

# Comparison of Two ELISA Methods for the Laboratory Diagnosis of Acute Leptospirosis

Hamid Reza Honarmand<sup>1</sup>, Gholamreza Abdollahpour<sup>2</sup>, Seyyed Saeed Eshraghi<sup>3</sup>

## Abstract

**Background:** Leptospirosis is the most common zoonosis widespread in tropical and temperate countries with low social-economic status. We aimed to compare an ELISA kit with an in-house ELISA assay to test the serum samples of the patients who were suspicious of leptospirosis according to their clinical symptoms.

**Methods:** A total of 282 serum samples of patients suspicious of leptospirosis admitted to hospitals in Rasht city (north of Iran) were examined for sero-diagnosis of leptospirosis. Blood samples were obtained with mean time of 6.36 days after the onset of the symptoms. Antibodies were detected using a commercial qualitative and by an in-house semi quantitative IgM and IgG ELISA and the results were compared with microscopic agglutination test (MAT) as the gold standard. All specimens with titers  $\geq 320$  against a pathogenic serovar in MAT were considered positive for leptospirosis.

**Results:** The results of MAT have demonstrated that 70 serum samples (24.8%) had a positive reaction with one of the leptospira serovar. The sensitivity, specificity, positive and negative predictive values were 87.1% , 91.0 % , 67.8%, and 95.5% for in-house ELISA assay, respectively, and 100%, 42.9%, 36.6%, and 100 % for commercial IgM ELISA assay, respectively.

**Conclusion:** Our results showed that IgM ELISA assay is a reliable and sensitive method for the laboratory diagnosis of acute leptospirosis. In-house semi quantitative IgM ELISA was more specific and commercial IgM ELISA was more sensitive.

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**Keywords** • Leptospirosis • zoonosis • ELISA

## Introduction

Leptospirosis is a common zoonosis in most tropical and temperate countries.<sup>1-3</sup> In temperate climates, the risk of acquiring the disease is strongly associated with occupational or recreational exposures, whereas in tropical countries and subtropical regions the risk of infections is more widespread and occurs through indirect contact with the urine of infected host animals.<sup>4,5</sup> In Iran, human leptospirosis is prevalent and endemic in Guilan province, a flat area located in the north of Iran, and south of the Caspian Sea, with a humid temperate climate, where the rice farming is the main agricultural activity in villages and cattle husbandry is also common.<sup>6,7</sup> The

<sup>1</sup>Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Guilan, Iran.

<sup>2</sup>Leptospira Research Laboratory, Faculty of Veterinary Medicine, University of Tehran,

<sup>3</sup>Department of Pathobiology, Faculty of Health, Tehran University of Medical Sciences, Tehran, Iran.

### Correspondence:

Seyyed Saeed Eshraghi,  
Department of Pathobiology, Faculty of Health, Tehran University of Medical Sciences, P.O. BOX 6446  
Tehran 14155, Iran.

**Tel:** + 98 21 88994823

**Fax:** + 98 21 88954913

**Email:** eshraghs@tums.ac.ir

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area harbors a variety of feral animals (especially boars and jackals) including an abundance of rodents.<sup>8</sup> Most farmers have domestic animals (cattle, horses, and dogs) in or at the premises of their houses.<sup>9</sup>

Early diagnosis of leptospirosis is important, since the mortality rate is high among patients with most severe forms of the disease.<sup>10,11</sup> However, clinical diagnosis is difficult during the early stages of the disease, when it may be confused with many other common febrile illnesses, such as dengue fever,<sup>1,12</sup> malaria,<sup>13</sup> typhoid,<sup>14</sup> and viral hepatitis.<sup>15,16</sup> Diagnosis of leptospirosis is often made by serological tests, because culture of the organism is time-consuming and expensive.<sup>17,18</sup> Performance of the reference serological test, the microscopic agglutination test (MAT), requires significant expertise, and MAT is rarely performed by routine diagnostic laboratories.<sup>19,20</sup> Several alternative serological methods for the early diagnosis of leptospirosis have been described, including the slide agglutination assay,<sup>21</sup> enzyme-linked immunosorbent assay (ELISA),<sup>22,23</sup> indirect hemagglutination assay (IHA),<sup>24,25</sup> immunofluorescence,<sup>26,27</sup> and detection of immunoglobulin M (IgM) antibodies.<sup>12,28</sup> In some studies, sensitivity of ELISA is reported from 77.8% to 100% which is mostly related to the time of blood sampling.<sup>29,30</sup>

In recent years, several rapid ELISA kits for the easy and reliable detection of anti-leptospira antibodies in the patients' blood have become commercially available and few in-house ELISA assays were introduced. The aim of the present investigation was to compare one ELISA kit with an in-house ELISA assay to test serum samples of the patients who were suspicious of leptospirosis according to their clinical symptoms.<sup>31</sup>

## Materials and Methods

In an experimental study, we evaluated the reliability of two ELISA assays in the laboratory diagnosis of acute leptospirosis. A total of 282 blood samples were obtained from 300 patients admitted to Razi and Imam Hospitals in Rasht (Guilan province, north of Iran). Eighteen blood samples were excluded from the study because of contamination. All of the patients were suspicious of leptospirosis according

to WHO criteria,<sup>32</sup> including fever, severe headache, conjunctiva suffusion, myalgia, arthralgia, icterus, general malaise, stiff neck, and history of close contact with wild or domestic animals, working in rice farms and/or contact with surface waters. Ten mL of venous blood were taken from all the patients. The serum samples were stored in  $-20^{\circ}\text{C}$ .

A commercial non-quantitative IgM and IgG ELISA (Serion ELISA classic *Leptospira* IgG/IgM, Serion GmbH, Germany) contained two separate kits for detection of IgM and IgG was used for testing the serum samples. An in-house semi quantitative ELISA (WHO/FAO/OIE Leptospirosis Reference Center of KIT Biomedical Research),<sup>31,32</sup> was used for the comparison. Plates were coated with Wijnberg strain (serovar Copenhagen, serogroup Icterohaemorrhagiae BioRad, France). IgM and IgG titers were determined by this assay.<sup>31</sup> In this test, all specimens with titer  $<1:80$  were considered negative, titers equal to  $1:80$  were regarded borderline and the serum samples with titers  $\geq 1:160$  were considered positive. For both methods, automatic ELISA washer was used for washing plates and the same peroxidase conjugate for IgM and IgG determination was used.

MAT was performed by using a microbial panel containing 25 pathogenic and three non-pathogenic strains.<sup>31</sup> All the serum samples with titers  $\geq 320$  against at least one pathogenic serovar were considered positive and other specimens, which might be obtained from the patients with *Salmonella* or viral diseases, were considered as negative.<sup>17,20</sup>

## Results

The results of the MAT demonstrated that 70 serum samples (24.8%) with titers  $\geq 320$  were positive and 212 serum samples (75.2%) with titers  $<160$  were negative. The comparative results of the in-house and commercial ELISA assays are presented in table 1. The comparative results of both IgM and IgG ELISA assays with MAT as reference test are shown in tables 2 and 3 respectively. The sensitivity, specificity, positive and negative predictive values of IgM ELISA and IgG ELISA assays are shown in table 4.

**Table 1:** Comparison of the results of in-house and commercial IgM & IgG ELISA assays

Type of ELISA	Positive No (%)	Negative No (%)	Borderline No (%)
In-house IgM ELISA	89 (31.6)	164 (58.2)	29 (10.1)
In-house IgG ELISA	110 (39.0)	145 (51.4)	27 (9.6)
Commercial IgM ELISA	191 (67.7)	75 (26.6)	16 (5.7)
Commercial IgG ELISA	107 (37.9)	123 (43.6)	52 (18.5)

**Table 2:** Comparison of the results of microscopic agglutination tests with the in-house and commercial IgM ELISA assays

Results of MAT Number of specimens	Results of ELISA assays			
	In-house IgM ELISA		Commercial IgM ELISA	
	Positive No	Negative No	Positive No	Negative No
Positive=70	61	9	70	0
Negative=212	19	193	121	91

**Table 3:** Comparison of the results of microscopic agglutination tests with the in-house and commercial IgG ELISA assays

Results of MAT Number of specimens	Results of ELISA assays			
	In-house IgG ELISA		Commercial IgG ELISA	
	Positive No	Negative No	Positive No	Negative No
Positive=70	60	10	59	11
Negative=212	38	174	49	163

**Table 4:** Comparison of the sensitivity, specificity, positive and negative predictive values of IgM and IgG ELISA assays

Type of ELISA	Sensitivity	Specificity	Positive predictive value	Negative predictive value
In-house IgM ELISA	87.0%	91.0%	76.0%	96.0%
Commercial IgM ELISA	100%	43.0%	37.0%	0.0%
In-house IgG ELISA	86.0%	82.0%	61.0%	95.0%
Commercial IgG ELISA	84.0%	77.0%	55.0%	94.0%

The results demonstrated that 70 positive samples were identified using the MAT assay which is the gold standard and reliable reference assay for detecting positive and negative samples. Serogroup of *Sejroe* with 27 cases and *Pomona* with three cases displayed the maximum and minimum positive reactions respectively. Ten serum samples reacted with two or more serogroups. The false positive and false negative samples identified by in-house IgM ELISA were 38 and 10 respectively, whereas commercial IgM ELISA identified 49 and 11 false positive and false negative samples, respectively. Approximately, 87.2% of the samples tested by in-house IgM ELISA and all of the samples tested by commercial IgM ELISA were real positive.

## Discussion

Delay in the diagnosis of leptospirosis may lead to kidney failure. Some untreated patients could develop kidney damage, meningitis, liver failure, and respiratory distress and in rare cases death occurs.<sup>2,3</sup> The diagnosis based on clinical symptoms is not reliable; therefore, laboratory support is an important tool in the diagnosis of the disease.

Because the culture of the organism is time-consuming and expensive, several rapid assays have been developed recently that can be used for screening of acutely ill patients.<sup>2,33</sup> Direct diagnosis using dark field microscopy is very difficult and does not have sensitivity and specificity; therefore, serological diagnosis is the best alternative. Detection of IgM against surface antigens is possible after day 5 of the disease onset. IgG can be detected from the

third week and will be more stable for months.<sup>34</sup> MAT is an available and the most reliable reference assay in diagnosis of acute leptospirosis. Unfortunately, laboratory running of MAT is difficult and requires a collection of standard and endemic strains. Periodic sub-culture of the microbial panel is initially necessary to have a fresh and well growth of *leptospire*s in cultures. It requires extensive work.

Among several other serological methods that were introduced for the early diagnosis of acute leptospirosis including the slide agglutination assay, IHA, immunofluorescence, and ELISA, the latter is easier, more reliable, and more common.<sup>24,31,34</sup> But the sensitivity of ELISA is mostly related to the time of blood sampling.<sup>29,30,35</sup>

The sensitivity and specificity of ELISA are dependent to many parameters, particularly the time of sampling and the coating antigen. Since leptospirosis is an acute bacterial disease, the diagnosis is based on detection of antigen-specific IgM that is detectable 6 days after the onset of infection. Thus, the sensitivity of IgM ELISA is low during the first week of infection but increases thereafter.<sup>36-38</sup>

Sensitivity and specificity of the assay could be improved by the coating of pure and specific antigens isolated from one or preferably multiple locally dominant pathogenic species, instead of the standard or non-pathogenic bacterial isolates.<sup>31</sup> The ELISA assay has been employed in many studies. Brandão and colleagues used the IgM ELISA for evaluating 108 serum samples from patients with leptospirosis and 245 seronegative samples as a control.<sup>39</sup> Positivity of the patients' samples was confirmed by a 4-fold antibody titer increase in MAT assay. Sensitivity and specificity of this

**Table 5:** Validity of ELISA in the diagnosis of leptospirosis in some similar studies

Ref. No	Study	Type of ELISA	Sensitivity	Specificity
29	Italy	Pan Bio IgM ELISA	100%	95%
35	U.S.A	Micro plate IgM ELISA	86.5%	97%
40	Thailand	Sonicated Antigen IgM ELISA	97.6%	96.5%
		Deoxicolate Extracted IgM ELISA	97.6%	94.1%
41	India	IgM ELISA day 2-3	28.1%	—
		IgM ELISA day4-5	54%	—
		IgM ELISA day6-7	77.8%	—

IgM ELISA assay was reported to be 99%. A similar study was conducted by Smiths and colleagues using 187 patients' samples confirmed by MAT and 245 seronegative control samples.<sup>42</sup> Sensitivity, specificity, positive predictive value and negative predictive value were 85.5%, 97.9%, 87.6%, and 97.4%, respectively. Vital and co-workers have also used the IgM ELISA assay on 19 MAT confirmed samples isolated from patients with leptospirosis and reported a 100% sensitivity and specificity.<sup>29</sup> In a comparative study, Ooteman and colleagues, investigated 125 samples from patients with leptospirosis using MAT, polymerase chain reaction, and IgM ELISA techniques. The IgM ELISA assay showed 96.6% sensitivity and 93.3% specificity.<sup>20</sup> Finally, Banjani and colleagues used four different techniques including IgM ELISA, IHA, IgM dipstick assay (LDS), and IgM dot-ELISA dipstick test (DST) to evaluate 128 serum samples from patients with leptospirosis and 642 samples from healthy individuals. They reported 86.5% sensitivity and 97% specificity for the IgM ELISA.<sup>35</sup>

The validity of different types of ELISA assays in diagnosis of leptospirosis in some similar studies are presented in table 5.

The main reason for attempting an early diagnosis of leptospirosis is to facilitate appropriate treatment, particularly for selection of an appropriate antibiotic treatment. The diagnosis can be guided by laboratory test results because some common infectious diseases are listed in the differential diagnosis of leptospirosis. A limitation to the use of single serum samples for sero-diagnosis (such as our study) is the persistence of the antibodies. Anti-leptospiral IgM antibodies are also persistent, but the rate of the decline shows marked variation.<sup>30,35</sup> Thus, a single IgM positive sample taken during an acute febrile illness with symptoms suggestive of leptospirosis is presumptive evidence of infection, but this finding requires confirmation by testing a convalescent sample, preferably by the use of an alternative method.<sup>30</sup>

Sensitivity of the serodiagnostic assays in acute-phase disease is very important. IgM antibodies have been detected as early as the

second day after the onset of symptoms, while IgG antibodies are detectable in the 7th day of the illness.<sup>43</sup>

In the present study, a commercial non-quantitative and an in-house semi quantitative IgM ELISA assay for diagnosis of acute leptospirosis were compared. The sensitivity, specificity, positive and negative predictive values of both assays were determined using MAT as reference test. The sensitivity of commercial non-quantitative IgM ELISA was somewhat more than sensitivity of in-house semi quantitative IgM ELISA but its specificity, positive and negative predictive values were significantly lower than in-house semi quantitative IgM ELISA. In our study, in-house semi quantitative IgM ELISA was somewhat less sensitive but more specific than commercial non-quantitative IgM ELISA. We believe that the higher sensitivity of the commercial ELISA may be caused by a low level cut-off that leads to decreased specificity. The results of IgG ELISA were highly compatible with the results of IgM ELISA. The diagnostic values of IgM ELISA in other similar studies are compatible with our study. Therefore, the sensitivity of IgM ELISA is related to the time of blood sampling.

## Conclusion

IgM ELISA assay is a reliable and sensitive method for diagnosis of acute leptospirosis and can be used for early diagnosis of the disease. However, a positive result requires confirmation by further testing of the patients' serum sample. Our results also showed that in-house semi quantitative IgM ELISA was more specific and commercial non-quantitative IgM ELISA was more sensitive.

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**Conflict of Interest:** None declared

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