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Original Article

Determination of Cut-off for *Leptospira* Immunoglobulin M Enzyme-linked Immunosorbent Assay in South India

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Abstract

Background: Leptospirosis is diagnosed by dark-field microscopy, culture, antigen detection, molecular methods, and serology. Among the serological methods, microscopic agglutination test is the reference serological test, while immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) is the most widely used test for the diagnosis of leptospirosis. The objective of the study was to revalidate the diagnostic cut-off of ELISA used for confirming a clinical diagnosis of leptospirosis.

Materials and Methods: This study was done in a tertiary care hospital in South India during a period of 2 years (2012–2014). Plasma samples from 100 healthy donors were initially used for the determination of the cut-off for *Leptospira* IgM ELISA by Panbio, Virion Serion, and Inbios kits. The final cut-off was determined by testing serum samples from 50 patients having scrub typhus with eschar, 20 sepsis, 20 enteric fever, 17 malaria, and 17 dengue samples. All ELISAs were performed according to the manufacturer's instructions.

Results: The final cut-off for *Leptospira* IgM ELISA by PanBio was taken as 20 PanBio units, that of *Leptospira* Virion Serion was 0.7 OD, and Inbios *Leptospira* was 1 OD.

Conclusion: All ELISAs have to be properly validated, and a cut-off has to be determined for these tests depending on the local prevalence of disease. The centers in South India can use the above cut-off values for performing IgM ELISA for *Leptospira* by these kits.

Key words: Leptospirosis, cut-off, IgM ELISA, Validation

INTRODUCTION

Leptospirosis is a zoonosis of ubiquitous distribution, caused by infection with pathogenic *Leptospira* species.[1] The different methods of the diagnosis of leptospirosis are microscopy, antigen detection, isolation, serology, and molecular methods. The serological methods of diagnosis include microscopic agglutination test (MAT), enzyme-linked immunosorbent assay (ELISA), and other rapid tests.[1]

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MAT is the reference method for serological diagnosis of leptospirosis.[1] The drawbacks of MAT are that it is complex, time-consuming procedure; interpretation is difficult and hazardous because of the risk of exposure to the live pathogen. Difficulty in maintaining the serovars necessitates continuous weekly subculturing of the strains which requires periodic verification of the strains. It gives false negative reactions in delayed seroconversion, which sometimes occurs by 30 days after infection. High degree of cross-reactions between different serogroups and cross-reactions in other unrelated infections such as syphilis, viral hepatitis, HIV, relapsing fever, Lyme's disease, legionellosis, and autoimmune diseases is due to the persistence of Immunoglobulin M (IgM) antibodies for long time. It is insensitive, particularly, in acute phase and in patients with severe disease who die before seroconversion.[1]

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ELISA: IgM ELISA, IgM capture ELISA, and IgM dot ELISA-dipstick to detect IgM antibodies against leptospires have been developed for diagnosis. IgM detection is more sensitive than MAT in early disease. The advantage of ELISA is that it can be performed easily with less infrastructure and technical expertise and is inexpensive compared to MAT. In addition, the ELISA can be automated, the result is objective, and especially, once a diagnostic cut-off has been decided upon, therefore having less interobserver/intraobserver variation. Since many other diseases have similar clinical manifestations, the ELISA should be validated. The usage of well-characterized archived specimens, consisting of those with disease and other clinically similar diseases, allows rapid assessment of the proof of concept and validation of the new assay.[2]

IgM ELISA is the most widely used method for the diagnosis of leptospirosis. Hence, the ELISA should be validated and the cut-off should be determined in the different regions based on the local prevalence of the disease in that particular region.

The objective of the study was to revalidate the diagnostic Cut-off of ELISA used for confirming a clinical diagnosis of leptospirosis.

MATERIALS AND METHODS

This study was done in Christian Medical College, Vellore, a tertiary care hospital in South India for 2 years (March 2012–February 2014). After obtaining the Institutional Ethical Clearance (IRB Min No 8109 dated 5.12.2012), for validation of IgM ELISA, samples were collected to estimate the cut-off in normal population and then validation of cut-off of ELISA was determined. The sample used to estimate the cut-off in normal population was 4 ml of blood from healthy blood donors. Blood was collected in ethylenediaminetetraacetic acid tubes (BD Vacutainer, Franklin Lakes, NJ, USA) for obtaining plasma. Adults ≥18 years of age and body weight ≥45 kg who had no acute illness in the past 30 days, who were negative for HIV, Hepatitis B, Hepatitis C, malaria, and syphilis, and who without any history of tuberculosis in the past 1 year were accepted as voluntary blood donors. 4 ml of blood was collected from 20 healthy donors in serum tube with clot activator (BD Vacutainer, Franklin Lakes, NJ, USA) for obtaining serum. For validation of cut-off of ELISA determined, serum samples were collected from proven cases of scrub typhus, sepsis, malaria, enteric fever and dengue. The details of these are given in Table 1.

These patients had no other illness except the one mentioned above. 4 ml of blood was collected from the patients in serum tube with clot activator (BD Vacutainer, Franklin Lakes, NJ, USA). These sera were tested for IgM to *Leptospira* by ELISA. The different tests performed are given in Table 2.

Serum was separated by centrifugation at 3000 rpm for 10 min at 4°C. The serum was stored at −70°C in two aliquots. Plasma (from blood of healthy donors) was separated by centrifugation at 3000 rpm for 10 min at 4°C. All ELISAs were performed and the reading was taken according to the manufacturer's instructions. The serum was diluted with diluent containing absorbent for rheumatoid factor. The results were noted, and calculations were done according to the manufacturer's instructions. All data were entered in Excel spreadsheet (Microsoft, USA), and the analysis was performed using STATA version 13. The data were summarized using mean along with standard deviation for continuous variables and frequency along with percentages for categorical variable.

RESULTS

Cut-off was determined with 100 plasma samples from adult healthy blood donors who were negative for HIV, hepatitis B, hepatitis C, syphilis, and malaria [Table 3].

Serum samples from 20 healthy donors had similar optical density (OD) values as the plasma.

The *Leptospira* ELISA was validated using serum samples from 50 patients having scrub typhus with eschar, 20 sepsis, 20 enteric fever, 17 malaria, and 17 dengue samples [Table 4].

The final cut-off for IgM ELISA for *Leptospira* by PanBio is taken as 20 PanBio units, that of *Leptospira* Virion Serion is 0.7 OD, and Inbios *Leptospira* is 1 OD.

Table 1: Samples for the validation of ELISA

Disease	Number of sample
Scrub typhus patients having eschar	50
Sepsis patients (blood culture positive)	20
Enteric fever patients (blood culture positive)	20
Malaria smear positive patients	17
Dengue serology-positive patients	17

ELISA: Enzyme-linked immunosorbent assay

Table 2: ELISA tests performed

Test for leptospirosis	Kit
IgM ELISA	PanBio, (PanBio Ltd., Brisbane, Australia)
IgM ELISA	Virion Serion (Serion Immundiagnostics, Wurzburg, Germany)
IgM ELISA	InBios (InBios International Inc., Seattle, WA)

ELISA: Enzyme-linked immunosorbent assay

Table 3: ELISA cut-off value of healthy blood donors

Measurement	Leptospira PanBio IgM (PanBio units)	Leptospira Virion Serion IgM (OD)	Leptospira InBios IgM (OD)
GM	3.974	0.198	0.017
SD	3.5	0.157	0.035
3 SD	10.5	0.471	0.105
GM+3 SD	14.474	0.669	0.122
Cutoff	15	0.7	0.2

GM: Geometric mean, SD: Standard deviation, ELISA: Enzyme-linked immunosorbent assay

Table 4: Diagnostic cut-off for ELISA

Measurement	Leptospira PanBio IgM (PanBio units)	Leptospira Virion Serion IgM (OD)	Leptospira InBios IgM (OD)
GM	3.771	0.145	0.063
SD	5.141	0.129	0.313
3 SD	15.423	0.387	0.939
GM+3 SD	19.194	0.532	1.002
Final cut-off	20	0.7	1

GM: Geometric mean, SD: Standard deviation, ELISA: Enzyme-linked immunosorbent assay

DISCUSSION

It is recommended that the Cut-off values for infectious disease diagnosis using serological assay like ELISA should be arrived at by testing sera from healthy individuals, those with proven disease, and also others with clinically similar illness. The usage of well characterized archived specimens, consisting of the aforementioned groups, allows the rapid assessment of the clinical utility of the new assay.[2] It is to be noted that, if the Cut-off is too low, it will overestimate, whereas if the Cut-off is too high, it will underestimate the burden of disease in the given community or region.

The prevalence of antibodies in the plasma samples of healthy blood donors was determined in this study. The plasma and serum samples of 20 healthy donors showed similar results; therefore, it was concluded that plasma values could be extrapolated to serum. Serum samples of patients who present with an illness such as scrub typhus, sepsis, enteric fever, malaria, and dengue (clinically indistinguishable from leptospirosis) were used to determine and validate the ELISA cut-off.

Validation of a diagnostic cut-off for a serological assay is necessary[2] in countries, especially South Asian and South-East Asian nations. This is because infections are a major cause of morbidity and mortality in these regions and is supported by evidence provided by various studies, discussed here. In a study undertaken by Desakorn *et al.* from Thailand, the PanBio IgM ELISA was evaluated by testing sera from healthy blood donors. Using the manufacturer's cut-off, which is 11 PanBio units, the sensitivity and specificity of the ELISA on paired sera were 90.8% and 55.1%, respectively. When the cut-off was placed at 20 PanBio units, the sensitivity fell to 76.1%, whereas the specificity improved to 82.6%.[3] In another study done by Tanganuchitcharnchai *et al.*, using a cut-off of OD ≥0.75 for IgM antibodies by ELISA (Standard Diagnostics, Inc., Gyeonggi-do, Republic of Korea), a sensitivity of 95% and specificity of 41% were observed in normal sera. On validation of the ELISA, using serum samples of patients with fever, the cut-off was found to be at OD of 1.7, the sensitivity decreased to 70%, and specificity increased to 78%.[4] The results of these two studies prove that a cut-off for serological diagnosis of leptospirosis has to be determined before its deployment for routine identification of cases. This has been emphasized by Surujballi and Mallory who validated the Cut-off of ELISA before performing the tests.[5]

In this study, the diagnostic cut-off for the *Leptospira* PanBio IgM ELISA (PanBio Ltd, Brisbane, Australia) was determined to be ≥20 PanBio units. The other IgM ELSA for *Leptospira* such as Virion Serion (Serion Immunodiagnostics, Wurzburg, Germany) and InBios (InBios International, Seattle, WA, USA) was also validated, and an OD ≥0.7 and ≥1.0 were considered significant. The three different ELISA kits evaluated had three different cut-off values for the detection of IgM antibodies to *Leptospira*. This demonstrates that the cut-off obtained for one kit cannot be used for another kit though it detects the same parameter.

In a study done by Chandrasekaran *et al.,* it was found that sensitivity of Serion ELISA for *Leptospira* IgM antibody was 33.3%.[6] Another study showed that Serion IgM ELISA was positive in 61% of the sera which were polymerase chain reaction positive and 90% of the sera which were MAT positive. Serion IgM ELISA was negative in 82% of MAT-negative samples. Agreement with the Panbio kit is 61%. There was discrepant result seen in 19/49 cases as these were positive by Panbio IgM but negative by Serion IgM. Serological cross-reactions were seen with syphilis, Borrelia and IgM to Epstein–Barr virus and to influenza virus antibodies.[7] In a study done by Sekhar *et al.*, it was seen that the sensitivity, specificity, and efficiency of the *Leptospira*

PanBio ELISA were 54.2%, 96.9%, and 71.3%, respectively.[8] Blacksell *et al*. showed that Panbio ELISA sensitivity of 60.9% and specificity of 65.6% were compared to the *Leptospira* MAT.[9] Effler *et al*. evaluated eight different tests for leptospirosis and showed that the sensitivity of *Leptospira* IgM ELISA was 36% and Serion ELISA *Leptospira* was 48%.[10] Compared to MAT, another study showed that *Leptospira* PanBio ELISA had a 90% sensitivity and 94% specificity.[11] These findings also emphasize the need for validation of Cut-off of ELISA based on the local prevalence. A well-defined cut-off improves the diagnostic capability of a test, especially, when performed on a single acute sample.

CONCLUSION

The final cut-off for *Leptospira* IgM ELISA by PanBio is taken as 20 PanBio units, that of *Leptospira* IgM by Virion Serion is 0.7 OD, and Inbios *Leptospira* IgM is 1 OD. All ELISAs have to be properly validated, and a cut-off has to be determined for these tests depending on the local prevalence of disease. This information can be used by other centers where in-house evaluation is not possible for logistic reasons.

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REFERENCES

1. Levett PN. Leptospirosis. Clin Microbiol Rev 2001;14:296-326.
2. TDR Diagnostics Evaluation Expert Panel, Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, *et al*. Evaluation of diagnostic tests for infectious diseases: General principles. Nat Rev Microbiol 2010;8:S17-29.
3. Desakorn V, Wuthiekanun V, Thanachartwet V, Sahassananda D, Chierakul W, Apiwattanaporn A, *et al*. Accuracy of a commercial IgM ELISA for the diagnosis of human leptospirosis in Thailand. Am J Trop Med Hyg 2012;86:524-7.
4. Tanganuchitcharnchai A, Smythe L, Dohnt M, Hartskeerl R, Vongsouvath M, Davong V, *et al*. Evaluation of the standard diagnostics leptospira IgM ELISA for diagnosis of acute leptospirosis in Lao PDR. Trans R Soc Trop Med Hyg 2012;106:563-6.
5. Surujballi O, Mallory M. Competitive enzyme-linked immunosorbent assay for detection of leptospira interrogans serovar Pomona antibodies in bovine sera. Clin Diagn Lab Immunol 2001;8:40-3.
6. Chandrasekaran S, Gomathi S. A standard screening test for the early and rapid diagnosis of leptospirosis. Indian J Med Microbiol 2004;22:23-7.
7. Trombert-Paolantoni S, Thomas P, Hermet F, Clairet V, Litou N, Maury L, *et al*. Leptospirosis screening: Performance of the serion elisa classic leptospira IgM KIT. Pathol Biol (Paris) 2010;58:95-9.
8. Sekhar WY, Soo EH, Gopalakrishnan V, Devi S. Leptospirosis in kuala lumpur and the comparative evaluation of two rapid commercial diagnostic kits against the MAT test for the detection of antibodies to *Leptospira interrogans*. Singapore Med J 2000;41:370-5.
9. Blacksell SD, Smythe L, Phetsouvanh R, Dohnt M, Hartskeerl R, Symonds M, *et al*. Limited diagnostic capacities of two commercial assays for the detection of leptospira immunoglobulin M antibodies in Laos. Clin Vaccine Immunol 2006;13:1166-9.
10. Effler PV, Bogard AK, Domen HY, Katz AR, Higa HY, Sasaki DM, *et al*. Evaluation of eight rapid screening tests for acute leptospirosis in Hawaii. J Clin Microbiol 2002;40:1464-9.
11. Zochowski WJ, Palmer MF, Coleman TJ. An evaluation of three commercial kits for use as screening methods for the detection of leptospiral antibodies in the UK. J Clin Pathol 2001;54:25-30.

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