



A COMPARATIVE STUDY OF A RAPID DIAGNOSTIC KIT WITH MSAT AND IgM ELISA IN THE DIAGNOSIS OF SYMPTOMATIC LEPTOSPIROSIS IN CHILDREN

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Abstract : BACKGROUND Leptospirosis is the most widespread zoonosis in the world with most prevalence in tropical, semitropical, and temperate regions and is usually endemic in humid warm areas. The disease is most common in adult men and women. The disease is also high in children in urban areas. The laboratory diagnosis of leptospirosis is fraught with several problems. Isolation of *Leptospira* is laborious, requires trained personnel, time consuming and has low sensitivity. Microscopic agglutination test (MAT) is a gold standard method but it requires trained personnel and darkfield microscope. AIM The study was undertaken to compare the efficiency of rapid diagnostic card test with MSAT (Macroscopic Slide Agglutination Test) and ELISA. Materials and Methods Blood samples were collected from 100 patients less than 15 years of age with clinical suspicion of Leptospirosis attending the outpatient department and admitted as inpatients in Government General hospital Chennai. A total of 100 serum samples were tested by rapid card test, IgM ELISA and MSAT. Results Of the 100 cases studied, positivity rate was 15 for leptospira rapid card test, 24 for MSAT (Macroscopic Slide Agglutination Test), and 22 for IgM ELISA respectively. Conclusion Early diagnosis is the first basis element of the strategy to control the disease. Though Rapid *Leptospira* card test is simple and easy to perform, it has a low sensitivity and is not a suitable rapid screening test for diagnosis of leptospirosis. The results must be further confirmed by ELISA and MAT.

Keyword :Macroscopic Slide Agglutination Test, IgM ELISA, Rapid *Leptospira* card test. <!-- /* Font Definitions */ @font-face {font-family:Calibri; panose-1:2 15 5 2 2 2 4 3 2 4; mso-font-charset:0; mso-generic-font-family:swiss; mso-font-pitch:variable; mso-font-signature:-520092929 1073786111 9 0 415 0;} @font-face {font-family:Cambria; panose-1:2 4 5 3 5 4 6 3 2 4; mso-font-charset:0; mso-generic-font-family:roman; mso-font-pitch:variable; mso-font-signature:-536870145 1073743103 0 0 415 0;} /* Style Definitions */ p.MsoNormal, li.MsoNormal, div.MsoNormal {mso-style-parent:""; margin-top:0in; margin-right:0in; margin-bottom:10.0pt; margin-left:0in; line-height:115%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:Calibri; mso-fareast-font-family:"Times New Roman"; mso-bidi-font-family:Calibri; mso-ansi-language:EN-CA; mso-fareast-language:EN-CA; mso-bidi-language:AR-SA;} h2 {mso-style-link:"Char Char3"; mso-style-next: Normal; margin - top: 12.0pt; margin-right:0in; margin-

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INTRODUCTION:

Leptospirosis is a global re-emerging zoonotic infection caused by pathogenic *Leptospira* spp. The disease is often misdiagnosed because of its vague clinical symptoms. The diagnosis is based on laboratory tests rather than clinical symptoms.

Human infection can occur either through direct contact with infected animals or, much more commonly through indirect contact with water or soil contaminated by urine of infected rodents or animals. *Leptospira* can survive for long periods in the renal tubules of infected animal without causing illness. Most human infections occur in young adult men and children as a result of occupational or environmental exposure. Infection is commonly associated with certain occupational workers such as farmer, sewage worker, veterinarian and animal handler.⁸

In its mild form, leptospirosis may present as an influenza-like illness with headache and myalgia. Severe leptospirosis, characterized by jaundice, renal dysfunction, and hemorrhagic diathesis, is referred to as Weil's syndrome.³ Clinical manifestation can be divided into two distinct clinical syndromes, such as patients presenting with mild anicteric febrile illness (90%) and severely ill with jaundice and other manifestations of Weil's disease (10%).¹⁰

The clinical diagnosis of leptospirosis is inaccurate and frequently gets confused with other similar febrile illnesses. The early diagnosis of the disease reduces the mortality and morbidity. Enormous progress has been made in laboratory diagnosis of leptospirosis in the last few decades. But the need for quick, simple, reliable and affordable diagnostic test for leptospirosis is still elusive.⁹

In leptospirosis, antibodies usually appear within five to seven days after onset of symptoms and persist in detectable levels for many months. From the patient and also physician point of view early detection of disease is desirable. The most commonly used tests are IgM ELISA and MSAT.⁴ These tests are sensitive and specific, but are expensive and laborious to perform. Hence, there is need to develop a simple, rapid test for screening of anti-leptospiral antibodies.⁵

The objective of the present study was to compare the efficiency of a Rapid diagnostic test with the commercially available IgM ELISA and Macroscopic Slide Agglutination Test (MSAT).

Study population

This cross sectional study was undertaken at the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital during September 2011 to February 2012. The study population consisted of 100 patients attending outpatient department and admitted as inpatients with complaints of fever in children below 15 years were included in the study.

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Inclusion Criteria

Patients with suspected symptomatic leptospirosis in children less than 15 years were included in this study.

Exclusion criteria

Children suffering from fever with specific etiology like Malaria, Typhoid, Dengue were excluded

MATERIALS AND METHODS

Blood samples were collected from 100 patients less than 15 years of age with clinical suspicion of Leptospirosis. Faine's criteria was used for clinical selection of suspected cases of leptospirosis (Faine's criteria for diagnosis of leptospirosis is on the basis of clinical, epidemiological and laboratory data, a score between 20 and 25 suggests a possible but unconfirmed diagnosis of leptospirosis)². Patients with fever, headache, jaundice, cough and breathlessness, sub conjunctival suffusion, signs of meningeal irritation and convulsion were included in this study. In these 100 patients other causes of prolonged fever like typhoid, dengue, hepatitis and malaria were excluded by doing Widal test, Rapid card test for dengue, Rapid card test for Hepatitis-B Surface Antigen and QBC test respectively.

Sample Collection and procedure

5 ml of blood was collected aseptically from the patients and transported immediately to the laboratory as per standard operative procedure. All the samples were subjected to Macroscopic Slide Agglutination Test for leptospirosis, IgM capture ELISA and Rapid Card Test.

MACROSCOPIC SLIDE AGGLUTINATION TEST (MSAT)

The standard serodiagnostic test for leptospirosis is MSAT which was performed as per the method of Mazzonelli et al. Different serovars of *leptospira* were grown in EMJH liquid medium for 7 days at 30C in a shaking incubator. After checking for growth and purity the leptospires were killed in formalin (0.5 ml of formaldehyde in 100ml culture). After 30minutes the killed leptospira culture was kept in boiling water bath for 30 minutes. The culture was rotated every 15 minutes. After cooling at room temperature, the cultures were centrifuged for 30 minutes at 10,000 rpm. The supernatant was used as antigen. A pooled suspension of locally prevalent serovars namely *icterohaemorrhagiae*, *autumnalis*, *australis*, *louisiana*, *grippotyphosa*, *hebdomadis*, *canicola* and *pomona* were used as antigens in MSAT⁵. The required cultures were obtained from Andaman Nicobar Island, National reference laboratory for leptospirosis and maintained in our lab in EMJH (Ellinghausen, McCullough, Johnson, Harris) liquid medium^{3,5}.

Preparation of phosphate buffer saline

Sodium Chloride	-8gm
Dipotassium hydrogen Phosphate	-1.21 gm
Potassium dihydrogen phosphate	-0.34 gm mixed
	with 1 litre of distilled water -pH7.2 ⁵

Procedure of MSAT

7µl of Phosphate Buffer Saline + 12 ml of MSAT antigen + 6µl of patient serum were rotated in aVDR L shaker at 120 rpm/min for 8 minutes. The slide was examined under dark ground microscope for agglutination.

The agglutination was graded as 1+, 2+, 3+ and 4+.

Clumps of agglutination with complete clearing of leptospiral antigen	4+,
obvious agglutination but partial clearing of antigen suspension	3+,
50% agglutination	2+,
25% agglutination	1+
No agglutination but uniform serum antingen mixture	Negative.
Significant	> 2 + ^{3,5}

IgM ELISA

It was performed using the Panbio leptospira IgM Microwell ELISA test as per the manufacturer's instructions. The validity of the kit was checked by running Positive and Negative controls as per the manufactures instructions⁴.

A negative result was defined as an absorbance of 0.0-0.3 optical density (OD) units, an equivocal result as 0.5 to ≤ 1 units and a positive result as > 1.0 OD units. Equivocal result indicates low positivity. In this study none of the samples showed equivocal result.

ACCUCARE™ LEPTOSPIRA IgG+IgM card test

Principle

The Accucare Leptospira IgG + IgM Rapid Card Test (RCT) is a qualitative test for the detection of both IgG and IgM antibodies to Leptospira organism in human serum, plasma or whole blood.

PROCEDURE Serum is dispensed with sample buffer. The Gold anti-Human IgG+IgM conjugate will bind to anti-Leptospira IgG/IgM antibodies in the specimen sample which in turn will bind with Leptospira antigen coated on the membrane in the test region as the reagent moves across the membrane. The Leptospira antigen on the membrane will bind the IgG+IgM antibody complex at the test line causing pale or dark pink line to form at the test line region of the test membrane. The appearance of pink line in the test region is considered as positive for IgG+IgM antibodies.

RESULTS

Table-1: Leptospirosis Positivity among 3 tests (n=100)

Procedure	Positive Cases	Percentage
MSAT	24	24%
ELISA	22	22%
Rapid Card Test	15	15%

Out of 100 samples tested 15 turned out to be positive by Leptospira IgG+IgM rapid test, 22 were positive by IgM ELISA method and 24 were positive by MSAT

Table-2: Sex Distribution of study population

Gender	No. of Cases n=100	ELISA Positive n=22	MSAT Positive n=24	Rapid Card test Positive n=15
Male	63	15	18	9
Female	17	7	6	6

Male children were more affected than the females¹.

Table-3: Area wise Distribution of study population

Area	No. of Cases n=100	ELISA Positive n=22	MSAT Positive n=24	Rapid Card test Positive n=15
North Chennai				
1. Washermenpet	24	5	7	2
2. Thiruvottiyur	12	3	3	2
3. Mint	18	3	2	3
Avadi	12	3	4	2
Saidapet	14	2	3	2
Triplicane	6	2	3	1
Puliyanthope	14	4	2	3

Leptospirosis was more commonly spotted among the people of North Chennai^{8,12}.

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Table-4 : Comparison of Rapid Card Test (RCT) with ELISA (n=100)

	ELISA		Total
	Positive	Negative	
Rapid Card Positive	10	5	15
Rapid Card Negative	12	73	85
	22	78	100

Sensitivity of RCT: 45.45% Specificity of RCT : 93.58%
Positive predictive value:66.66% Negative predictive value:85.88%

Pearson Chi-square : 20.517 p Value : 0.000 significant

The relative sensitivity and specificity of Leptospira IgG+IgM rapid test when compared with IgM ELISA was 45.45 and 93.58% respectively.

Table-5 : Comparison of MSAT with ELISA (n=100)

	ELISA		Total
	Positive	Negative	
MSAT Positive	17	7	24
MSAT Negative	5	71	76
	22	78	100

Sensitivity of MSAT: 77.27% Specificity of MSAT: 91.00%
Positive predictive value : 70.83% Negative predictive value : 93.42%

Pearson Chi-square : 43.885p Value : 0.000 significant

The relative sensitivity and specificity of MSAT when compared with IgM ELISA was 77.27 and 91.00% respectively.

DISCUSSION Leptospirosis is a disease of tropical countries, where often it is endemic, but may also occur as epidemics. Laboratory diagnosis is necessary to confirm the diagnosis of clinically suspected leptospirosis due to its varied symptoms. The laboratory tests are mainly based on serological methods, and the most widely used reference standard method is MAT (Microscopic Agglutination Test).⁴ Though it is a gold standard method for serodiagnosis of leptospirosis but it can be done only reference centres. It determines agglutinating antibodies in the serum of a patient by mixing it in various dilutions with live leptospirae. It is very specific, but has the following disadvantages. (i) facilities for culturing and maintaining live leptospirae are needed (ii) the method is technically demanding and time-consuming,¹² and need dark field microscope (iii) need specialised equipments.

IgM ELISA is valuable in endemic situations and during outbreaks, where a large number of patients have to be tested. It is one such test, which is popularly done for the diagnosis of acute leptospirosis. The cost of the test and requirement of specialized equipments still restricts the use of IgM ELISA even in well equipped laboratories. It is a costly procedure and require experienced lab technician and need ELISA reader and washer. It can't be done in the primary health centre and rural centre level. It needs standardisation for every test procedure.⁴

The Leptospira IgG+IgM Rapid card test potentially can be used outside the laboratory and can be done for individual samples without the need of batch testing. It can be used at Primary Health Centre, Rural Centre Level and District Hospital. It does not require any trained person, special lab and sophisticated equipment. Results available within 10-20 minutes.

Test procedure is simple and rapid easily performed in multipurpose health worker level. Though the test is simple and it detects both IgG & IgM antibodies but it does not differentiate acute or chronic infection.

Macroscopic slide agglutination test (MSAT) procedure is simple but it needs killed antigen of locally prevalent serovars required experience personnel and dark field microscopy^{3,5}. It cannot be done in the primary health centre level, rural centre. It can only be done at the reference centres. It detects both IgG & IgM antibodies and do not differentiate acute or chronic infection.

In this study more number of leptospirosis cases were reported during the rainy season especially in October & November. This may be due to the polluted environment which is an important epidemiological risk factor⁸. This correlates with Sumathi G et al study (2004-2006)¹¹. Sharma KK et al study also showed the highest incidence of leptospirosis was during the rainy season.

This study shows that male children are most commonly (54%) affected when compared to female children (Table 2). This may be attributed due to more exposure to contaminated water. This correlates with the Pappachan et al study in which 58.9% of cases were men⁷. North Chennai, a heavily populated and congested area with poor sanitary conditions showed more number of leptospirosis cases⁸ (Table 3). In this study, all the children presented with fever and the frequency of other symptoms reported among study population was as follows headache (48%), vomiting (33%), calf tenderness (29%), conjunctival suffusion (13%), abdominal pain (21%), diarrhoea (17%). In Sriharan M et al study all the patients had fever with chills and myalgia (100%)¹⁰. In De A et al study fever was present in all cases (100%), myalgia in 51.35%, followed by jaundice and conjunctival suffusion. The result of Leptospira IgG+IgM Rapid card test showed less sensitivity (45.45%) and specificity (93.58%) when compared with IgM ELISA. The positive predictive value is low 66.66% and negative predictive value is 85.88% (Table 4). The result of MSAT showed sensitivity 77.27% and specificity 91.00% when compared with IgM ELISA (Table 5). The positive predictive value is 70.83% and negative predictive value is 93.42%. This correlates with Sumathi G et al study (2004-2006)². Where sensitivity was 78.42% & Specificity was 92.73% respectively.

In this study only children suffering from acute fever suspected with leptospirosis were taken. Screening test for diagnosing using Rapid Card Test is not enough and it needs gold standard procedure for confirmation as low sensitivity and specificity.

Out of the three tests performed in this study, IgM ELISA showed good sensitivity and specificity. The study done by Senthilkumar et al also showed similar finding⁹. A study by I.M. El Jalli showed ELISA was 100% sensitive when compared to the Microscopic Agglutination Test⁴. This implies that this test could be a good alternative in laboratories where facilities and resources for preparation of multiple antigens is not possible for MSAT⁵. Rapid card test & MSAT detect both IgG & IgM antibodies and do not differentiate acute or chronic infection whereas ELISA detects only IgM antibodies which indicates acute infection⁵. So, for diagnosing acute infection in children IgM ELISA can be used as a valuable tool.

CONCLUSION:

Leptospira IgG&IgM Rapid Card Test does not require trained personnel or specialised equipment and can be performed even in the peripheral centres. Though the Rapid Card Test gives a quick result, it is not a suitable test for diagnosis of Leptospirosis as sensitivity is very low. It should be further confirmed by standard tests like ELISA and MAT¹². All these tests detect antibodies, but if a rapid test that detects antigen becomes available, the purpose of diagnosis at the peripheral level can be fulfilled.

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