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Microscopic Agglutination Test for Diagnosis of Leptospirosis by Using Filter Paper-Dried Serum Samples

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ABSTRACT

Background and objectives: Microscopic agglutination test is the gold standard sero-diagnostic method for detection of leptospirosis. Moreover, it helps identify serovars and their titers in serum samples. For obtaining accurate titer results, proper sampling, collection, storage, and transportation of samples are crucial while maintaining the cold chain. Since storage for long periods and the subsequent deterioration of samples may affect the final titers, we proposed an alternative method of MAT testing using filter paper-dried serum samples. We also evaluated sensitivity and specificity of the MAT test by using filtered-dried serum samples compared with the conventional MAT test.

Methods: This experimental study was performed on human and animal serum samples that were sent to a reference leprospirosis laboratory in 2020. Overall, 142 positive samples (with 289 titers for different strains) and 15 negative samples were used for MAT test using filtered-dried serum. For this purpose, each sample was dried on a filter paper (Whatman 903, GE Healthcare) at room temperature (20-30 °C) and kept for four days. On the fifth day, the filter papers were cut into small pieces, soaked in phosphate buffer saline, vortexed, and slowly mixed on shaker for two hours to elute antibodies. The MAT tests were performed simultaneously and under the same environmental conditions.

Results: The new MAT test using dried serum samples showed 79% sensitivity and 100% specificity. The test also had positive predictive value of 92% and negative predictive value of 24% when compared with the gold standard MAT test.

Conclusion: Filter-dried serum can be used for MAT test to overcome serum storage and transportation problems.

Keywords: Agglutination Tests, Leptospirosis, Diagnosis.

INTRODUCTION

Leptospirosis is a potentially life-threatening zoonosis caused by pathogenic serovars of *Leptospira spp*., spirochete bacteria with good affinity to areas where heavy rainfall results in waterlogging of land. Human leptospirosis is common in several states of India such as the Gujarat, Maharashtra, Karnataka, Kerala, Tamil Nadu, and Andaman Nicobar islands where survival of bacteria is possible. The infection can also occur in other mammals. The clinical spectrum is broad and not specific varying from mild flu-like illness to a wide variety of clinical syndromes including hepatic failure, renal failure, jaundice, severe pulmonary hemorrhage, disseminated intravascular coagulation, and meningitis (1, 2). Thus, early diagnosis and differentiation of leptospirosis from other acute febrile illnesses is essential to reduce the risk of mortality (3-5). The diagnostic modalities include clinical assessment, serological test, isolation of *Leptospira* from clinical samples, and molecular methods. The use of isolation technique is limited due to the need for long incubation periods, specific culture medium, and proper culture conditions. Compared to the culture method, serological tests are rapid, sensitive, and specific. Molecular methods are faster to detect leptospirosis compared to other diagnostic methods, but they are costly (6). Microscopic agglutination test (MAT) for leptospirosis not only has epidemiological advantage, but also helps to determine serotype/serovar-specific antibodies. However, live strains of different *Leptospira* serovars are required as antigen for MAT. On the other hand, maintaining live strains of *Leptospira* can be done in a reference leptospirosis laboratory where facilities for preservation of the strains are available.

For MAT test, transportation with proper cold chain maintenances and storage of samples are crucial. Serum dried on filter paper is the alternate method for storage and transportation of samples. Drying serum samples do not affect test results, do not require immediate refrigeration, occupy little space, and simplify transportation; therefore, they may be used for the diagnosis of leptospirosis in locations where laboratory resources are limited. This study aimed to validate the results of MAT by using filtered dried serums.

MATERIALS AND METHODS

This experimental study was performed on human and animal serum samples that were sent to a reference leprospirosis laboratory in 2020.

Serum samples from different districts of the south Gujarat region, which are endemic for leptospirosis, are sent to the laboratory for routine serosurvey every year. The study received ethical approval from the institutional Ethical review board (ethical code: GMCS/STU/ETHICS/APPROVAL/6494/21).

Samples with low volume and leakage were excluded. All MAT-positive and 1% of MAT-negative samples were included in the study. As positive samples showed titers with more than one serovar, instead of testing only one serovar, which is predominant in the test samples, we tested all serovar titers. Overall, 142 positive samples (with 289 titers for different strains) and 15 negative samples were used for MAT using filtered-dried serum samples. For this purpose, 40 µl of each sample were dried on a filter paper (Whatman 903, GE Healthcare) at room temperature (20-30 °C). The filter papers were stored at room temperature for four days.

On the fifth day, the filter papers were cut into several pieces and placed in test tubes containing phosphate buffer saline (PBS, pH 7.4).

The tubes were vortexed for a few minutes and kept on shaker for two hours at slow speed to elute antibodies from the filter paper. Next, the samples were centrifuged for 15 minutes at 10,000 rpm (25 °C).

The resulting supernatant equivalent to 1:100 dilution of serum was separated (7). The routine MAT using serum and filtered-dried serum samples was performed simultaneously in the same environmental conditions. The MAT test using conventional serum samples was carried out according to the method described by Faine et al. 1999 (8). In brief, serum samples were serially diluted by two-fold dilution in PBS, added to wells containing antigen and incubated.

Positive results were obtained when observing at least 50% agglutination under a dark-field microscope. Results of the two MAT test methods were compared in terms of sensitivity, specificity as well as positive and negative predictive values.

RESULTS

Results of the standard MAT and new MAT method for differ
ent titers are shown in table 1. Out of 289 titers, 227 were in the acceptable range of one-fold dilution above and below the gold standard MAT results and counted as true positive. Moreover, 43 titers (15%) were false negative and 19 (7%) titers were false positive. All 14 negative samples were tested negative. This suggests overall sensitivity and specificity of 79% and 100%, respectively. Positive predictive value was 92% and negative predictive value was 24% when compared with the gold standard MAT test.

Table 1- Comparison of results of gold standard MAT and new MAT tests form different titers of positive samples

Standard MAT titer	No. of titers tested	No. of titers with acceptable tires results by filter dried serum	False negative	False positive
50	7	5	1	1
100	167	122	35	10
200	78	69	4	5
400	37	31	3	3
Total positive MAT tested	289	227 (79%)	43(15%)	19(7%)
Total negative MAT tested	14	14 (100%)	0	0

DISCUSSION

Serological tests for leptospirosis are essential for detection of infection, serovars, and their titers, which requires proper collection, transportation, and storage. In the present study, the MAT test using filtered-dried serum samples had 79% sensitivity and 100% specificity. We dried serum samples collected in 2020 since older samples may have been deteriorated. The drying procedure at room temperature lasted 2-4 days. To elute antibodies, the filter papers were cut into two small pieces, soaked into PBS, vortexed, and slowly mixed on a shaker for two hours. All steps were performed at room temperature.

In the present study, we stored the filter papers at room temperature for 2-4 days as this would be the most practical method for the collection of samples from remote areas. Our findings indicated a sensitivity of 79% and specificity of 100% for the new MAT method compared with the conventional MAT method. In a study by Blanco and Romero, the filter papers were stored for 1, 7, 30, and 365 days at 4 °C and room temperature. They reported 100% specificity and 100% sensitivity for the MAT test using filter-dried samples compared to the conventional MAT method after a storage period of 1 and 7 days at room temperature and at 4 °C (7).

Desvars et al. used filter-dried whole blood samples from 22 seropositive and 30 seronegative patients. In their study, of 22 seropositive patients, 11 were seropositive at titer 1/200 and 11 were seropositive at titer 1/6400 using the conventional MAT technique (9).

Filter-dried sera have been used in several sero-diagnostic studies for detection of *Trypanosoma cruzi* (10), Newcastle disease virus (11), dengue virus (12), *Echinocossusgranulosus* (13), *Rickettsia* (14), measles (15), and HIV-1 (16), which showed that antibodies remain stable in filter-dried sera during storage or transportation.

CONCLUSION

Our findings indicate that performing the MAT test using filter paper-dried serum samples offers convenience in the collection, storage, and shipping of samples. This method can be useful for samples from remote areas with poor facilities and inappropriate transportation and storage conditions. The MAT test results using filtered-dried sera had a sensitivity of 76% and specificity of 100% compared with the standard MAT method. It is recommended to determine sensitivity and specificity of the method by using other elution methods such as soaking in Sorensen buffer instead of PBS or soaking in PBS for longer periods and at temperature of 4 °C.

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CONFLICTS OF INTEREST

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.The authors declare that there is no conflict of interest.

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