory control run is made on the same day that patient specimens are tested.

If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.

EXPECTED VALUES

Cryptococcal antigen in the CSF or serum of untreated patients indicates active disease. Declining titers indicate a positive response to chemotherapy in the treated patient. Failure of titers to decline indicates inadequate therapy. Occasionally, however, low titers may persist for an indefinite period in the presence of nonviable fungus.

LIMITATIONS OF THE PROCEDURE
A negative CALAS test does not preclude diagnosis of cryptococcosis, particularly if only a single specimen has been tested and the patient shows symptoms consistent with cryptococcosis.

One false positive reaction due to an antigen of *Trichosporon beigelii* which crossreacts with the *Cryptococcal neoformans* capsular polysaccharide has been reported.[13] The reaction occurred in a serum specimen from a patient with disseminated *Trichosporon* infection.

Although the presence of nonspecific interference can invalidate the CALAS test results, this does not exclude the possibility of cryptococcal infection since cryptococcosis can occur concomitantly with other conditions (see BIOLOGICAL PRINCIPLES).

SPECIFIC PERFORMANCE CHARACTERISTICS
The CALAS Pronase procedure has been shown to effectively reduce: 1) rheumatoid factor (RF) reactions, 2) prozone effects at high antigen concentrations, and 3) false negative results due to apparent masking of antigen following specific antibiotic therapies. Although these types of specimens are relatively rare in the overall population, selected populations may contain significant numbers.

A selected group of 85 problematic patient specimens (containing 16 RF sera and several sera of the "prozone" and "masked antigen" interference types described above) were assayed with both the CALAS kit (with pronase) and a reference EIA procedure. The EIA utilized an anticryptococcal monoclonal and was not affected by RF's or other nonspecific interference. The data in Table 1 show that only one result was discrepant when the EIA and CALAS procedures were compared directly.

Table 1 – Results of Comparison Between the CALAS kit (with Pronase) and Monoclonal Based EIA Procedure

		EIA +	EIA -	
CALAS	+	54	1*	100% sensitivity
	-	0	30	100% specificity

*The discrepant result was resolved as a low positive (1:8 titer) in favor of the CALAS Pronase procedure when the specimen was reassayed by the CDC latex kit. Thus, the sensitivity and specificity of the CALAS kit (with pronase) procedure were both 100% in this study.

Seventy-seven of these problematic specimens were also assayed by the CALAS kit without pronase treatment. CALAS results without pronase treatment included four false negative, two false positive, and five indeterminant results. The overall sensitivity and specificity of the CALAS kit without the pronase procedure were 91% and 92%, respectively.

When the CALAS kit (with pronase) procedure was compared directly with the original pronase procedure described by Stockman and Roberts[12] there was 100% agreement with 18 positive and 16 negative specimens tested. Titers were not significantly different (within a two-fold dilution) between these pronase procedures. Thus, the pronase procedures were demonstrated to be equivalent and reproducible.