

Original Research Article

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## A Leptospirosis Workup by Evaluation of Efficiency of Two Rapid Immunodiagnostic Tests in Comparison with Microscopic Agglutination Test

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### ABSTRACT

#### Keywords

Immunochromatography test, ELISA, Leptospirosis, Microscopic agglutination tests.

#### Article Info

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Serological tests play an important role in the diagnosis of leptospirosis as many clinical manifestations simulate diseases like dengue fever, hepatitis, Enteric fever and Malaria. Laborious and time consuming MAT, a gold standard test led to the introduction of Immunochromatography based tests, card tests and various ELISA kits for rapid diagnosis of acute leptospirosis by peripheral laboratories. The objective of our study was to know the efficacy of the tests like Leptocheck WB IgM(ICT), PanBio IgM ELISA(ELISA), with MAT. Hundred and eleven serum samples from 54 patients with clinical suspicion of leptospirosis and 50 serum from asymptomatic subjects was tested for IgM antibodies by the kits. Out of 54 serum collected, 42.59% were positive by ICT, 48.14% by ELISA, and 53.7% by MAT. ICT on comparison with MAT showed sensitivity of 75 % and 77.4%, Specificity of 96% and 95.6%, Positive Predictive value (PPV) 95.7% and 96%, Negative Predictive Value (NPV) 77.4% and 75.9% respectively and Kappa value 0.707. ELISA recorded a sensitivity of 89.7% and 90.3%, specificity of 100%, PPV of 100%, NPV 89.3% and 88.46 respectively and Kappa value 0.889 with MAT. As ELISA was more sensitive and specific in picking up leptospiral cases early in the infection when compared to ICTs, thus should be included with ICTs in laboratories to increase the detection of suspected cases of acute leptospirosis with history of 4-6 days of febrile illness.

### Introduction

Laboratory diagnosis of Leptospirosis is a vital part in the diagnosis of this disease as many clinical manifestations simulate other diseases like dengue haemorrhagic fever, serum Hepatitis, Enteric fever, Malaria and many others. Serological tests play a main role in differentiating these diseases (Faine, 1982; Levett, 2001).

Microscopic Agglutination Test is the standard test employed in centres for

confirming the cause of the disease (Levett, 2001; WHO, 2003). This test requires a panel of locally prevalent live leptospire which has to be maintained for routine procedures. The procedure is very laborious, time consuming and may cause cross infections in laboratories (WHO, 2003; Cumberland, 1999), following which many peripheral laboratories employ Immuno-chromatography based tests, card tests, spot tests and various ELISA kits for rapid diagnosis of acute leptospirosis (Adler

*et al.*, 1980; Terpstra *et al.*, 1980; Winslow *et al.*, (1997); Gussenhoven *et al.*, 1997; Smits *et al.*, (2001).

The objective of the study was to know the efficacy of routinely used tests like LeptocheckWB IgM, PanBio IgM ELISA, with respect to the gold standard MAT.

## Materials and Methods

Hundred and eleven serum samples were obtained from 54 patients who were clinically suspected for leptospirosis(test group).First Serum samples from 50 fifty asymptomatic subjects(controlgroup) were also screened for leptospiral antibodies by these serological tests.rapid tests were conducted at K.S.Hegde Medical Academy and Hospital Mangalore and the MAT test was done at TANUVAS, Zoonosis Research Laboratory Chennai.The serum samples were collected during the period July 2011 to October 2012.The study was done only after obtaining institutional ethical clearance and informed consent from the patients.

## Inclusion criteria

Test group: included patients' with clinical suspicion of leptospirosis following Faines' criteria that included fever, headache, nausea,vomiting, abdominal pain, calf pain, conjunctival suffusion, pedal edema and renal failure (Faine, 1982). Also includes the environmental factors like floods, animal reservoirs and state of occupation.Control group included patients with absence of fever and no complaints of acute leptospirosis.

## Exclusion criteria

Other causes of acute febrile diseases such as enteric fever, dengue and malaria.

A. Collection of serum sample: first sample

on admission (Acute sera)(54nos).(2-7 days),  
Second sample(54 nos.) -(8-14 days)

B. Serological Tests done:

1.Genus specific tests:

- a. Leptocheck -WB IgM card test(ICT).
- b. PanBio ELISA IgM(ELISA)

2.Serogroup/Serovar specific tests:

Microscopic Agglutination test(MAT)

C. Methodology:

The procedure was followed according to the manufacturer's insert in the kits for Leptocheck-WB IgM and PanBio IgM ELISA (Winslow *et al.*, and Gussenhoven, 1997). MAT procedures were followed from research articles with minor modifications. A group of 13 serogroup/serovars of *Leptospira* were included for screening the serum.

Serovars used are Australis australis (Ballico), Autumnalis autumnalis (Rachmati), Ballum ballum (S102),Canicola canicola (HondUtrechtIV), Grippotyphosa grippotyphosa (MoskvaV), Hebdomadis hebdomadis, Icterohaemorrhagia icterohaemorrhagiae (Copenhageni), Javanic javanica (Veldrat Bat. 46), Pomona pomona (Pomona), Pyrogenes pyrogenes(Salinem), hardjo Sejroe(Sejroe), Tarassovi tarassovi (Perepelitsin),Semaranga patoc (patoc 1) (World Health Organization, 2003 and Cumberland *et al.*, 1997 and Cole *et al.*, 1973).

D. Statistical analysis :Sensitivity(Sn), Specificity (Sp), Positive Negative value (PPV), Negative Predictive value(NPV), Degree of agreement(kappa value) calculated with respect to MAT using SPSS 16.

**Results and Discussion**

The efficacy of two methods were carried out in this study, of which two were commercial test kits (Leptocheck and PanBIO ELISA), and the gold standard MAT. Out of the 54 serum collected during the admission of clinically suspected cases, (42.59%) were positive by Leptocheck WB IgM, (48.14%) by PanBIO ELISA, and (53.7%) by MAT. The LeptocheckWB on comparison with MAT showed Sn of 75 %, Sp of 96%, PPV 95.7%, NPV 77.4% and Kappa value (Value of Agreement) 0.707. PanBio IgM ELISA

recorded a Sn of 89.7%, Sp 100%, PPV 100%, NPV 89.3% and Kappa value was 0.889.with MAT (Table 1).

A report of second sera collected is as follows. Leptocheck WB IgM showed positivity of 25 (46.3%), 28(51.9%) by PanBIO ELISA and 57.4% by MAT. The immunochromatography based test showed a sensitivity of 77.4% (24/31), Specificity 95.7% (22/23), PPV 96.0% (24/25), NPV 75.9 % ( 22/29) and Measure of agreement- Kappa value-0.707 with MAT(Table1).

**Table.1** Comparison of Leptocheck WB and ELISA with MAT (Admission and second sample)

Acute sample (1-7 days) median-4 days		Leptocheck WB IgM		Panbio ELISA IgM		MAT	
		Clinically suspected	Asymptomatic	Clinically suspected	Asymptomatic	Clinically suspected	Asymptomatic
	<b>Total cases</b>	54	50	54	50	54	50
	<b>Positive</b>	23	02	26	03	29	06
	<b>Negative</b>	31	48	28	47	25	44
	<b>Sensitivity</b>	75%		89.7%		64.8%	
	<b>Specificity</b>	96%		100%		88%	
	<b>PPV%*</b>	95.7%		100%		82.86%	
	<b>NPV%#</b>	77.4%		89.3%		63.79%	
	<b>Kappa value with MAT</b>	0.707		0.889			
<b>Second sample</b>	<b>Total cases</b>	54	00	54	00	54	00
<b>Days 8-13</b>	<b>Positives</b>	25	-	29	-	31	-
<b>Median -11 days</b>	<b>Negatives</b>	29	-	25	-	23	-
	<b>Sensitivity</b>	77.4%	-	90.3%	-	-	-
	<b>Specificity</b>	95.7%	-	100%	-	-	-
	<b>PPV%*</b>	96%	-	100%	-	-	-
	<b>NPV%#</b>	75.9%	-	88.46%	-	-	-
	<b>Kappa value</b>	0.707	-	0.888	-	-	-

\*PPV-positive predictive value, #NPV- Negative predictive value

**Table.2** Leptocheck WB/Other ICTs

Author and year	Admission sample				Second sample			
	Sn	Sp	PPV	NPV	Sn	Sp	PPV	NPV
Blacksell et al. 2006	47.3 %	75.5%	21.6%	91.1%	-	-	-	-
Panwala Tanvi et al 2011	98.36	86.95	86.95	98.36	87.87	88	90.82	84.61
Goris MG et al 2013	78.0	98.0	-	-	-	-	-	-
Panwala tanvi et al 2015	84.8	37.3	40	83.3	-	-	-	-
Niloofa et al 2015	84.6	73.3	60.9	90.6	80.8	76.9	70.3	85.6
Our study	75%	96%	95.7%	77.4%	77.4%	96%	76%	75.9%

Sn-Sensitivity :Sp-Specificity,PPV-Positive Predictive value ; NPV- Negative predictive value.

**Table.3** PanBio ELISA/Verion Serion

Author and year	Admission sample				Second sample			
	Sn	Sp	PPV	NPV	Sn	Sp	PPV	NPV
Zochowski <i>et al.</i> ,.(2001)	90%	94%	-	-	-	-	-	-
Levett <i>et al.</i> ,.2002	70%	-	-	-	95.5%	89.5%	-	-
Blacksell <i>et al.</i> ,.2006	60.9%	65.6%	20.0%	92.2%	65.2%	45.4%	14.4%	90.2%
Panwala Tanvi <i>et al.</i> , 2011	96.82	88.05	88.40	96.72	91.42	95.5	96.96	88
Desakorn varunee <i>et al.</i> ,2012	52.3	66.4	-	-	90.8	51.1	-	-
Niloofa <i>et al.</i> ,2015	86.0	84.5	73.3	92.5	80.2	88.5	82.6	86.9
Our study	89.7%	100%	100%	89.3%	90.3%	100	100	88.46%

PanBio IgM ELISA recorded Sensitivity 90.3%(28/31),Specificity 100%(23/23), PPV 100%(28/28),NPV 88.46%(23/26) and Kappa value was 0.888 with MAT(Table 4). Seropositivity for leptospiral antibodies was seen in 2,3 and 6 cases by Leptocheck,

PanBio ELISA and MAT respectively out of 50 asymptomatic cases.(Table 1).

Leptocheck-WB IgM and PanBio-ELISA IgM, are genus specific serological tests for diagnosis of Leptospirosis.These tests

reported IgM antibodies as early as 3-5 days of infection (Levett, 2001; WHO, 2003). Varying degree of sensitivity and specificity was shown by ICTs in previous studies (table 2). Our study recorded a sensitivity of 75% and specificity of 95% with admission sample for Leptocheck WB in comparison with the reported data (Goris *et al.*, 2013). But some of the earlier reports showed a higher sensitivity and lower specificity rate (Panwala *et al.*, 2011; Panwala Tanvi *et al.*, 2015 and Niloofa *et al.*, 2015). This would be due to geographical distribution of leptospira serovars and time of blood collection.

Many published data cited PanBio ELISA to be highly sensitivity in early stages of infection which was in agreement with our study (Panwala *et al.*, 2011; Niloofa, 2015 and Zochowski *et al.*, 2001). Certain studies reported a lower rate of sensitivity and specificity (Blacksell *et al.*, 2006 and Desakorn *et al.*, 2012). In our study the ELISA test picked up more number of cases when tested with second sample (median -11 days) which was in accordance with other studies (table 3). In our study, Pan Bio Elisa was more sensitive in picking up leptospiral cases when compared to ICTs as reported by other studies (Panwala *et al.*, 2011; Panwala Tanvi *et al.*, 2015, Niloofa *et al.*, 2015 and Zochowski *et al.*, 2001).

Both the commercial kits showed relatively similar specificities with MAT where ELISA test recorded 100%, in contrast to other studies (table 2 and 3). Both the tests showed great agreement with MAT (a kappa value of 0.707 and 0.888 for ICT and ELISA test respectively).

Microscopic agglutination test is the gold standard in the diagnosis of leptospirosis, but it requires maintenance of live strains of *Leptospira* for use as antigens. The test procedure is very laborious and also leads to

cross infections among lab personnels. Therefore it is maintained only in reference laboratories. The sensitivity of MAT was found to increase with repeat serum samples collected during the course of infections for which paired sera is mandatory. Due to these limitations of MAT, rapid tests like ICTs and ELISA tests should be used as screening tests in tertiary hospital labs that do not have facilities for maintaining live strains.

In conclusion, in our study ICT and ELISA test were equally sensitive when compared with MAT. Our study also recorded high specificity of ELISA test with MAT. Though MAT is a standard test is not feasible for small laboratories because of its limitations. Therefore combining the results of two rapid tests could increase the detection of suspected cases of acute leptospirosis with history of 4-6 days of febrile illness and thus preventing complications in future.

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