

Evaluation of Lepto Dri Dot as a rapid test for the diagnosis of leptospirosis

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SUMMARY

Lepto Dri Dot is a new card agglutination test developed by the Dutch Royal Tropical Institute for the rapid diagnosis of leptospirosis. We evaluated the test in field conditions in The Andaman Islands. Patients suspected of leptospirosis who attended three primary health centres were included in the study. The test results were compared with blood culture or microscopic agglutination tests on paired serum samples; 74 of 124 patients were diagnosed as having leptospirosis based on these criteria. Lepto Dri Dot had a sensitivity of 67·6% (50/74) and a specificity of 66·0% (33/50) during week 1. During weeks 2–4 the values increased to 85·5% (47/55) and 80% (40/50) respectively. An IgM ELISA was also performed on the serum samples for comparison and this was marginally less sensitive, but more specific, during the first week of illness. The positivity rates for the Dri Dot test during days 2–3, 4–5 and 6–7 were 53·1% (17/32), 75·0% (18/24) and 83·3% (15/18), respectively. The corresponding values for ELISA were 28·1% (9/32), 54% (13/24) and 77·8% (14/18). Both Dri Dot and ELISA showed good agreement with the standard diagnostic criteria after the first week of illness ($\kappa = 0·65$ and $0·74$, respectively). The overall concordance of the two tests was 89·5% ($\kappa = 0·79$). The test does not require special storage or sophisticated equipment and can be performed by relatively low skilled personnel.

INTRODUCTION

Leptospirosis is an emerging infectious disease of worldwide distribution. It can affect any of the organ systems and leads to a variety of clinical syndromes and manifestations. In the acute stage, the symptoms and signs are indistinguishable from other acute bacterial and viral infections. The disease, if not diagnosed and treated at an early stage when organ damage has not taken place, can cause fatal complications such as pulmonary haemorrhage, adult respiratory distress syndrome, renal failure, etc. Hence, early confirmation of the diagnosis improves the prognosis. For a definite diagnosis, isolation of the

organism from a clinical specimen is required. However, seroconversion or a fourfold or higher rise in the serum antibody titre in the microscopic agglutination test (MAT) [1, 2] is supportive of the diagnosis. Leptospire are slow growing organisms and it takes several days or weeks for cultures to yield results. MAT is laborious as it requires the maintenance of several leptospiral strains to be used as antigens and it can be performed only by skilled personnel. Although an IgM ELISA is often used as an alternative to MAT, an ELISA reader and micropipettes make this technique only suitable for reference centres or well-established laboratories.

A number of immunoassays including an indirect haemagglutination test [3], microcapsule agglutination test (MCAT) [4] and Lepto Dipstick [5] have recently become commercially available. These tests

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are easy to perform and can be carried out with minimum facilities. However, they require at least 3 h of incubation [6, 7] before results can be obtained. Lepto Dri Dot is a card agglutination test recently developed by the Dutch Royal Tropical Institute in Amsterdam [8]. The assay is based on the binding of leptospira specific antibodies in patients' serum to the broadly reactive antigen coated on latex particles leading to a fine agglutination. Results can be obtained within 60 s. We evaluated the test in a clinical setting in The Andaman Islands, where leptospirosis has been endemic since the early part of the 20th century [9].

MATERIALS AND METHODS

Patients

The study was conducted at three primary health centres in South Andaman during the period May 2000 to July 2001. All suspected cases of leptospirosis attending the outpatient department of these hospitals during the study period were included. The criteria for suspecting leptospirosis was acute onset of fever, headache and generalized body ache associated with any of the following signs and symptoms: calf muscle tenderness, haemorrhagic manifestations including sub-conjunctival haemorrhage, jaundice, cough, breathlessness and haemoptysis or oliguria.

Blood samples were collected from patients on the day of reporting to the hospital. Follow-up samples were collected after an interval of 2–4 weeks of onset of the disease.

Laboratory tests

Isolation of leptospira was attempted from all first blood samples using Ellinghausen, McCullough, Johnson and Harris (EMJH) semi-solid medium (Difco Laboratories, USA) following standard procedures [1].

The MAT was performed as recommended [1] using 10 live serovars of *Leptospira interrogans* that are commonly encountered in India as antigens. These were australis (strain Ballico), ballum (strain Mus127), canicola (strain H.Utrecht IV), grippotyphosa (strain Moskva V), hebdomadis (strain Hebdomadis), icterohaemorrhagiae (strain RGA), pomona (strain Pomona), rachmati (strain Rachmat), poi (strain Poi), and hardjo (strain Hardjopraj). MAT was performed on all serum samples in doubling dilutions from 1 in 25 to 1 in 400. Those samples giving a positive result at 1 in 400 were titrated to their end point titres.

IgM ELISA plates were prepared in house by coating the wells with broadly reactive antigen (boiled culture supernatant) prepared from strain Wijnberg (serovar copenhageni, serogroup Icterohaemorrhagiae), following the procedure of Terpstra et al. [10]. An IgM ELISA was performed on all samples in doubling dilutions from 1 in 10 to 1 in 80. This ELISA system was standardized with a negative to positive cut-off titre of 80.

The Lepto Dri Dot test kits were supplied by the Royal Tropical Institute (KIT), Amsterdam, The Netherlands. The kit contains coloured (blue) latex particles, activated with broadly reactive leptospira antigens prepared from pathogenic strain Lely 607 (serovar hardjo, serogroup Sejroe), which is dried onto an agglutination card. The test was performed according to the recommended procedure [8]; 10 μ l serum was added to the card near to the blue dot and mixed with the latex particles using the flat end of the plastic spatula included in the kit to obtain a homogenous suspension spread uniformly over the card. The card was rotated slowly and gently and results recorded as positive or negative agglutination after 30 s. The Lepto Dri Dot test was performed at the laboratories of the respective primary health centres while the MAT and IgM ELISA tests were carried out at the National Leptospira Reference Laboratory.

The standard criteria for a diagnosis of leptospirosis were isolation of the organism from blood, seroconversion from negative to a minimum titre of 100 in the MAT or a fourfold or greater rise in titre in this test.

The results of the Dri Dot Test and IgM ELISA were compared with the standard diagnostic criteria to calculate the sensitivity, specificity, predictive value positive (PVP) and predictive value negative (PVN). These indices were calculated for the test during week 1 of illness and weeks 2–4. The kappa value (κ) for agreement between test results and standard diagnostic criteria, standard error of κ , Z statistic and the corresponding P values [11] were calculated. The percentage agreement between Dri Dot and ELISA and κ value and Z statistic was also calculated. These statistical calculations were performed using the Epi-Info program of Epi-Info version 6.3 [12].

RESULTS

A diagnosis of leptospirosis was made in 74 of 124 clinically suspected patients based on the standard diagnostic criteria. The remaining 50 patients did not

Table 1. Sensitivity, specificity, positive and negative predictive values of Lepto Dri Dot and IgM ELISA at different stages of illness

Test and duration of illness	Sensitivity (%)	Specificity (%)	PVP* (%)	PVN† (%)	κ	Z value	P
Lepto Dri Dot							
≤ 7 days	67.6 (50/74) [55.6–77.7]	66.0 (33/50) [51.1–78.4]	74.6 [62.3–84.1]	57.9 [44.1–70.6%]	0.328	3.68	0.0001
> 7 days	85.5 (47/55) [72.8–93.1]	80.0 (40/50) [65.9–89.5]	82.5 [69.6–90.8]	83.3 [69.2–92.0]	0.655	6.72	0.0000
IgM ELISA							
≤ 7 days	48.6 (36/74) [37.0–60.5]	78.0 (39/50) [63.7–88.0]	76.6 [61.6–87.2]	50.6 [39.1–62.1]	0.245	3.00	0.0135
> 7 days	89.1 (49/55) [77.1–95.5]	84.0 (42/50) [70.3–92.4]	86.0 [73.7–93.3]	87.5 [74.1–94.8]	0.732	7.51	0.0000

* Predictive value positive; † Predictive value negative. Figures in parentheses are numbers used for calculating the index and figures in brackets are 95% confidence intervals.

fulfil the criteria and were considered as controls. Among the 74 confirmed patients, isolation of leptospire was successful in 34 and for the remaining 40 patients diagnosis was based on MAT results on paired sera. Of these 40 patients, 26 showed seroconversion and 14 exhibited a fourfold or greater rise in antibody titre. The indices of validity for the week 1 were based on the results of the tests done on all 124 samples. As 74 of them met the standard criteria for diagnosis, the pretest probability, which is the prevalence of disease among those screened with the test, was 59.7%. During weeks 2–4, samples from 19 culture positive patients were not available. Hence the pretest probability was 52.4% (55/105).

Table 1 shows the sensitivity, specificity and predictive values of Dri Dot and ELISA during week 1 and weeks 2–4. The sensitivity and specificity of Dri Dot was 67.6% (95% CI: 55.6–77.7%) and 66.0% (95% CI: 51.1–78.4%) respectively during week 1 of illness. The increase in the sensitivity during weeks 2–4 was statistically significant ($\chi^2 = 5.41$, $P = 0.020$). Although the specificity increased to 80% during the weeks 2–4, it was not statistically significant ($\chi^2 = 2.49$, $P = 0.1149$). PVP and PVN were 74.6% and 57.9%, respectively during week 1 and these increased subsequently to above 80%. The indices for the IgM ELISA were slightly higher except for a lower sensitivity during week 1 of illness. The agreement of both the tests with the standard criteria was low ($\kappa < 0.40$) but higher than that expected by chance during week 1. During the weeks 2–4 agreement was good to excellent ($\kappa = 0.66$ and 0.72 , respectively).

Among the 19 culture positive cases whose second serum samples were not available 12 gave positive

Table 2. Positivity of Lepto Dri Dot and IgM ELISA at different time intervals during the first week of illness

Duration of illness in days	Number of patients	Positivity rates (%)	
		Dri Dot	IgM ELISA
2–3	32	17 (53.1) [35.0–70.5]*	9 (28.1) [14.4–47.0]
4–5	24	18 (75.0) [52.9–89.9]	13 (54) [33.2–73.8]
6–7	18	15 (83.3) [57.7–95.6]	14 (77.8) [51.9–92.6]

* 95% confidence interval.

results in the Dri Dot test. Their serum samples were taken during week 1 and the positivity rate (63.1%) was not statistically different from the sensitivity recorded week 1 of illness ($\chi^2 = 0.13$, $P = 0.716$).

Table 2 shows the sensitivity of Dri Dot and ELISA at different days intervals during week 1. It was 53.1% during days 2–3 of illness; 75.0% during days 4–5 and 83.3% by the end of week 1. The agreement between the results of Dri Dot and IgM ELISA was 89.5% ($\kappa = 0.791976$, $Z = 12.17$, $P < 0.0001$).

The post-test probability of a negative test, which is the prevalence of disease among those the test identified as negative, is equal to the rate of false negative results. This was 34% during week 1 and 20% during weeks 2–4. The probability of disease among those screened and those found negative is an indicator of the usefulness of the test. This was 25.9% (59.7–34%) during week 1 and 42.4% (52.4–20.0%) during weeks 2–4.

DISCUSSION

The Dri Dot test detected antibodies in 67.6% on the samples collected within week 1 of illness and its sensitivity was higher than that of IgM ELISA at all intervals during this period. However, the specificity of the Dri Dot was lower (66.0%) than that of the IgM ELISA. Both Dri Dot and IgM ELISA showed higher sensitivity during weeks 2–4 of illness when the antibody level usually reaches its peak following infection [2]. This was expected as in the majority of the leptospiral infections antibodies usually reach detectable levels within week 1 or as early as day 3 or 4 of illness, attain peak levels during weeks 3 or 4 and then slowly decline [2, 13, 14].

The study subjects were consecutive suspected cases of leptospirosis attending a primary health centre. Hence the pretest probabilities were close to real-life situations and the predictive values give useful information about the utility of the test. The Dri Dot test recorded only 74.6% PVP during week 1, indicating that one-quarter of the positive tests during the acute stages would be false. The PVN (57.9%) was lower, and a negative test cannot be used to rule out leptospirosis during week 1 of illness. The IgM ELISA also showed similar characteristics, although the predictive values were marginally better than that of the Dri Dot. Although the utility of the test appears to be low during week 1, the low values might result from pooling the data for the whole of week 1. PVN is dependent on the sensitivity of the assay and by day 4 or 5 of illness this reached 75%, which might be acceptable. Any test that detects antibodies would not give reliable information before this, as it takes at least 3–4 days for the development of detectable levels of antibodies [13, 15].

During weeks 2–4 the PVN of both the tests increased to values above 80%. There was a large difference between pre-test probability (prevalence of disease among the study subjects) and post-test probability of negative test (52.4 *vs.* 20%) during weeks 2–4. Thus the test gives useful information for diagnosis of leptospirosis during this period. Both Dri Dot and IgM ELISA showed good agreement, which was expected as in both tests the antigens used were broadly reactive.

The patients considered as controls were those with symptoms similar to leptospirosis, but who were culture negative and unresponsive in the MAT assay. Although the MAT performed on paired samples has high sensitivity, it is possible that a few of the cases

might have been missed by both the tests and were misclassified as controls. Since it is improbable that the positivity rate of the Dri Dot among MAT negative patients is higher than that among MAT positive patients, an underestimation of sensitivity is less likely but the specificity might have been underestimated marginally. This method of selecting controls was used to retain the proportion of true patients among the study subjects as it would occur in clinical settings, where the test would be used to screen for leptospirosis.

The IgM ELISA gave more specific results than the Dri Dot as it specifically detects IgM antibodies, the presence of which is generally accepted to indicate current infection. Agglutination may result from both IgM and IgG antibodies and in areas with high endemicity, the background seroprevalence can be an interfering factor. However, as IgM molecules are more agglutinating than IgG, the false positivity rate may not be as high as the seroprevalence rate. This is evident from the present data, where the difference in specificity between IgM ELISA and Dri Dot was 12% during week 1 and 4% during weeks 2–4, although the seroprevalence in the area is about 40–50% [16]. The cut-off titre used for the IgM ELISA was 80, which has been found to be ideal for the prevalence rates existing in The Andaman Islands. With this cut-off point, the test had values of 89% for sensitivity and 84% for specificity after week 1 of illness.

The Dri Dot test is simple to perform and results can be obtained within 1 min. The test kit can be stored at room temperature with no special equipment required and it can be performed by persons with relatively few skills. It need not be done in batches as in the case of ELISA and the result on a single sample gives reasonably reliable information by about the fifth day of illness. These advantages make Dri Dot an ideal test for use even in peripheral laboratories with minimum laboratory facilities.

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