

## Macroscopic slide cell agglutination test for rapid diagnosis of Leptospirosis

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Tirawatnpong S, Chirathaworn C. Macroscopic slide cell agglutination test for rapid diagnosis of Leptospirosis. Chula Med J 1999 Mar; 43(3): 141-6

- Objective** : *To evaluate whether the macroscopic slide cell agglutination test (MCA) is suitable as a screening test for leptospirosis.*
- Setting** : *Immunology unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University*
- Design** : *Comparative study of diagnostic tests*
- Subjects** : *Serum samples from patients suspected of leptospirosis, healthy donors and patients with other infectious diseases*
- Methods** : *Macroscopic slide cell agglutination test (MCA) and the microscopic agglutination test (MAT)*
- Results** : *MCA is as sensitive as MAT and the specificity of the test is 84.2%*
- Conclusion** : *MCA should therefore be used as a screening test for leptospirosis as it is a simple, rapid and more cost effective which provides a comparative results to MAT*
- Key words** : *Leptospirosis, Macroscopic slide cell agglutination, MCA, MAT.*

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Received for publication. November 15, 1998.

สุรนนท์ ตีระวัฒนพงษ์, จินตนา จิรถาวร. การใช้ *macroscopic slide cell agglutination test (MCA)* เพื่อช่วยวินิจฉัยโรคติดเชื้อ *Leptospirosis* ได้อย่างรวดเร็ว. *จุฬาลงกรณ์เวชสาร* 2542 มี.ค; 43(3): 141-6

- วัตถุประสงค์** : ประเมินวิธี *macroscopic slide cell agglutination test (MCA)* เพื่อใช้ในการช่วยวินิจฉัยโรคติดเชื้อ *Leptospirosis*
- สถานที่ทำการศึกษา** : หน่วยวิทยานิพนธ์ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
- รูปแบบการศึกษา** : เปรียบเทียบการตรวจวินิจฉัยโรคด้วยวิธีทางห้องปฏิบัติการ
- กลุ่มตัวอย่าง** : น้ำเหลืองจากผู้ป่วยที่สงสัยมีการติดเชื้อ *Leptospira*, และน้ำเหลืองกลุ่มควบคุม ซึ่งแบ่งเป็นกลุ่มคนปกติ และกลุ่มผู้ป่วยโรคติดเชื้ออื่นๆ
- วิธีการ** : วิธี *macroscopic slide cell agglutination test (MCA)* และ *microscopic agglutination test (MAT)*
- ผลการศึกษา** : วิธี *MCA* มีความไวเท่ากับวิธี *MAT* และมีความจำเพาะ 84.2%
- สรุป** : วิธี *MCA* ควรนำมาใช้เป็น *screening test* สำหรับช่วยวินิจฉัยโรคติดเชื้อ *Leptospirosis* ได้ เนื่องจากเป็นวิธีที่ทำงาน ได้ผลรวดเร็ว ภายใน 4 นาที และมีราคาถูกกว่าวิธี *MAT*

Leptospirosis, a disease with a worldwide distribution, is infectious and caused by bacteria of the genus *Leptospira*. The causative organism can induce mild to severe clinical manifestations which include fever, myalgia, muscle tenderness, headache, hepatomegaly, jaundice, and renal failure. The disease can be misdiagnosed because it may be confused with other common febrile diseases such as dengue fever, malaria, typhoid and viral hepatitis. Proper diagnosis relies on the isolation of causative organisms. However, it can take up to 2 months to isolate bacteria in this genus because the bacteria grow slowly and isolation is not always successful. The laboratory diagnosis is mainly based on serological methods. The microscopic agglutination test (MAT) is a reference method for the detection of antibodies to leptospira. However, few laboratories perform MAT because the maintenance of living organisms is required for this assay, and this creates a risk of laboratory personnel being infected by the organism. Also, the test is time consuming and the interpretation of antibody titers requires microscopic examination and expertise.<sup>(1)</sup>

Several groups have developed other immunological assays including an enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination (IHA), macroscopic slide cell agglutination (MCA) and indirect immunofluorescence (IF) to use as an alternative to MAT.<sup>(2-7)</sup> The laboratory diagnosis of leptospirosis in Chulalongkorn hospital still relies on MAT. The problem is that it takes about a week to get the result. We are interested in setting up an assay that is sensitive, simple and rapid to use as a screening test before confirmation by MAT. Here we demonstrate that the macroscopic slide cell agglutination test (MCA) is as sensitive as MAT (100%), and showed

84.2% specificity when compared with MAT. This assay showed some cross reactivity with sera from patients positive for anti-treponemal antibody (syphilis), Widal test (salmonella infection) and anti-*Mycoplasma pneumoniae* antibody (*M. pneumoniae* infection). And additional, MCA showed no reactivity with sera from patients who had positive for anti-streptolysin O (streptococcal infection), Weil-Felix test (*Rickettsia* disease) and normal healthy individuals. It is easy to perform the MCA test and the results can be seen within 4 minutes. We hope to use MCA as a screening assay for detecting antibodies to leptospira in the near future.

## Materials and Methods

### Sera:

Sixty-one sera collected from the outpatient unit of Chulalongkorn Hospital were from patients that leptospirosis was suspected. Those sera were assayed for antibodies specific to leptospira by both MAT and MCA. In addition, 37 sera from healthy blood donors and 50 from patients with other infections [Syphilis (10 sera), Salmonella infection (10 sera), Streptococcal infection (10 sera), *Mycoplasma pneumoniae* infection (10 sera) and *Rickettsia* (10 sera)] were tested by MCA in order to determine normal antibody titer and cross-reactivity of this assay. All sera were stored at -20°C until they were assayed.

### Macroscopic slide cell agglutination test (MCA)

The kit for the macroscopic slide cell agglutination test was purchased from Diagnostics Pasteur (Sanofi, France). The antigen used in this kit was heat-stable leptospiral antigen extracted from *Leptospira biflexa* serovar Patoc I which is a non-

pathogenic strain. The assay was performed according to the manufacturer's instructions. Briefly, 10 µl of a 1:2 dilution of each serum sample was mixed with 10 µl leptospira antigen on a clean slide. The slide was rotated for 4 minutes at room temperature before immediately reading the agglutination. Further dilutions were performed in the serum sample that gave the positive result to determine the antibody titer.

### Microscopic agglutination test (MAT)

The assay was performed at the Leptospirosis Center, Faculty of Tropical Medicine, Mahidol University. The serial serum dilutions starting at 1:100 were incubated with live *Leptospira interrogans* different serovars for 2 hours at 28-30°C and examined for agglutination by a dark-field microscope. The antibody titer was the highest dilution giving 50% agglutination. The significant titer was 1:100.

### Results

Sensitivity and specificity of the MCA compared to the MAT are shown in Table 1. The 4 of 61 sera from patients that leptospirosis was suspected were positive by both MCA and MAT. There was no sera that was positive only by MAT. The data suggests

that MCA is as sensitive as MAT (100%). All 48 out of 57 sera negative by MCA were also negative by MAT, which suggests that the specificity of the MCA test is 84.2% compared with the MAT test. Nine sera positive only by MCA test had antibody titers range from 1:2 to 1:32 (1:2 2 samples, 1:4 4 samples, 1:8 2 samples and 1:32 1 sample). The correlation between MCA and MAT was 85% (52 of 61).

By using different serovars of lived *Leptospira interrogans* as antigens incubated with patients' sera dilution, in MAT test, Table 2 shows the reciprocal antibody titers by MAT test compared with reciprocal antibody titers by MCA test. This data shows that all of 4 sera positive by MAT test had antibody titers in MCA test much higher than 1:8 (range from 1:64 to >1:512).

The positive reactivity in control individuals and patients with other infections determined by MCA test are shown in Table 3. Only 1 of 10 sera from patients with syphilis gave reaction of titer 1:2, 5 of 10 patients with Salmonella infection showed reactions from titer of 1:2 to 1:8, and 2 of 10 patients with *Mycoplasma pneumoniae* infection were also gave reaction of titer 1:4. All sera from 37 healthy donors, 10 patients with Streptococcal infection and 10 patients with Rickettsia disease were negative by this test.

**Table 1.** Sensitivity and specificity of the MCA test compared to the MAT test.

MCA test	MAT test	
	positive (n = 4)	negative (n = 57)
positive (n = 13)	4	9
negative (n = 48)	0	48

total = 61 sera

MCA test : sensitivity [ 4 / 4 ] x 100 = 100 %

specificity [ 48 / 57 ] x 100 = 84.2 %

**Table 2.** Reciprocal antibody titers by MAT compared to MCA of 4 positive sera.

Serum No.	Reciprocal antibody titers	
	MAT test ( <i>L.interrogans</i> serovars)	MCA test
1	1:100 ( <i>L. wolffi</i> )	>1:512
2	1:300 ( <i>L. bratislava</i> )	1:64
3	1:1000 ( <i>L. bratislava</i> )	1:128
4	1:100 ( <i>L. bratislava</i> )	1:256

**Table 3.** Reactivity of MCA test with sera from healthy donors and patients with other diseases.

Diseases (n)	No. positive (%)	Reciprocal titers (n)
Healthy donors (37)	0 (0 %)	-
Syphilis (10)	1 (10 %)	2 (1)
Salmonella infection (10)	5 (50 %)	2 (2), 4 (2), 8 (1)
Streptococcal infection (10)	0 (0 %)	-
<i>Mycoplasma pneumoniae</i> infection (10)	2 (20 %)	4 (2)
Rickettsia infection (10)	0 (0 %)	-

### Discussion

The macroscopic slide cell agglutination test (MCA) is not only easy to perform but also can be done in facilities that do not have laboratory equipment. Early diagnosis of leptospirosis is important since the infection can be deadly in some cases. The MCA test obtains results within 4 minutes and can be done at bedside. We have shown preliminary data demonstrating that this assay is as sensitive as MAT and may be used as a screening test for leptospirosis. The cut-off titer for MCA selected based on this study is 1:8. Sera that are positive by MCA will be confirmed by MAT which is still a reference method and can indicate which serovar the antibody is specific to. The MCA test reveals cross-reactivity with sera from patients

with other febrile illness. However, a study showed that MCA detected antibody in 22% of sera from patients which were negative by MAT.<sup>(7)</sup> It is possible that MCA can detect antibodies earlier than MAT. In addition to the MCA test, we are attempting to establish an ELISA assay to use as another laboratory diagnosis for leptospirosis. ELISA can indicate whether the antibodies detected are leptospira-specific IgM which eliminates the need of testing paired sera if IgM antibodies are detected in the first serum sample.

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