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Comparison between latex and microscopic agglutination test for detection of human leptospiral antibodies

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ABSTRACT

Leptospirosis, notably Weil's syndrome, is an often severe, acute febrile illness caused by microorganisms of the genus *Leptospira*. The microscopic agglutination test (MAT) is the basic method for the sero-diagnosis of leptospirosis, as the test has a high sensitivity and can be used for classification, but has some disadvantages. Therefore, we have explored latex agglutination test (LAT) for use as a practical and rapid for sero-diagnosis of human leptospirosis and compared the applicability of commercial tests MAT and LAT in the detection of specific antibodies against *Leptospira interrogans* in human.

Introduction

Leptospirosis is zoonotic disease caused by pathogenic *Leptospira* species.[1] Incidence is the highest in tropical regions, including the Asia, Africa and Latin America.[2] The laboratory diagnosis of human leptospirosis relies mainly on serological assays aimed at the detection of leptospiral antibodies in serum samples. Sensitivity and specificity of serological techniques used in the diagnosis of leptospirosis are different. To make a reliable diagnosis, it is necessary to use a number of techniques, together.[3] The reference test for serologic diagnosis of leptospirosis is the microscopic agglutination test (MAT), wherein suspected sera are reacted with live antigen of leptospiral and then examined under dark-field microscopy. The end point titer is determined as the highest serum dilution showing approximately 50% free, unagglutinated leptospires compared to the control. Considerable effort is required to reduce the subjective effect of observer variation. The MAT has some disadvantages and is a complex test to perform and interpret which indicate the need for an alternative test for routine diagnosis of leptospirosis. One of the problems is its use of live organisms as antigens. This requires the continuous culture and handling of the bacteria in laboratories. Other drawbacks of MAT include the continuous risk of contamination of the antigen cultures, a high degree of cross-reaction between different serogroups, and the subjective assessment of results can also make quality assurance of the MAT difficult.[4] Moreover, the repeated subculture of large numbers of serovars had some risks for laboratory technicians. Latex agglutination test (LAT) has been explored for use as a practical and rapid diagnostic technique of human leptospirosis and compared the applicability of MAT and LAT in the detection of specific antibodies against *Leptospira interrogans* in human.

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Key words: Human leptospirosis; latex agglutination test; microscopic agglutination test.

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Ethical approval

All ethical considerations were considered carefully, and all procedures performed in studies involving human participants were approved by the Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran.

Materials and Methods

Sample collection and processing

A total of 213 serum samples (62 men and 151 women) were collected from Kerman province, from December (2014) to February (2015). Sera were sep-

arated by centrifugation of blood at 3000 g for 10 min at room temperature and transferred into 1.5 mL sterile micro tube (Eppendorf) and were kept at –20°C until required. These samples were submitted to the *Leptospira* Research Laboratory of the Faculty of Veterinary Medicine at the University of Tehran, Iran.

Microscopic agglutination test

Microscopic agglutination test was performed mainly as described by Turner (1968) with some modification as follows: a 10-day-old culture of *L. interrogans* in liquid medium was used as antigen. The density of leptospires was adjusted to 2×10^8 leptospires/mL.[5] Five reference strains of *Leptospira interrogans* which were used as antigen includes: *hardjo*, *pomona*, *icterohaemorrhagiae*, *grippotyphosa*, and *canicola*. All serum samples were serially diluted, starting from 1 in 50 dilution, using 2-fold dilution (1 in 100, 200, 400, 800 and 1600). Then, 10 µL of serum dilution was added to 10 µL of appropriate antigen on a microscopic slide, and incubated at 30°C for 90 min. Finally, the slide was examined under dark-field microscope (Olympus BX50; Olympus Corp., Tokyo, Japan). One antigen control and two (positive and negative) standard serum controls were used each time. Titers 1:100 or greater were considered positive. The end-point titer was determined as the highest serum dilution showing agglutination of at least 50% of the leptospires.

Latex agglutination test

The latex agglutination test assay was performed mainly as described by Kelen (1960) with a commercial kit (Zist Faravard Pars, Guilan, Iran) by placing one drop of a serum sample on a white agglutination card.[6] Subsequently, the serum drops were mixed with equal volumes (10 µL) of control and test latex. Latex and serum were mixed with the disposable tip of a pipette. The card was then shaken gently for 5 min. Samples were considered negative if no agglutination was observed within 2 min, positive when agglutination became visible less than 1 min and suspected when slight agglutination visible between 1 and 2 min.

Results

Microscopic agglutination test

Number and frequency of positive and negative samples were shown in each sex among 213 sera in Table 1. Antibodies were detected at least against one serovar of *Leptospira interrogans* in 21 sera (9.86%) among 213 samples at a dilution 1:100 or greater. Positive titers against more than one serovar were detected in 4 sera of the positive samples (Table 1). Therefore, there were 25 positive reactions against different serovar of *L. interrogans*. Positive titers were recorded against serovar *pomona* (11 samples), *hardjo* (8 samples), *grippotyphosa* (3 samples), *icterohaemorrhagiae* (2 samples) and *canicola* (1 samples) (Tables 2 and 3).

Latex agglutination test

Table 4 shows number and frequency of positive, negative and suspected samples in each sex among 213 sera by LAT.

Comparison between the MAT and LAT showed that some positive sera by MAT, were negative by

Table 1. Number and frequency of positive and negative samples in men and women among 213 sera.

Sera	Positive samples	Negative samples	Total
Men	9 (4.22%)	53 (24.88%)	62 (29.10%)
Women	12 (5.63%)	139 (65.27%)	151 (70.90%)
Total	21 (9.85%)	192 (90.15%)	213 (100%)

Table 2. Frequency and number of positive sera by microscopic agglutination test at a dilution 1:100, in terms of number of serovars among 213 samples.

Number of serovars	Number of positive sera	Frequency (%)
One serovar	17	7.98
Two serovars	2	0.94
Three serovars	1	0.47
Four serovars	1	0.47
Total	21	9.86

LAT and some positive or suspected sera by LAT were negative by MAT.

The kappa statistic method was used to determine the overall agreement value between MAT and LAT. According to Table 5, number of observed agreement is 198 (92.96% of the observations) and number of agreements expected by chance is 167.9 (78.83% of the observations). Accordingly, 0.512 <κ value ± standard error (SE)=0.667±0.079 <0.823 (95% confidence interval), so, there is *Good* agreement (high correlation) between two tests. A number of serological techniques are used in the diagnosis of leptospirosis, each having its own sensitivity and specificity. According to Table 6, the test has a sensitivity of 85.71%, specificity of 93.75%, positive predictive value of 60% and a negative predictive value of 98.36%, and the κ value ±SE of agreement is 0.667±0.079.

Discussion

Leptospirosis is now identified as one of the emerging infectious diseases, exemplified by recent large outbreaks in Nicaragua,[7] Brazil, India,[8] Southeast Asia, the United States,[9] and most recently in Malaysia.[10]

In leptospirosis, antibodies usually appear within

Table 3. Number and frequency of serum samples with positive titer against each serovar, at different dilutions.

Serovar	Dilutions 1:100 Number (#)	1:200 Number (#)	1:400 Number (#)	1:800 Number (#)	1:1600 Number (#)
pomona	11 (44)	2 (8)	1 (4)	1 (4)	1 (4)
hardjo	8 (32)	1 (4)	0 (0)	0 (0)	0 (0)
grippotyphosa	3 (12)	0 (0)	0 (0)	0 (0)	0 (0)
icterohaemorrhagiae	2 (8)	0 (0)	0 (0)	0 (0)	0 (0)
canicola	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Total	25 (100)	3 (12)	1 (4)	1 (4)	1 (4)

#, frequency (%) of positive serum samples.

Table 4. Number and frequency of positive and negative samples in each sex among 213 sera.

Sera	Positive samples	Negative samples	Suspected samples	Total
Men	7 (3.28%)	50 (23.47%)	5 (2.35%)	62 (29.10%)
Women	10 (4.69%)	133 (62.44%)	8 (3.75%)	151 (70.90%)
Total	17 (7.97%)	183 (85.91%)	13 (6.10%)	213 (100%)

Table 5. Number of positive and negative serum samples of 213 samples by microscopic and latex agglutination test.

	LAT positive or suspected	LAT negative	Total
MAT positive	18	3	21
MAT negative	12	180	192
Total	30	183	213

LAT, latex agglutination test; MAT, microscopic agglutination test.

Table 6. The test sensitivity, specificity, positive predictive value and negative predictive value.

Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
85.71	93.75	60	98.36

less than one week after the onset of the clinical findings and in a significant proportion of patients, antibodies persist in detectable quantities for many months.[11] Serological testing is the most widely used means for diagnosis of leptospirosis, and the MAT is the gold standard serological test. The MAT using live bacteria is the most widely used serological test. It is the reference test against which all other serological tests. The sensitivity of the MAT can be improved by using local serovars rather than reference strains, but reference strains assist in the interpretation of results between laboratories. The specificity of the MAT is good, therefore, there is not cross-react with Leptospira to a significant extent. However, there is significant serological cross-reactivity between serovars and serogroups of *Leptospira* and an animal infected with one serovar is likely to have antibodies against the infecting serovar that cross-reacts with other serovars in the MAT.[12] The major advantage of the MAT is its high specificity and important disadvantages are the need for facilities to culture and maintain panels of live leptospires. Furthermore, the test is both technically demanding and time-consuming.[13] The results of present study show the same efficacy between the MAT and LAT and demonstrate almost equally high sensitivities of the latex agglutination assay for detecting infections (Table 5). These results show that the latex agglutination assay will be a valuable tool in the diagnosis of leptospirosis. Mentioned assay is simple and rapid to perform, and can be performed without the need for training or special or expensive equipment. This method has a reasonable sensitivity and specificity, and results are in agreement with those of the MAT, in particular for samples collected early in the disease. So, these test characteristics make the assay suitable for use in situations where facilities or resources to perform more complicated tests are not available. The method also gives a quick result, which can be important in the management of patients, especially when attention must be given to a large number of patients.

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