International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Comparative Evaluation of Dot Blot and ELISA for the Detection of Anticysticercal Antibodies in the Cerebrospinal Fluid for the Immunodiagnosis of Neurocysticercosis

Shripad A. Patil[1], Anupama R.Pai[1], M. Veerendrakumar[2]

[1]Departments of Neuromicrobiology and [2]Neurology,
National Institute of Mental Health and Neurosciences, Hosur Road, Bangalore-560029, INDIA

Corresponding Author: Shripad A. Patil

Received: 28/11/2014 *Revised: 16/12/2014* *Accepted: 02/01/2015*

ABSTRACT

Neurocysticercosis (NCC) is one of the major parasitic infections of the human central nervous system (CNS). Dot- blot has been established for the quick diagnosis of Neurocysticercosis (NCC) by analyzing the cerebrospinal fluid (CSF) from patients for anti-cysticercal antibodies. The assay is compared with indirect Enzyme linked immunosorbent assay (ELISA) for the efficacy. A total homogenate (scolices, membrane and fluid) of *Taenia solium* cysts obtained from infected pigs was used as the antigen in the assays. CSF samples were collected from chronic meningitis cases which were suspected for NCC, tuberculous meningitis (TBM) and other chronic meningitis cases apart from controls from viral meningitis, pyogenic meningitis and non-neurological controls at National Institute of Mental Health and Neurosciences (NIMHANS) Bangalore, India. Anticysticercal antibodies were detected in 68 of 285 (23.86%) tested CSF samples by ELISA and in 53 of 285 (18.6%) samples by dot blot. Results of the present study revealed that some of the CSF samples which showed non-specific reactivity by ELISA (false positivity) probably due to the sticky component(s) in them is overcome by dot blot. This finding is also confirmed by western blot. Therefore dot blot may be used not only for wider screening of samples but can also be used for rapid diagnostic purpose in poorly equipped laboratories in endemic regions of NCC.

Keywords: Dot blot, ELISA, Immunodiagnosis, Neurocysticercosis, Cerebrospinal fluid

INTRODUCTION

Cysticercosis is a health problem in developing countries due to poor hygiene. Neurocysticercosis (NCC) is one of the major parasitic infections of the human central nervous system (CNS). It is caused by metacestode larvae of the tapeworm, *Taenia solium*. According to WHO reports, approximately 50 million people suffer with NCC and it is responsible for about 50,000 deaths per year around the world. WHO declared NCC as "an important, neglected disease". [(1,2)] The disease is endemic in sub Saharan Africa, Latin America, Asia and many other developing countries. [(3)] NCC is now emerging in developed countries like USA, UK and also in other European countries, due to increased travelling to and

from endemic regions. [2] Incidence of cysticercosis has been reported from various regions in India by various workers and India is referred as endemic for cysticercosis. [2-6]

The parasite enters the brain as microscopic oncospheres breach the blood brain barrier and develop into the metacestode stage (cysticerci), causing NCC. [7] The symptoms of NCC are nonspecific and varied from mild to severe, which may include epilepsy, headache, hydrocephalus, intracranial hypertension, chronic meningitis and psychiatric disturbances. [1,8,9] NCC has been identified as the major etiologic agent for epileptic seizures in 30-40% late-onset epilepsy cases in endemic countries. [1,10]

As the clinical manifestation depends mainly on the number, location and size of lesions, accurate diagnosis of NCC requires neuroimaging methods such as Computed Tomography (CT) or Magnetic Resonance Imaging (MRI). In developing countries where *T. solium* is endemic, CT and MRI facilities are not easily available. Further, in certain instances, tuberculosis, neoplasia, toxoplasmosis, brain abscess and fungal infections may also cause lesions which can be confused with NCC. In such cases, the diagnosis can be supported by clinical, epidemiological and immunological findings and by the response to cysticidal treatment. [11,12]

Several immunological tests have been developed for the demonstration of specific antibodies in the serum and cerebrospinal fluid (CSF) of patients with NCC. Different techniques like ELISA, [13] latex agglutination, [14] Western blot and Enzyme linked immunoelectrotransfer blot (EITB) [15,16] assay are in use.

ELISA and EITB assay which employs total antigens or partially purified antigens are most widely used antibody detection tests. However, the EITB assay requires technical expertise and hence not so convenient for epidemiological purposes. There is a need for a simplified technique which can be used even in less equipped laboratories or in field conditions.

Dot ELISA, a rapid and easy immunodiagnostic technique, has been widely used in the diagnosis of many infections like visceral leishmaniasis, [17] malaria, cystic echinococcosis, fascioliasis, [18] schistosomiasis [6] and tuberculous meningitis. [19] The dot ELISA has nitrocellulose membrane as a carrier of protein where more antigen binds than to microtitre plates and the results can be read visually without any instrument.

In the present study, attempt has been made at this tertiary neurological care center to establish Dot ELISA as an immunodiagnostic test for NCC and compare the results with conventional ELISA technique for the detection of anticysticercal antibodies in the CSF of chronic meningitis cases suspected for neurocysticercosis.

MATERIALS AND METHODS

CSF samples used in the present study are collected for establishing the diagnosis by immunological means as per the ethical guidelines of the institute. A total of 285 CSF samples [NCC =68; TBM =88; Viral meningitis =19; Pyogenic meningitis =30; other chronic meningitis =61 (fungal, neurosyphilis, carcinomatous); non neurological controls n=19] were collected aseptically from patients and controls at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India, and were stored at -70 ºC till further use (Table.1)

Case selection criteria

Neurocysticercosis: The criteria of Del Brutto[12] were followed for the classification of NCC cases where radiological findings, clinical presentation and positive for

cysticercal antibodies by ELISA were considered.

Table.1 Number of CSF samples analysed by ELISA and Dot blot technique for anticysticercal antibodies.

CSF tested from different subjects	No. of Samples	Positive for anti-cysticercal antibodies	
		ELISA	Dot blot
NCC	68	68	53
TBM	88	3	3
Viral meningitis	19	0	0
Pyogenic meningitis	30	0	0
Other chronic meningitis*	61	0	0
Non neurological control	19	0	0
Total	285	71	56

*Fungal, Carcinomatous, Neurosyphilis cases

Tuberculous meningitis (TBM): TBM cases were diagnosed on the basis of clinical diagnosis such as subacute presentation, fever, headache, vomiting, kerning signs with or without pulmonary involvement or a source case and positive for anti-TB antibodies and or culture positive for *M.tuberculosis.*

Viral meningitis: Cases were diagnosed on the basis of clinical presentation followed by positivity for antiviral antibodies in the CSF and or positive by PCR.

Pyogenic meningitis: Cases were positive for CNS bacterial infection by culture or smear.

Fungal infections: Cases were diagnosed by culture or by India ink staining for *Cryptococci*.

Neurosyphilis: Cases were diagnosed on the basis of positivity by VDRL test which was confirmed by *Treponemapallidum* Particle agglutination assay.

Carcinomatous meningitis: Cases were diagnosed by clinical suspicion and cytospin studies suggestive of the condition.

Non neurological cases: Subjects who underwent spinal anesthesia for non-neurological cause like hernia, hydrocoel or surgical delivery.

Preparation of Cysticercal Antigen

Cysticercal antigen was prepared by obtaining the cyst infected pork from slaughter house in cold condition soon after the sacrifice of the pig. The ham was put at -70 ºC in case of delay in processing for a day or two till processed further. Cysts were plucked with the help of sterile forceps from the pork by making gentle incisions and collected in ice cold phosphate buffer saline with preservatives (Thiomersal, 0.002%). The cysts were later finely grinded in a sterile glass homogeniser with excess of PBS and the homogenised solution was centrifuged at 5000 rpm in cold centrifuge. The clear supernatant was measured for protein strength by Lowry's method and was used as total cyst antigen in the ELISA.

ELISA

The presence of cysticercal antibodies in the CSF were detected by ELISA. Briefly, microtitre plates (TPP, Trassadingen, Switzerland) were coated with 50 µl/well (1µg/ml) of cysticercal antigen dissolved in Phosphate Buffered Saline (PBS), by overnight incubation at 4 ºC in a moist chamber. The plates were then washed with PBST (PBS containing 0.05% Tween 20) to remove any unbound antigen and tap dried. Freshly prepared 1% (w/v) PBST milk (Anikspray, nonfat dry milk, India) was added (100 µl/well) to block free binding sites. Plate was later tap dried and stored at -70 ºC in dry condition until use.

CSF samples were diluted 1:10 in PBST milk 1% (w/v) and added (50 µl/well) to the antigen coated ELISA plate in duplicates. Positive and Negative controls were also added each time to compare the results. Plate was later incubated at 37ºC in a moist chamber, for 90 min. After the incubation, plate was washed 5 times using PBST and tap dried. Horse radish peroxidase (HRP) conjugated polyclonal rabbit anti-human IgG (Dako, Denmark,1:3000 dilution with PBST milk) was then added (50 µl/well) and plate was

re- incubated at 37ºC for 60 minutes. At the end plate was washed 5 times with PBST and tap dried. Later O-phenylene-diaminechromogen (75 µl/well), dissolved in phosphate citrate buffer (0.1 M, pH 5) containing 0.01% hydrogen peroxide, was added to each well. Plate was incubated in dark for 10 min and checked for colour development. Reaction was then stopped by adding 50 µl of 2N sulphuric acid to each well. The absorbance values (OD) were obtained by reading the plate in an ELISA reader (Sunrise Magellan, Tecan-AG, Austria Gmbh) at 492 nm. The cut off value was calculated by taking the absorbance of CSF samples from non-diseased controls + 3 SD values of the O.D.

Dot Blot

Nitrocellulose (NC) membrane strips (Microdevices Ambala, India) stuck to plastic surface for ease of handling was used for dot blot procedure. Using a micropipette, 5 µl of antigen (1 µg/ µl in PBS), was spotted on the strips towards one end to form a dot. Strips were then allowed to dry at ambient temperature and stored at -20 ºC until used for detection of anti-cysticercal antibodies in CSF.

The antigen coated strips were dipped in CSF samples which were diluted 1:10 with freshly prepared PBST milk (1% w/v) and incubated for 30 min with continuous shaking. Strips were then washed 5 times with PBST and were incubated with peroxidase conjugated (HRP) rabbit polyclonal secondary antibody (IgG) diluted 1:1000 in PBST milk, at ambient temperature for 15 min. Strips were again washed 5 times with PBST and were developed with 4-chloronaphthol chromogen (Sigma, USA) for 10 min. The reaction was then stopped by soaking the strips in distilled water and then dried. The results were read (blind coded) according to the colour intensity of the dot and compared with positive and negative controls.

RESULTS

In the present study, anticysticercal antibodies were detected by ELISA in 68 of 285 (23.86%) CSF samples from chronic meningitis and controls whereas dot blot detected antibodies in 53 of 285 (18.6%) CSF samples. The cut off value (Mean ± 3SD of controls) observed was around 0.200. The samples in which OD value is between 0.100 to 0.200 are considered border line positive (considered positive in analysis) and those having OD values > 0.200 are considered clear positive by ELISA.

The CSF samples which showed a clear violet colored dot at the site of antigen applied on NC membrane were considered reactive by Dot blot assay (Fig.1). This assay was performed for a total of 285 samples, of which 56 samples were positive by the dot blot based on colour development (Fig.2).

Further, two CSF samples (one reactive and other non-reactive by dot blot) when tested by western blot showed similar reactivity on nitrocellulose strip revealing the appropriate reaction by dot blot (Fig.3).

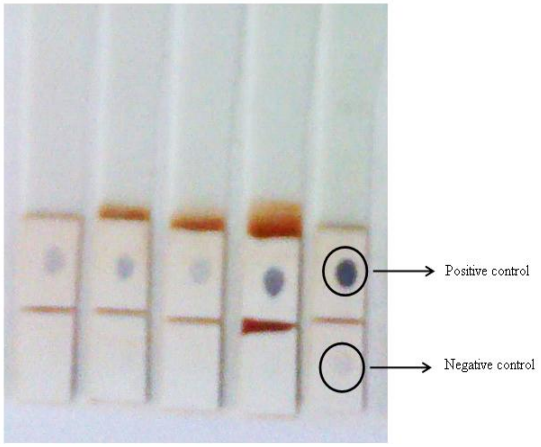

Fig.1. Dot blot reactivity reveals anticysticercal antibodies in CSF samples.

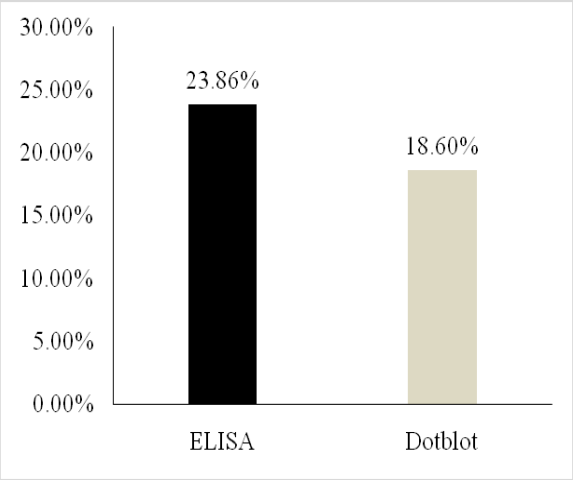

Fig.2 Number of CSF samples positive by ELISA and Dot blot technique for anti-cysticercal antibodies. (n=285).

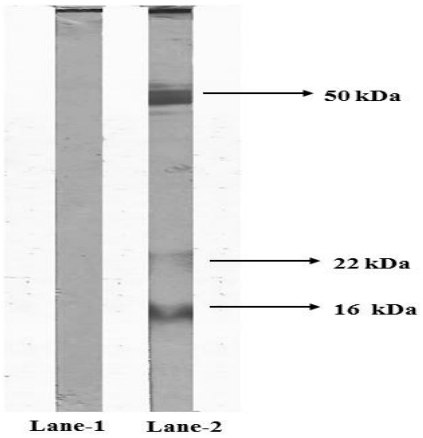

Fig.3. Two of the samples tested with Dot blot were analysed by Western blot.

Lane-1: Nonreactive by both dot blot and western blot; Lane-2: Reactive by both Dot blot and Western blot.

DISCUSSION

NCC, the most common parasitic infection of the CNS, is the major cause of active epilepsy (26.3% to 53.8%) in developing countries(5). In India, NCC has been reported from various parts including, Bihar, Uttar Pradesh, Delhi, Chandigarh, Pondicherry, Bangalore and other parts of South India. [(2-6)]

There are a number of immunoassays developed for detection of antibodies against cysticercal antigens in CSF of NCC patients. In a preliminary study by Biswas et al [(6)] dot blot using the complete homogenate of cysticercal cyst was found to be 56.25% sensitive compared to plate ELISA which showed sensitivity of 43.75% for diagnosis of NCC using serum.

Another preliminary study conducted to detect anticysticercal antibodies in CSF with antigens dotted on a synthetic polyester fabric impregnated with polymerized resin (dot-RT) reported that 14 out of 15 cases of NCC were positive by dot ELISA with titres from 1 to 128. Vaz et al reported high degree of sensitivity and specificity using membrane and scolex (M+S-Cc) antigens of *Cysticercus cellulosae.* vesicular fluid (VF) antigen of *Cysticercus longicollis* was also reported to be highly antigenic. [(20,21)]

There is lack of information on comparative evaluation of ELISA and Dot blot. In the present study attempt has been made to evaluate these 2 assays for the diagnosis of NCC. The present study revealed slightly lesser percentage positivity by dot blot compared to ELISA. This could be because of higher sensitivity of ELISA than the dot blot assay or because of non-specific binding of some of the CSF samples to ELISA plate due to alleged sticky component (s) seen in our past experience. The non-specific binding of CSF in ELISA is also noticed in our recent study in CNS tuberculosis. [(19)] Such binding might lead to some degree of false positivity in ELISA which is not noticed in the dot blot as the nitrocellulose membrane gives a clear dot at the point of application of antigen. It is also noticed that some of the CSF samples showing around 0.100 OD by ELISA (borderline positive) have shown reactivity by dot blot suggesting dot blot is equally sensitive. However, with our experience of about two decades we always keep some degree of false positivity in mind while analyzing CSF ELISA results.

Among TBM cases, 3 samples also showed reactivity for cysticercal antigen by ELISA as well as by Dot blot. The co-infection in these cases could not be ruled out due to poor follow-up. None of the samples from 68 controls showed any reactivity either by ELISA or by Dot blot assay.

In a comparative evaluation of ELISA and dot blot assay using a recombinant cysticercal antigen Tsol-p27, the sensitivity was reported to be 86.7% for both the tests using serum samples. [22]

ELISA and enzyme immuno transfer blot (EITB) have been used most frequently for the detection of anticysticercal IgG antibodies in serum or CSF. Different authors have used ELISA for immunodiagnosis of NCC and reported varied sensitivities of 85%, [23] 93%, [24] 41%, [25] 86.7% [22] using serum and 71%, [25] 90% [23] using CSF. The specificities of ELISA by various studies are reported to be 100% with CSF, [23] 94.6% using serum, [22] and 95.7% using CSF or serum. [22] In the present study, ELISA and Dot blot results were more or less comparable. Further, the test reactivity seen by western blot confirmed the reaction noticed on dot blot. The present study which compares both ELISA and dot blot using total extract of the cyst has revealed robust reaction in dot blot compared to ELISA. In ELISA, the antigen gets coated onto the plate in the well, whereas in dot blot it gets concentrated on to the NC membrane as a dot which reveals clear visible reaction for antibody. This study is first to our knowledge which has analysed varieties of CSF samples by both dot blot and ELISA.

CONCLUSION

Dot blot is a rapid, easy-to-perform and less expensive technique unlike ELISA. Further it does not require any instrument to analyse the reaction as it can be read visually. The assay can be done even in poorly equipped laboratories in remote areas by imparting a basic training to the laboratory staff. The assay would be convenient in epidemiological studies where large number of samples need to be analysed in short time. The dot-blot assay may thus be used not only for wider screening of the samples but also for immunodiagnostic purpose.

ACKNOWLEDGEMENT

The study is approved by the ethical committee of NIMHANS. The Director is thanked for his permission and support to carry out the study. The help rendered by the Neurologists, Neurosurgeons and staff of Department of Neuromicrobiology of NIMHANS at various stages is kindly acknowledged. Part of the funding for the study is from UGC.

Funding: This work was supported by National Institute of Mental Health and Neurosciences, Bangalore and University Grants Commission, India.

Conflict of interest: There is no conflict of interest academically or monetarily.

Ethical approval: Approved by Institutional ethical committee vide no. NIMH/EC-XXI/2001-02.

REFERENCES

1. White AC, Jr. Neurocysticercosis: a major cause of neurological disease worldwide. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1997; 24 (2):101-13; quiz 14-5.
2. Garcia HH, Del Brutto OH, Cysticercosis Working Group in P. Neurocysticercosis: updated concepts about an old disease. The Lancet Neurology. 2005;4(10):653-61.
3. Rajshekhar V, Joshi DD, Doanh NQ, van De N, Xiaonong Z. Taenia solium taeniosis/cysticercosis in Asia: epidemiology, impact and issues. Acta tropica. 2003;87(1):53-60.

4. Agarwal V, Kumar P, Chadda RK. Neurocysticercosis presenting as psychiatric illness. Indian journal of pediatrics. 2001;68(11):1073-4.
5. Prasad KN, Prasad A, Verma A, Singh AK. Human cysticercosis and Indian scenario: a review. Journal of biosciences. 2008;33(4):571-82.
6. Biswas R, Parija SC, Narayan SK. Dot-ELISA for the diagnosis of neurocysticercosis. Revista do Instituto de Medicina Tropical de Sao Paulo. 2004;46(5):249-52.
7. Saenz B, Ramirez J, Aluja A, Escobar A, Fragoso G, Morales J, et al. Human and porcine neurocysticercosis: differences in the distribution and developmental stages of cysticerci. Tropical medicine & international health: TM & IH. 2008;13(5):697-702.
8. Chandramuki A, Nayak P. Sub acute and chronic meningitis in children--an immunological study of cerebrospinal fluid. Indian journal of pediatrics. 1990;57(5):685-91.
9. Del Brutto OH. Neurocysticercosis: a review. The Scientific World Journal. 2012; 2012:159821.
10. Lescano AG, Garcia HH, Gilman RH, Gavidia CM, Tsang VC, Rodriguez S, et al. Taenia solium cysticercosis hotspots surrounding tapeworm carriers: clustering on human seroprevalence but not on seizures. PLoS neglected tropical diseases. 2009;3(1):e371.
11. Rajshekhar V, Chandy MJ. Validation of diagnostic criteria for solitary cerebral cysticercus granuloma in patients presenting with seizures. Acta neurologica Scandinavica. 1997;96 (2):76-81.
12. Del Brutto OH, Rajshekhar V, White AC, Jr., Tsang VC, Nash TE, Takayanagui OM, et al. Proposed diagnostic criteria for neurocysticercosis. Neurology. 2001;57 (2):177-83.
13. Diwan AR, Coker-Vann M, Brown P, Subianto DB, Yolken R, Desowitz R, et al. Enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to cysticerci of Taenia solium. The American journal of tropical medicine and hygiene. 1982;31(2):364-9.
14. Rocha SM, Suzuki LA, Silva AD, Arruda GC, Rossi CL. A rapid latex agglutination test for the detection of anti-cysticercus antibodies in cerebrospinal fluid (CSF). Revista do Instituto de Medicina Tropical de Sao Paulo. 2002;44(1):57-8.
15. Gottstein B, Tsang VC, Schantz PM. Demonstration of species-specific and cross-reactive components of Taenia solium metacestode antigens. The American journal of tropical medicine and hygiene. 1986;35(2):308-13.
16. Tsang VC, Brand JA, Boyer AE. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (Taenia solium). The Journal of infectious diseases. 1989;159(1):50-9.
17. Pappas MG, Hajkowski R, Hockmeyer WT. Dot enzyme-linked immunosorbent assay (Dot-ELISA): a micro technique for the rapid diagnosis of visceral leishmaniasis. Journal of immunological methods. 1983;64(1-2):205-14.
18. Romia SA, Youssef ME, Handoussa AE, Rizk HM, Sallam SM. Dot-ELISA as a diagnostic test in hydatid disease. Journal of the Egyptian Society of Parasitology. 1992;22(3):603-10.
19. Patil SA, Kavitha AK, Madhusudan AP, Netravathi M. Comparative evaluation of ELISA and dot-blot for the diagnosis of tuberculous meningitis. Journal of immunoassay & immunochemistry. 2013;34(4):404-13.
20. Vaz AJ, Ferreira AW, Camargo ME, Nakamura PM, Camargo ED. DOT-ELISA for detection of anti-Cysticercus cellulosae antibodies in human cerebrospinal fluid using a new solid-phase (resin-treated polyester fabrics). Preliminary report. Revista do Instituto de Medicina Tropical de Sao Paulo. 1990;32(5):355-9.

21. Vaz AJ, Nakamura PM, Camargo ME, Camargo ED, Ferreira AW. Dot-ELISA for the detection of anti-Cysticercus cellulosae antibodies in cerebrospinal fluid using a new solid phase (resin-treated polyester fabric) and Cysticercus longicollis antigens. Revista do Instituto de Medicina Tropical de Sao Paulo. 1996;38(6):391-6.
22. Salazar-Anton F, Tellez A, Lindh J. Evaluation of an immunodot blot technique for the detection of antibodies against Taenia solium larval antigens. Parasitology research. 2012;110 (6): 2187-91.
23. Espinoza B, Ruiz-Palacios G, Tovar A, Sandoval MA, Plancarte A, Flisser A. Characterization by enzyme-linked immunosorbent assay of the humoral immune response in patients with neurocysticercosis and its application in immunodiagnosis. Journal of clinical microbiology. 1986;24(4):536-41.
24. Sloan L, Schneider S, Rosenblatt J. Evaluation of enzyme-linked immunoassay for serological diagnosis of cysticercosis. Journal of clinical microbiology. 1995;33(12):3124-8.
25. Proano-Narvaez JV, Meza-Lucas A, Mata-Ruiz O, Garcia-Jeronimo RC, Correa D. Laboratory diagnosis of human neurocysticercosis: double-blind comparison of enzyme-linked immunosorbent assay and electroimmunotransfer blot assay. Journal of clinical microbiology. 2002; 40(6):2115-8.

How to cite this article: Patil SA, Pai AR, Veerendrakumar M. Comparative evaluation of dot blot and ELISA for the detection of anticysticercal antibodies in the cerebrospinal fluid for the immunodiagnosis of neurocysticercosis. Int J Health Sci Res. 2015; 5(2):75-82.

****

International Journal of Health Sciences & Research (IJHSR)

Publish your work in this journal

The International Journal of Health Sciences & Research is a multidisciplinary indexed open access double-blind peer-reviewed international journal that publishes original research articles from all areas of health sciences and allied branches. This monthly journal is characterised by rapid publication of reviews, original research and case reports across all the fields of health sciences. The details of journal are available on its official website (www.ijhsr.org).

Submit your manuscript by email: editor.ijhsr@gmail.com OR editor.ijhsr@yahoo.com