RESEARCH NOTE

# COMPARISON OF A SLIDE AGGLUTINATION TEST, LEPTOTEK DRI-DOT, AND IgM-ELISA WITH MICROSCOPIC AGGLUTINATION TEST FOR *LEPTOSPIRA* ANTIBODY DETECTION

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Abstract. A slide agglutination test (SAT), LeptoTek Dri-Dot and IgM-ELISA were compared with a microscopic agglutination test (MAT) for the detection of *Leptospira* antibodies. Paired sera from 10 patients whose leptospirosis was clinically suspected and diagnosed by MAT, were evaluated in this study. Our data, especially from acute samples, demonstrate the SAT and Dri-Dot were more sensitive as initial screening tests than MAT. IgM-ELISA has an advantage over MAT, SAT, and Dri-Dot since the results can be interpreted from a single serum testing if the results of the test are positive. Eight of the ten cases could be diagnosed by IgM-ELISA. Our data suggest that IgM-ELISA may be used for the diagnosis of leptospirosis. However, the agglutination test is useful for screening and for secondary infection cases for which IgM antibodies may be undetectable. MAT can be performed as a reference test and when information regarding the causative serovar is required.

## INTRODUCTION

Leptospirosis is a zoonosis in which the symptoms vary from subclinical infection to severe or fatal illness. Conventional laboratory diagnosis is based on serological assays since the successful *Leptospira* culture rate is very low. The microscopic agglutination test (MAT) is the reference serological assay. However, MAT is a laborious and time consuming technique (Ahmad *et al*, 2005; McBride *et al*, 2005). A highly specific, rapid, easy to perform test is needed since leptospirosis occurs in areas when MAT is often not available. Various serological diagnostic assays, sush as macroscopic agglutination, indirect hemagglutination, ELISA, dot-ELISA and dipstick assays, have been developed (Brandão *et al*, 1998; Tirawatnapong *et al*, 1999; Chirathaworn *et al*, 2001; Vijayachari *et al*, 2002; Bajani *et al*, 2003; Vitale *et al*, 2004 ; Boonyod *et al*, 2005; Ooteman *et al*, 2006). ELISA is a technique known for its sensitivity and specificity. The subclasses of immunoglobulin can be identified by this technique. Detection of IgM antibody specific to an organism suggests acute infection. Assays that cannot identify subclasses of immunoglobulin, such as the MAT and slide agglutination test (SAT), require paired sera to demonstrate infection is caused by the suspected organism. However, assays based on the agglutination technique have been widely used since they

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are easy to perform (Brandão *et al*, 1998; Tirawatnapong *et al*, 1999; Vijayachari *et al*, 2002; Bajani *et al*, 2003).

In this study, we compared MAT, ELISA-IgM, SAT and Dri-Dot, for detecting antibodies to *Leptospira* in pairs of sera from 10 patients in whom leptospirosis was suspected.

## MATERIALS AND METHODS

### Serum samples

Paired sera from 10 patients in whom leptospirosis was clinically suspected, were evaluated. The selected paired sera had MAT titers ≥400 or a four-fold rise between acute and convalescent samples. Each convalescent titer sample was collected 3-16 days after the acute sample.

### Microscopic agglutination test (MAT)

The MAT was performed at the National Institute of Health and Department of Livestock Development, Thailand. The assay was done by incubating a diluted serum sample with live *Leptospira* and agglutination was observed under a dark-field microscope. Sera with a titer ≥ 1:100 were considered positive on MAT.

### IgM-ELISA

IgM antibody detection was performed using the *Leptospira* IgM-ELISA kit from Panbio (Maryland, USA) according to the manufacturer's instructions. Briefly, serum diluted to 1:100 was added into a leptospiral antigen coated microtiter well followed by anti-human IgM conjugated to horseradish peroxidase and then TMB substrate. Positive and negative controls were performed in the same manner as samples. Results were interpreted by comparing absorbance of samples with a cut-off value.

### Slide agglutination test (SAT)

*Leptospira* antigen purchased from Biorad (Marnes-la-Coquette, France) was mixed with two-fold diluted serum. The antibody titer was the highest dilution of serum in which agglutination was observed. An antibody titer ≥ 1:8 was considered positive on the SAT (Tirawatnapong *et al*, 1999).

### LeptoTek Dri-Dot

LeptoTek Dri-Dot (bioMérieux, Netherland), a card agglutination test kit, was kindly provided by Dr George Watt. Undiluted serum (10 μl) was spotted and mixed with dried antigen on the card. The presence of antibodies in the serum was demonstrated by agglutination of antigen coated latex particles.

## RESULTS

The results of *Leptospira* antibody detection are shown in Table 1. Ten pairs of sera from 10 patients designated patients A through J are shown in Table 1.

### SAT and LeptoTek Dri-Dot assays showed good overall agreement

All sera positive by SAT (titer ≥ 1:8) were also positive by Dri-Dot. In three cases, patients, D, I and J, whose first samples were MAT negative (but were IgM-ELISA positive), both the SAT and Dri-Dot gave positive results. In patient F, whose MAT titer on the first serum was 1:100, antibody was detected on the SAT. However, the SAT titer was lower than the cut-off titer. The Dri-Dot and IgM-ELISA gave negative results for this sample. All tests gave positive results when the second sample of this patient was tested. Both the SAT and MAT demonstrated 4-fold rising antibody titers.

### Detection of IgM antibodies by ELISA as an indicator for acute infection

Samples from all cases included in this study could be laboratory confirmed by IgM-ELISA. Although the first set of samples in two patients, F and G, were negative, the presence of antibodies was demonstrated when the second set of samples were examined. In addition, the first set of samples in three patients, D, I and J, were MAT negative but IgM-ELISA positive.

Table 1
Leptospira antibody detection by SAT, LeptoTek Dri-Dot, IgM-ELISA and MAT.

| Patient #[a] | SAT titer | Dri-Dot | IgM-ELISA | MAT titer |
|---|---|---|---|---|
| A1 | 512 | Positive | Positive | 3,200 |
| A2 | 512 | Positive | Positive | 3,200 |
| B1 | 128 | Positive | Positive | 400 |
| B2 | 64 | Positive | Positive | 400 |
| C1 | 32 | Positive | Positive | 400 |
| C2 | 256 | Positive | Positive | 6,400 |
| D1 | 16 | Positive | Positive | <100 |
| D2 | 512 | Positive | Positive | 3,200 |
| E1 | 512 | Positive | Positive | 6,400 |
| E2 | 256 | Positive | Positive | >12,800 |
| F1 | 4 | Negative | Negative | 100 |
| F2 | 32 | Positive | Positive | 800 |
| G1 | 4 | Negative | Negative | <100 |
| G2 | 256 | Positive | Positive | 6,400 |
| H1 | 8 | Positive | Positive | 100 |
| H2 | 128 | Positive | Positive | 1,600 |
| I1 | 32 | Positive | Positive | <100 |
| I2 | 16 | Positive | Positive | 200 |
| J1 | 64 | Positive | Positive | <100 |
| J2 | 256 | Positive | Positive | 800 |

[a]1 indicates the first sample; 2 indicates the second sample

## DISCUSSION

In this study, two rapid assays and an IgM-ELISA were compared with a reference method, MAT. Although, the number of samples tested in this study was not high enough to determine statistical difference, the rusults of antibody detection could still demonstrate the advantage of each assay.

In other studies inwhich leptospirosis was diagnosed on the basis of a fourfold or greater antibody rising, sensitivity and specificity of IgM-ELISA, compared with MAT, were between 90-100% and 92-96%, respectively (Brandão *et al*, 1998; Levett and Branch, 2002). The slide agglutination test (SAT) is an inexpensive assay which can be performed quickly and easily. Arimitsu *et al* (1994) compared IgM-ELISA and SAT with MAT. They demonstrated that the overall results for antibody detection by those three assays were similar. However, the SAT and ELISA were statistically more sensitive as initial screening test. SAT detected 27 (44%) of 62 MAT-negative patients with the first serum sample. ELISA and SAT had very similar results. Lepto Dri Dot is a card agglutination test developed by the Royal Tropical Institute, Netherlands. Sensitivity and specificity for *Leptospira* antibody detection was compared with blood culture or microscopic agglutination tests on paired serum samples. Lepto Dri Dot had a sensitivity of 67.6% and a specificity of 66.0% by 1 week of infection. The values increased to 85.5% and 80%, respectively during weeks 2-4 (Vijayachari *et al,* 2002). This test does not require sophisticated equipment and can be performed easily and quickly.

Our data demonstrated that both the SAT and Dri-Dot were more sensitive than the MAT for early antibody detection in the course of

the disease. This suggests that both assays are suitable for leptospirosis screening. The results of the SAT and Dri-Dot tests were not different. Dri-Dot is more expensive than SAT, however, there is no need to do serum dilution when Dri-Dot is performed.

The IgM-ELISA used in this study could detect antibodies in all cases, however, two were negative when the first samples were tested. This confirms that when the result of the IgM-ELISA for the first sample is negative but the clinical findings are suggestive or the agglutination test is positive, the second sample for IgM-ELISA testing should be examined. Although, in patient F the IgM-ELISA was negative, the MAT titer was 1:100; this may be from a previous infection or early infection with leptospirosis. Testing of the second sample was still needed.

In conclusion, Although MAT is a reference technique for *Leptospira* antibody detection, in most laboratories where the MAT is not available, the slide agglutination test or Dri-Dot can be performed to provide physicians with preliminary and rapid results. it is very easy to perform the slide agglutination test or the Dri-Dot and they usually take less than 5 minutes to perform. IgM-ELISA gives more specific results than the SAT or Dri-Dot. The IgM-ELISA is useful when obtaining a second sample is not practical since a positive result on the first sample is suggestive of acute infection. Finally, samples positive on SAT, Dri-Dot or IgM-ELISA may be further subjected to MAT for confirmation and for information regarding serovars of *Leptospira*.

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