

Evaluation of point of care serum cryptococcal antigen by lateral flow immunoassay for diagnosis of cryptococcosis and cryptococcal meningitis in HIV-positive patients

Ulka Kamble, K. H. Dheeresh, Kakasaheb Bhosale, M. B. Indu, Brijesh Sharma, Anuradha Chowdhary¹
 Department of Medicine, Dr Ram Manohar Lohia Hospital and Post Graduate Institute of Medical Education and Research,
¹Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, New Delhi, India

Address for correspondence:

Dr. Brijesh Sharma, Department of Medicine, Dr Ram Manohar Lohia Hospital and Post Graduate Institute of Medical Education and Research, New Delhi, India. E-mail: brijeshsharma@gmail.com

Abstract

Background: Cryptococcal meningitis (CM) is the initial acquired immunodeficiency syndrome (AIDS) defining illness in 2% of patients with CD4 levels $<100/\mu\text{L}$ and a leading cause of mortality in AIDS in the developing world. It is the most common opportunistic infection of the central nervous system in AIDS in various Indian studies. Detection of serum cryptococcal antigen (SCRAG) is the most widely used diagnostic method for cryptococcosis. The presence of cerebrospinal fluid cryptococcal antigen (CSF CRAG) is diagnostic of CM. CRAG can be determined by latex agglutination (LAT), enzyme-linked immunosorbent assay and now, by lateral flow (LFA) immunoassay. LFA is a point of care test that rapidly detects CRAG. **Aims and Objectives:** This study compares LAT and LFA for the detection of serum CRAG and diagnosing CM. **Materials and methods:** Two hundred and ten patients of HIV/AIDS were submitted to SCRAG LFA by dipstick. A sample was also sent to laboratory for SCRAG by LAT. CSF examination was done for those who were positive for SCRAG LFA and those who had symptoms suggestive of meningitis. SCRAG by LFA was compared with SCRAG by LAT, CSF CRAG by LAT and LFA, CSF cryptococcal culture and CSF India ink examination for *Cryptococcus*. **Results:** Fifteen patients were found positive for SCRAG by LFA dipstick. All of them were also positive for SCRAG by LAT. Twelve of them had C. D4 count below below 100 cells/mm³. CSF CRAG was positive in all 12 SCRAG positive who were submitted to CSF examination. **Conclusion:** We found that serum detection of CRAG by LFA dipstick is as sensitive as CRAG detection in serum by LAT and CSF CRAG detection by LFA and LAT. It is thus a rapid test for diagnosing CM in HIV patients with low CD4 counts.

Key words: Antigen fungal, HIV infection, meningitis cryptococcal, point-of-care systems

INTRODUCTION

Cryptococcal meningitis (CM) is the initial acquired immunodeficiency syndrome (AIDS) defining illness in 2% of patients with CD4 levels $<100/\mu\text{L}$ and a leading cause of mortality in AIDS in the developing world.^[1] It is the most common opportunistic

infection of the central nervous system in AIDS in various Indian studies.^[2-4]

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Kamble U, Dheeresh KH, Bhosale K, Indu MB, Sharma B, Chowdhary A. Evaluation of point of care serum cryptococcal antigen by lateral flow immunoassay for diagnosis of cryptococcosis and cryptococcal meningitis in HIV-positive patients. *Indian J Sex Transm Dis* 2021;42:14-8.

Submitted: 07-Nov-2019

Revised: 07-Jan-2020

Accepted: 31-Aug-2020

Published: 03-May-2021

Access this article online

Quick Response Code:



Website:

www.ijstd.org

DOI:

10.4103/ijstd.IJSTD_94_19

Early diagnosis and treatment decreases CM-related mortality. The detection of serum cryptococcal antigen (SCRAG) is the most widely used diagnostic method for cryptococcosis. The presence of cerebrospinal fluid cryptococcal antigen (CSF CRAG) is diagnostic of CM. CRAG can be determined by latex agglutination (LAT),^[5] enzyme-linked immunosorbent assay^[6] and now, by lateral flow (LFA) immunoassay.^[7,8] LFA is a point of care test that rapidly detects CRAG. This study compares LAT and LFA for detection of serum CRAG and diagnosing CM.

MATERIALS AND METHODS

A cross-sectional observational study was done with 210 HIV/AIDS patients with or without signs of meningitis presenting to the department of medicine and/or anti-retroviral therapy (ART) clinic at a teaching hospital in New Delhi. The study included patients above the age of 18 years who had documented HIV infection, irrespective of CD4 counts and ART status. HIV infection in them was confirmed by series of 3 tests as per the National AIDS Control Organization guidelines. All patients with history of cryptococcosis and patients already on fluconazole therapy were excluded from the study.

Patients were then subjected to detailed history, examination, and laboratory investigations. At the time of examination serum CRAG-LFA with dipstick was done at bedside or in the clinic. Simultaneously, one or more samples were sent to laboratory for detection of CRAG by LAT.

In all CRAG LFA-positive patients, CSF testing was done for CRAG detection by LFA at bedside and same sample was sent to the laboratory for detection of CRAG by LAT. CSF culture and India ink examination was also done. CSF study and CRAG were also done in all patients with symptoms suggestive of meningitis.

We compared CRAG by LFA with CRAG by LAT and CSF CRAG by LFA with CSF CRAG by LAT. Titers of CRAG by both methods were also compared. We also compared SCRAG with CSF CRAG, CSF India ink and CSF cryptococcal culture for diagnosing CM. SCRAG-LAT was taken as the gold standard for serum cryptococcal antigenemia and CSF-CRAG LAT as the gold standard for diagnosing CM. The results obtained by LFA and LAT were also compared with clinical signs, symptoms, CD4 count, and ART status.

Lateral flow assay technique

The CRAG LFA provides both qualitative and semi quantitative results. The test is done as follows: First

a drop of LFA specimen diluent is taken in a tube, then 40 μ L of patient specimen is added to it. Next, the CRAG LFA test strip is inserted into the tube and incubated for 10 min to interpret the results. The presence of two lines (test and control lines), regardless of the intensity of the test line indicates a positive result. A single control line indicates a negative test result. If the control line does not appear, the results are invalid and the test should be repeated. For semi-quantitative results, the patient's titers are reported as the highest dilution that yields a positive test result.

Latex agglutination test

The LAT test was done using the commercial preparation PASTOREX TM CRYPTOPLUS (BIO-RAD, FRANCE), according to the instructions of producer. 120 μ l of CSF (1:2 diluted in glycine buffer) is mixed with 20 μ l of pronase and incubated at 56°C for 30 min. An aliquot of 25 μ l is transferred on the slide card on the designated rings and mixed with applicator sticks. The test is carried out with a positive control. The card is then rocked by hand for 5 min and agglutination read immediately. Results are then read out visually based on degree of agglutination and rated on a scale ranging from 0 to 4+. A reading of 2+ was considered positive.

Positive samples are then titrated by serial dilutions. Antigen titer value are defined as dilution prior to which antigen is not detected.

RESULTS

Of 210 HIV patients who underwent screening for cryptococcal infection, CRAG was positive in 15 patients by LFA. Of these 4 patients were asymptomatic and 11 had symptoms of meningitis [Table 1]. Nine patients had CD4 counts <50 cells/mm³, 3 had CD4 counts between 50 cells/mm³ and 100 cells/mm³ and 3 had counts between 100 cells/mm³ to 200 cells/mm³. None of the patients with CD4 above 200 cells/mm³ was CRAG positive [Table 2]. All those with CRAG positive by LFA had CRAG positive by LAT as well.

Out of 15 CRAG-positive patients, 3 asymptomatic patients did not give consent for lumbar

Table 1: Serum cryptococcal antigen relation with symptom status*

Status	Total (number of patients)	SCRAG +ve	SCRAG -ve
Symptomatic	51	11	40
Asymptomatic	159	4	155

*The P value is <0.00001. Significant at P<0.01. SCRAG=Serum cryptococcal antigen

puncture. Remaining 12 (11 symptomatic and one asymptomatic) were subjected to lumbar puncture and CSF study was done. All of them had CRAG positivity in CSF both by LAT and LFA. Four patients out of 12 patients with serum and CSF CRAG positivity had no growth on fungal culture. Five out of the 12 patients, positive for serum and CSF CRAG had CSF negative for India ink staining [Table 3].

None of the symptomatic patients who were SCRAG negative by Lateral flow assay (LFA) had CSF CRAG positive by LAT or LFA. CSF cryptococcal culture and India ink was also negative in all these patients [Table 4].

CRAG titers by LAT and LF were compared in 7 serum and 4 CSF samples [Table 5]. It was seen that consistently CRAG was positive in higher dilutions by LF.

DISCUSSION

As CM has a high prevalence (>3.2%) and the fact that cryptococcal subclinical infection is high, a

Table 2: Serum cryptococcal antigen relation with CD4 count*

CD4 count	Number of patients	
	Total	SCRAG (LF) +ve
<50	39	9
50-100	95	3
>100	76	3

*The *P* value is 0.000763. The result is significant at *P*<0.01. SCRAG=Serum cryptococcal antigen; LF=Lateral flow

Table 3: Cerebro spinal fluid results in 12 patients of serum cryptococcal antigen positive by lateral flow who were subjected to cerebro spinal fluid examination*

Result	CSF crag (LF)	CSF crag (LAT)	CSF India ink	CSF culture
Positive	12	12	8	9
Negative	0	0	4	3
Total	12	12	12	12

*3 asymptomatic patients who were SCRAG positive did not consent for CSF examination. SCRAG=Serum cryptococcal antigen; CSF=Cerebro spinal fluid; LF=Lateral flow; LAT=Latex agglutination test

Table 4: Cerebrospinal fluid results in 43 symptomatic patients who were serum cryptococcal antigen negative by lateral flow

Result	CSF crag (LFA)	CSF crag (LAT)	CSF India ink	CSF culture
Positive	0	0	0	0
Negative	43	43	43	43
Total	43	43	43	43

CSF=Cerebrospinal fluid; LFA=Lateral flow assay; LAT=Latex agglutination test

rapid diagnostic test needs to be introduced and evaluated. SCRAG by LFA is a point of care test to detect cryptococemia. In our study, LFA was compared against LAT, taking CRAG by LAT as gold standard. This was done as previous studies have shown superiority of LAT over other methods of cryptococcal detection.^{[9,10].}

The prevalence of cryptococcal antigenemia in our study was 7.1% among HIV-infected people, irrespective of CD4 counts and ART status and this was comparable to study by Park *et al.* (0.04%–12%)^[11] and Casadevall from New York (6.1–8.5%).^[11]

The mean age group of our study population was 38.8 years (19–78 years). Majority of them were males. Sexual transmission was the route of spread in almost all the cases. Most of the patients were diagnosed with HIV infection more than a month before being enrolled in our study. Hence, majority of them were on ART. These demographic characteristics of our study population were similar to studies conducted earlier by Chakraborty *et al.*,^[12] Amelu *et al.*^[13] and Ogba and Abia Bassey.^[14]

About 34.6% of our patients had one or the other symptoms suggesting meningitis. Among them, 15 patients were found to be CRAG positive. Fever was observed in 13 patients (86.67%), headache in 11 (73.33%), vomiting in 6 (40%), and neck rigidity in 10 patients (66.67%). Most of the CRAG-positive patients had symptomatic meningitis (80%; 12 out of 15). This was in agreement with study by Baradkar *et al.*^[15] and Clark *et al.*^[16] Our study also found that there is an increased chance of CRAG to be positive in patients with lower Glasgow Coma Scale (GCS). Zuger *et al.*^[17] had shown that patients with higher GCS at diagnosis had better prognosis in CM.

CRAG prevalence in our study was 7.1% (15/210). This was comparable to study by Park *et al.*^[11] but lower to those done outside India mainly in sub-Saharan countries where the cryptococcal fungal burden is higher than the rest of the world.

The CD4 count of our study population ranged from 6 to 780 cells/mm³. No patient receiving ART was detected to be CRAG positive. Of the patients positive for CRAG, 9 out of 15 (60%) had CD4 <50 cells/mm³; three out of 15 (20%) had CD4 between 50 and 100 cells/mm³ and the remaining three (20%) had CD4 >100 cells/mm³. Previous studies by Micol *et al.*,^[18] Pongsai *et al.*^[19] also found that there is decreasing cryptococcal prevalence with increasing CD4 counts and this was in agreement with the present study.

Table 5: Correlation of crag titers by latex agglutination test and lateral flow assay in serum and cerebrospinal fluid*

Serum crag titres (LAT)	Serum crag titres (LFA)
1:256	1:320
1:256	1:320
1:128	1:160
1:1024	1:1280
1:256	1:320
1:512	1:640
1:64	1:80
CSF crag titers (LAT)	CSF crag titers (LFA)
1:1024	1:1280
1:1024	1:1280
1:64	1:80
1:512	1:640

*R=1, the P value is <0.00001. The result is significant at P<0.01.

CRAG=Cryptococcal antigen; CSF=Cerebrospinal fluid; LFA=Lateral flow assay; LAT=latex agglutination test

In the present study, all patients (15/210) who were CRAG positive by LAT were also CRAG positive by LFA. Those with CRAG negative by LA were negative by LFA also. Thus, the sensitivity and specificity of CRAG LFA is 100% each and this was in agreement with several previously published studies by Huang *et al.*,^[20] Klausner *et al.*,^[21] McMullan *et al.*,^[22] Rugemalila *et al.*^[23] and Escandón *et al.*^[24] A study results showed the CRAG LFA had excellent agreement with the CRAG by LAT.

All 12 CRAG-positive patients who were subjected to lumbar puncture, had CSF CRAG positive by both, LA and LFA. This observation was in agreement with multicentric study by Rolfes M,^[25] Vijayan *et al.*^[21] and Kabanda *et al.*^[26]

In a study by Lourens *et al.*,^[27] high concentrations of the CRAG resulted in decreased visual intensity of the test lines (hook effect). This accounts for 100% sensitivity of LFA only on diluted CSF samples for the diagnosis of CM, especially when high organism load is present. However, in our study patients with CRAG positive in high dilutions also had CRAG positive by LFA. We detected CRAG positive by LFA and CSF CRAG positive by LFA in patients with titers as low as 1:10 as well as in very high titers as 1:10,000. Although antigenemia is highly predictive of the development of CM and an independent predictor of mortality, our study did not find any statistical significance between the two. This may be due to small sample size (15 samples) and short duration of our study.

Of 54 symptomatic patients whose CSF was analyzed, 12 patients were positive by both CRAG LFA and CSF LFA. Thus, CRAG LFA is 100%

sensitive and specific to diagnose CM. This agreed with study by Kozel and Bauman.^[28]

CSF of the above patients was also subjected to India ink staining and fungal culture. India ink and fungal cultures were negative in all those in whom CRAG was negative by LA and LFA. Of the CSF CRAG-positive cases, 66.6% were positive for CSF India ink staining and 75% were positive for CSF fungal culture. When LFA and LA were taken as gold standard for diagnosis of CM, sensitivity, specificity, positive predictive value, and negative predictive value of CSF fungal culture were 66.7%, 100%, 100%, 97.9%, and 58.3%, respectively. The corresponding values in case of India ink staining were 97.5%, 100%, 100%, respectively.

This is because India ink is highly operator dependable and the sensitivity of culture depends on quantity of CSF sample used for culture.^[25]

No specific importance was given to CSF volume inoculated in our study. Perhaps, larger volume of inoculation might yield better sensitivity for cryptococcal detection by fungal culture. Thus, conventional methods are inferior to CRAG LFA in detection of cryptococcal infection.

All cases of cryptococcal antigenemia in our study had more advanced disease with low CD4 counts. We had excluded previously diagnosed cases of cryptococcosis and our study had no cases of cryptococcal immune reconstitution inflammatory syndrome. None of our patients on ART had cryptococcal antigenemia and this is in concordance with previous studies that ART confers protection against cryptococcosis.

There were some limitations in our study. Only one asymptomatic patient who had CRAG positive was submitted to CSF examination. A larger group of asymptomatic CRAG positive patients need to be evaluated by CSF examination before treating all CRAG-positive patients as CM. Further studies on point of care CRAG by LFA need to focus on asymptomatic patients with low CD4 counts as they stand to gain maximum from this strategy.

CONCLUSION

Early diagnosis is the key to improve survivability in CM. CRAG is a rapid point of care test recommended by the WHO for diagnosing cryptococcal infection. The reports of LFA are obtained in <10 min.

Our study is the first study in India to evaluate CRAG detection by LFA method by comparing it against the LAT method. We found that serum detection of CRAG is as sensitive as CRAG detection in serum by LAT, CSF CRAG detection by LFA and LAT, thus making it possible to implement this method on a large scale even in resource poor, remote areas where access to electricity, well-equipped laboratory, cold chains is not possible. In future, LFA might replace LAT as diagnostic test of choice to diagnose CM.

Acknowledgments

None.

Financial support and sponsorship

AC was supported by the research grant from ICMR, Delhi, vide grant no OMI/13/2014-ECD-I.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 2009;23:525-30.
- Kumarasamy N, Solomon S, Flanigan TP, Hemalatha R, Thyagarajan SP, Mayer KH. Natural history of human immunodeficiency virus disease in Southern India. *Clin Infect Dis* 2003;36:79-85.
- Sobhani R, Basavaraj A, Gupta A, Bhavne AS, Kadam DB, Sangle SA, *et al.* Mortality and subclinical characteristics of hospitalised adult patients with HIV in Pune, India. *Indian J Med Res* 2007;126:116-21.
- Kumarasamy N, Vallabhaneni S, Flanigan TP, Mayer KH, Solomon S. Clinical profile of HIV in India. *Indian J Med Res* 2005;121:377-94.
- Gordon MA, Vedder DK. Serologic tests in diagnosis and prognosis of cryptococcosis. *JAMA* 1966;197:961-7.
- Gade W, Hinnefeld SW, Babcock LS, Gilligan P, Kelly W, Wait K, *et al.* Comparison of the PREMIER cryptococcal antigen enzyme immunoassay and latex agglutination assay for detection of cryptococcal antigens. *J Clin Microbiol* 1991 Aug;29(8):1616-9.
- Jarvis JN, Percival A, Bauman S, Pelfrey J, Meinjtes G, Williams GN, *et al.* Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. *Clin Infect Dis* 2011 Nov;53(10): 1019-23.
- Lindsley MD, Mekha N, Baggett HC, Surinthon Y, Auttheteinchai R, Sawatwong P, *et al.* Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. *Clin Infect Dis* 2011;44:321-5.
- Saha DC, Xess I, Biswas A, Bhowmik DM, Padma MV. Detection of *Cryptococcus* by conventional, serological and molecular methods. *J Med Microbiol* 2009;58:1098-105.
- Khyriem AB, Sujatha S, Das AK, Parija SC. Comparison of latex agglutination and co-agglutination for the diagnosis and prognosis of cryptococcal meningitis. *Indian J Med Microbiol* 2003;21:252-6.
- Currie BP, Casadevall A. Estimation of the prevalence of cryptococcal infection among patients infected with the human immunodeficiency virus in New York City. *Clin Infect Dis* 1994;19:1029-33.
- Chakraborty N, Mukherjee A, Santra S, Sarkar, RN, Banerjee D, Guha SK, *et al.* Current trends of opportunistic infections in HIV seropositive patient in Eastern India. *J Infect Dis* 2008;61:49-53.
- Amelu AS, Kempker RR, Tenna A, Smitson C, Berhe N, Fekede D, *et al.* High prevalence of cryptococcal antigenemia among HIV infected patients receiving antiretroviral therapy in Ethiopia. *PLoS One*. 2013;8(3):e58377.
- Ogba OM, Abia Bassey L. Cryptococcal antigenemia among HIV seropositive patients accessing care in antiretroviral therapy (ART) clinics in Calabar, South Southern Nigeria *Microbiol Discov* 2015;3:7.
- Baradkar V, Mathur M, De A, Kumar S, Rathi M. Prevalence and clinical presentation of Cryptococcal meningitis among HIV seropositive patients. *Indian J Sex Transm Dis AIDS* 2009;30:19-22.
- Clark RA, Greer D, Atkinson W, Valainis GT, Hyslop N. Spectrum of *Cryptococcus neoformans* infection in 68 patients infected with human immunodeficiency virus. *Rev Infect Dis* 1990;12:768-77.
- Zuger A, Louie E, Holzman RS, Simberloff MS, Rahal JJ. Cryptococcal disease in patients with the acquired immunodeficiency syndrome. Diagnostic features and outcome of treatment. *Ann Intern Med* 1986;104:234-40.
- Micol R, Lortholary O, Sar B, Laureillard D, Ngeth C, Dousset JP, *et al.* Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients. *J Acquir Immune Defic Syndr* 2007;45:555-9.
- Pongsai P, Atamasirikul K, Sungkanuparph S. The role of serum cryptococcal antigen screening for the early diagnosis of cryptococcosis in HIV infected patients with different ranges of CD4 cell counts. *J Infect* 2010;60:474-7.
- Huang HR, Fan LC, Rajbanshi B, Xu JF. Evaluation of a new cryptococcal antigen lateral flow immunoassay in serum, cerebrospinal fluid and urine for the diagnosis of cryptococcosis: A meta-analysis and systematic review. *PLoS One* 2015;10:e0127117.
- Vijayan T, Chiller T, Klausner JD. Sensitivity and sensitivity of a new cryptococcal antigen lateral flow assay in serum and cerebrospinal fluid MLO; *Med Lab Obs*. 2013;45(3):16-20.
- McMullan BJ, Halliday C, Sorrell TC, Judd D, Sleiman S, Marriott D, *et al.* Clinical utility of the cryptococcal antigen lateral flow assay in a diagnostic mycology laboratory. *PLoS One* 2012;7:e49541.
- Rugemalila J, Maro VP, Kapanda G, Ndaro AJ, Jarvis JN. Cryptococcal antigen prevalence in HIV infected Tanzanians: A cross sectional study and evaluation of a point of care lateral flow assay. *Trop Med Int Health* 2013;18:1075-9.
- Escandón P, Lizarazo J, Agudelo CI, Chiller T, Castañeda E. Evaluation of a rapid lateral flow immunoassay for the detection of cryptococcal antigen for the early diagnosis of cryptococcosis in HIV patients in Colombia. *Med Mycol* 2013;51:765-8.
- Rolfes M, Butler E, Von Hohenberg M, Nabeta H, Kwizera R, Rajasingham R, *et al.* Evaluation of a Novel Point of Care Lateral Flow Assay to Detect Cryptococcal Antigen in Plasma and CSF. In 19th Conference on Retrovirus and Opportunistic Infections. Seattle; 2012.
- Kabanda T, Siedner MJ, Klausner JD, Muzoora C, Boulware DR. Point-of-care diagnosis and prognostication of cryptococcal meningitis with the cryptococcal antigen lateral flow assay on cerebrospinal fluid. *Clin Infect Dis* 2014;58:113-6.
- Lourens A, Jarvis JN, Meintjes G, Samuel CM. Rapid diagnosis of cryptococcal meningitis by use of lateral flow assay on cerebrospinal fluid samples: Influence of the high-dose "hook" effect. *J Clin Microbiol* 2014;52:4172-5.
- Kozel TR, Bauman SK. CrAg lateral flow assay for cryptococcosis. *Expert Opin Med Diagn* 2012;6:245-51.