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## Latex Agglutination test for the early diagnosis of *Cryptococcal meningitis*

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**Abstract**

*Cryptococcal* infection which may cause meningitis is the most prevalent and common invasive fungal infection. Serodiagnosis is an important adjunct for rapid diagnosis of *Cryptococcosis*. In the present study, antigen detection using CALAS meridian diagnostic kit was evaluated along with other traditional methods in Nair Hospital, Mumbai. Out of 100 CSF samples, 20% positivity was observed by only culture method, 21% positivity by microscopy and culture method together, while 22% positivity was observed when all the three methods namely culture, microscopy & LAT were used for the testing of CSF. Microscopy, culture and antigen detection tests were well correlated in the study. *Cryptococcal* antigen detection from CSF was a useful method for an early diagnosis. The immunological diagnosis of *Cryptococcal meningitis* by CALAS meridian diagnostic kit was rapid and had 92%-95.5% sensitivity and specificity.

**Keywords:** *Cryptococcal meningitis*, Serodiagnosis, CALAS meridian diagnostic kit, CSF

**1. Introduction**

Several fungal pathogens are known to cause meningitis such as *Cryptococcus*, *Candida*, *Rhodotorula*, *Aspergillus* and many other filamentous fungi. *Cryptococcal* infection which may cause meningitis, is the most prevalent and common invasive fungal infection [1]. *Cryptococcosis* is increasing due to an ever rising population of immunocompromised subjects and now being regarded as one of the AIDS- defining infections. International community of health personnel have responded sharply to this "Awakening giant", and through international conferences on *Cryptococcus* and *Cryptococcosis* has tried to bring forth its awareness [2].

The detection of *Cryptococcal* polysaccharide antigen in the cerebrospinal fluid or serum by latex agglutination was first described by Bloomfield *et al* in 1963 and has become essential in the diagnosis and management of patients with *Cryptococcal* disease. It is by far the most valuable fungal serodiagnostic study in clinical practice and has become routine CSF study in many hospitals. The test is reported to have a sensitivity of approximately 90% and to be nearly 100% specific when specimen (CSF) are boiled and proper controls for nonspecific agglutination are performed [3]. The method is simple, requires only 20 min. and makes the Latex Test *Cryptococcal* antigen specifier'. Rarely, false positive tests may occur when a cross-reactive antigen is present such as the polysaccharide of *Trichosporon beigeli* or another micro-organism [4].

Serodiagnosis is an important adjunct for rapid diagnosis of *Cryptococcosis*. Antigen detection is very reliable with high degree of sensitivity and specificity<sup>2</sup>. The most commonly employed method for either screening or titration is the demonstration of muco polysaccharide antigen in serum and CSF by Latex agglutination test (LAT) [5]. In the present study, antigen detection using CALAS meridian diagnostic kit was evaluated along with other traditional methods.

**2. Material and methods**

This prospective longitudinal study was carried out over a period of three years, from January 1997 to December 1999 at the Department of Microbiology, after taking the

Permission from Institutional Ethics committee of T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai.

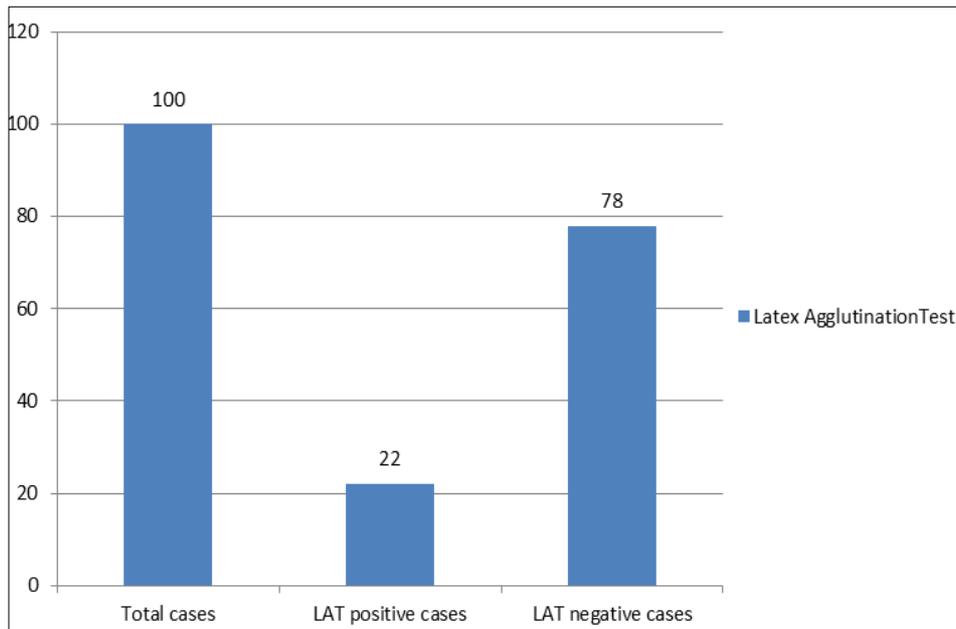
**2.1. Microbiological and serological analysis**

**2.1.1** Cerebrospinal Fluid (CSF) was obtained from 100 patients who were clinically suspected for *fungal meningitis* and were showing signs and symptoms of fever with chronic headache, body ache, Nausea, vomiting, staggering gait, altered sensorium and neck stiffness by trained clinicians by the standard procedure [6] and sent to Microbiology department for the further analysis.

**2.1.2** CSF samples were centrifuged at 3000 rpm for 10 minutes immediately and sediment was studied by microscopy (Wet mount, Modified India Ink Preparation, [7] and Gram staining) culture methods by using selective & differential media along with biochemical tests and blood culture [8] for confirmation of *Cryptococcal meningitis*

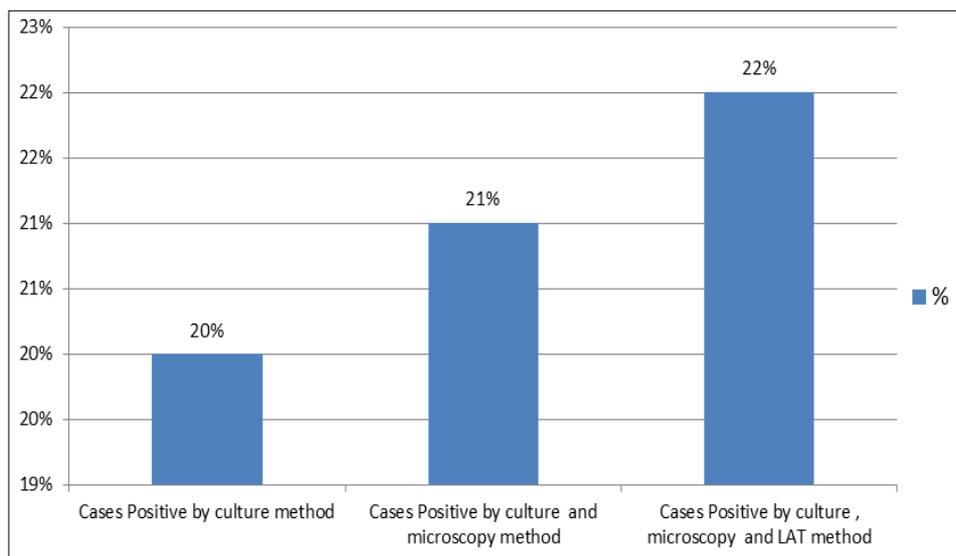
**2.1.3** Total 100 CSF samples were studied for the detection of Cryptococcal antigen by Latex agglutination test using CALAS meridian diagnostic kit. Test was carried as per manufacturer's instructions provided with the Kit.

**3. Results**



**Graph 1:** Cryptococcal antigen detection by Latex Agglutination Test (LAT) n=100

22% of the cases were positive for the LAT test, while 78% cases were negative



**Graph 2:** Percentage positivity of diagnosed cases of *Cryptococcosis* by various methods. n=100

Out of 100 CSF samples, 20% positivity was observed by only culture method, 21% positivity by microscopy and culture method together, while 22% positivity was observed

When all the three methods namely culture, microscopy & LAT were used for the testing of CSF. Microscopy, culture and antigen detection tests were well correlated in the study

#### 4. Discussion

Cryptococcosis has been termed the 'Awakening giant' among the mycoses since 1970 [9]. Serodiagnosis is an important adjunct for rapid diagnosis of *Cryptococcosis*. Antigen detection is very reliable with high degree of sensitivity and specificity [2].

Chapin *et al* had reported detection of *Cryptococcal* antigen from urine of patient with AIDS [10]. The serologic test for the diagnosis of *Cryptococcosis* is both specific and sensitive [5]. The reagents for the test are commercially available in kit form. Latex particles are coated with the specific hyper immune rabbit immunoglobulin and mixed with distributions of patients CSF, Serum or urine [11]. A positive agglutination at a dilution of 1:4 strongly suggests *Cryptococcal* infection. Titres of >8 usually indicate active disease and most patients with AIDS have higher antigen titres [12].

On rare occasion, the LAT may yield specious results in either serum or spinal fluid. Most false results are caused by the presence of rheumatoid factor which is eliminated by treating the specimen with pronase or dithiothreitol by boiling it with EDTA [10]. False positive tests can also be caused by contamination of the specimen with a minute amount of agar as agarose, which may occur if the Sample Pipetter or inoculating loop that is used to inoculate media for culture is reintroduced into the spinal fluid". Recently false-positive LAT of sera from HIV infected patients were limited with 2-mercaptoethanol but not with pronase [13]. Four different commercial kits for detection of *Cryptococcal* antigen from CSF or serum had been demonstrated. Their sensitivity and specificities were compared by Tanner *et al* in July 1994 [14]. Since the sensitivity and specificity and titers detected have been shown to vary among these kits, intra-laboratory controls are essential. Each laboratory should employ a kit from only one manufacture and check each row lot with reference reagents [11].

In the present study, antigen detection using CALAS meridian diagnostic kit gave above 90% sensitivity and specificity. In our study findings of microscopy, culture and antigen detection techniques were well correlated. In fact antigen detection could diagnose one additional *Cryptococcal meningitis* case which was otherwise could be missed by microscopy and culture methods and was well correlated with the clinical picture and therapeutic response of the patient. The patient was 3 months old child, with hydrocephalous as a presenting symptom and the mother of the child the was HIV positive. It was understood tha hydrocephalous could be an early or late manifestation of the *Cryptococcal meningitis* [15]. According to Deodhar *et al* [16], *Cryptococcal* antigen detection test is highly specific and has sensitivity more than 90 percent. The sensitivity and specificity of the CALAS kit used in the present study without the pronase procedure was 91% and 92% respectively. There were no false positive or false negative results obtained in the study.

However Howard *et al* in 1994 [17] had reported false positive results due to syneresis fluid by CALAS Meridian diagnostic Kit, Robert *et al* in 1981 [18] had reported false positive results due to Rheumatoid factor in serum, Blevins *et al* in 1995 [19] had reported false positive results due to Disinfectants and Soaps by Latex—*Crypto* Antigen Detection System, and false negative test results with culture positive cases were reported by Currie *et al* in 1993 due to low *Cryptococcal* antigen in CSF and were

dependent on the Latex Kit used [20]. But, Tanner *et al* [14] when compared five different commercial kits for detection of *Cryptococcal* antigen, concluded that Meridian LCAT appears to be the most sensitive and specific kit over all. All these kits were easy to use, although end point determination was easiest with the Meridian LCAT kit [15]. However Latex test is rapid, easy to perform, quite reliable and useful in the prognosis of the treatment for *Cryptococcal meningitis* [16]. Several Enzyme Immuno Assays have also been developed to detect either antigen or antibody. In comparison with LAT reading of Enzyme Immuno Assays (EIA) is less subjective, is unaffected by prozone reaction and may detect antigen earlier and in small amounts. Specimen does not require pretreatment with pronase as Enzyme Immuno Assays does not react with rheumatoid factor. However EIA requires more time to perform than the LAT. Both test detect all *Cryptococcal* serotypes [5]. Sensitivity of the Eiken LAT was enhanced by Pronase treatment which appeared to be useful in patients with pulmonary *Cryptococcal* disease, and its use may prevent unneeded lung biopsies [21]. Sedamoto *et al* [22] concluded that *C. neoformans* capsular polysaccharide antigen and anti-*C. Neoformans* antibody formed soluble immune complexes in patients sera which interfered with antigen detection by the LAT without protease treatment" Among patients with *Cryptococcal meningitis* false negative results are rare. Test for antibodies to *Cryptococcus* have prognostic rather than diagnostic value because they are not detected in active *Cryptococcosis*. Recently, Sujatha *et al* had concluded that the *Staphylococcal* coagglutination test is a very useful adjunct to direct microscopy in the rapid diagnosis of *Cryptococcal meningitis* [23].

There are several reproducible and accurate test using immuno fluorescent techniques for the detection of antibody to *Cryptococci*. The fixation of antibody to the antigen is detected by the subsequent addition of fluorescein labelled, rabbit antihuman globulin to make an immuno fluorescence "Sand witch". In addition to these procedures, Gordon and Lapa have developed a charcoal particle test to detect the presence of antibody, and immuno-diffusion had also been used. The value of serologic testing for following patients during treatment had been reviewed by Bardana. The difficulties encountered in preparation of antigen had been reviewed by some authors. [24]

#### 5. Conclusion

*Cryptococcal* antigen detection from CSF was a useful method for an early diagnosis .The immunological diagnosis of *Cryptococcal meningitis* by CALAS meridian diagnostic kit was rapid and had 92-95. 5% sensitivity and specificity.

#### 6. Acknowledgement

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