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'Leptorapide' – a one-step assay for rapid diagnosis of human leptospirosis

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SUMMARY

Leptospirosis is a globally important zoonotic infection caused by spirochaetes of the genus *Leptospira*. It is transmitted to humans by direct contact with infected animals or indirectly via contaminated water. It is mainly a problem of the resource-poor developing countries of the tropical and sub-tropical regions of the world but outbreaks due to an increase in travel and recreational activities have been reported in developed and more industrialized areas of the world. Current methods of diagnosis are costly, time-consuming and require the use of specialized laboratory equipment and personnel. The purpose of this paper is to report the validation of the 'Leptorapide®' test (Linnodee Ltd, Northern Ireland) for the diagnosis of human leptospirosis. It is a simple one-step latex agglutination assay performed using equal volumes of serum sample and antigen-bound latex beads. Evidence of leptospiral antibodies is determined within minutes. Agglutination is scored on a scale of 1–5 and the results interpreted using a score card provided with the kit. Validation has been performed with a large sample size obtained from individuals originating from various parts of the world including Brazil and India. The test has shown sensitivity and specificity values of 97·1% and 94·0%, respectively, relative to the microscopic agglutination test. The results demonstrate that Leptorapide offers a cost-effective and accurate alternative to the more historical methods of antibody detection.

Key words: Infectious disease, leptospirosis, spirochaetes, tropical diseases, zoonoses.

INTRODUCTION

Considered the most common zoonosis worldwide, leptospirosis is a bacterial infection caused by spirochaetes from the genus *Leptospira* [1–4]. The genus *Leptospira* includes both saprophytic and pathogenic species. The pathogenic family consists of 13 pathogenic species: *L. alexanderi, L. alstonii, L. borgpetersenii, L. inadai, L. interrogans, L. fainei, L. kirschneri, L. licerasiae, L. noguchi, L. santarosai, L. terpstrae, L. weilii*, and *L. wolffii*, with more than 250 serovars [5].

Leptospires persist in the kidneys and genital tracts of carrier wild and domestic animals and are excreted

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in urine and genital fluids. Survival outside the host is favoured by warm moist conditions [6]. Humans are generally considered to be incidental dead end hosts who can become infected by any parasitic leptospire, although recent information would indicate that under certain circumstances, humans can also maintain infection [7]. Transmission to humans occurs by direct contact with infected animals or indirectly, via water contaminated from urine shed by carrier animals such as rodents or domestic animals [1, 8, 9]. The most common route of entry is through abrasions in the skin or mucous membranes of the nose, eyes or throat [10].

Infected humans exhibit a wide spectrum of clinical responses to infection ranging from subclinical, acute flu-like symptoms, pyrexia of unknown origin, haemoptysis, acute renal disease, CNS involvement, to acute haemorrhagic fever [11, 12] none of which are pathognomonic for leptospirosis.

In the absence of a clinical diagnosis, there is a need for rapid supportive laboratory diagnoses especially in the face of an epidemic. Laboratory methods are based on the demonstration of the organism in the patient or the presence of an immune response. Organism detection methods are slow (culture) and expensive (culture and PCR) and require access to specialist laboratories [13, 14]. Antibody detection methods are often the only practical option but have a variety of limitations. Patients only develop a detectable serological response 10–14 days post-infection. The standard serological test – the microscopic agglutination test (MAT) – is not very cross-reactive, thus with the potential for a person to be infected by such a large number of serovars, the World Health Organization (WHO) [4] recommends the use of 19 different live antigens in the test making it impractical for all but specialist laboratories. The response to this problem has been the development of a variety of genus-specific tests – mainly IgM ELISAs, lateral flow tests and MATs. There are disadvantages inherent in some of these tests: expense (ELISA, lateral flow tests and whole cell antigen only MATs) or still the requirement for an element of laboratory access and expertise (ELISA tests). MATs, in which antigen is attached to a carrier matrix, offer the cheapest and simplest approach to diagnosis. The purpose of this paper is to describe the validation of a commercial latex agglutination test (Leptorapide®, Linnodee Ltd, Northern Ireland) for the rapid serological diagnosis of human leptospirosis.

MATERIALS AND METHODS

Sera

Fifty-five human sera samples were collected from the Leptospira Reference Unit, Hereford, UK. Twenty-six samples were confirmed positive for leptospirosis and 29 samples were confirmed negative using MAT. Fifty-two human sera samples were collected from the WHO/FAO/OIE Leptospira Reference Unit, KIT Biomedical Research Institute, The Netherlands (hereafter KIT, The Netherlands). Of these, nine were identified as positive and 43 identified as negative using MAT. Sera ($n=168$) from patients with clinical presentation suggesting leptospirosis in the southern Brazilian state of Rio Grande de Sul, was used to test Leptorapide in a population where the disease is endemic. Sera samples ($n=406$) were obtained from the endemic region of South Andaman in an independent study sponsored by the WHO and performed by the Indian Council of Medical Research (ICMR). Samples were obtained from patients who had fever along with any of the following symptoms: headache, body ache and muscle tenderness, jaundice, haemorrhagic tendency, cough with haemoptysis or breathlessness, oliguria or signs of meningeal irritation. Cases were identified during an outbreak in Middle Andaman. Ninety-six sera samples were obtained from a leptospirosis reference centre in Poland. All 96 samples were confirmed negative by MAT testing. To determine the efficacy of Leptorapide as a rapid screening test in relation to the MAT, 220 sera samples representing patients with clinical symptoms suggesting leptospirosis were tested by the Istituto Superiore di Sanita (ISS), Rome, Italy, and 472 clinically suspected patient samples were tested by the Centers for Disease Control and Prevention (CDC, USA).

IgM ELISA

The Panbio IgM ELISA (Panbio Pty Ltd, Australia) was used according to the manufacturer's instructions. A titre of $\geqslant$1:80 was considered positive for the presence of leptospiral antibody.

MAT

The MAT was performed according to standard WHO guidelines, described by Wolff [15], Palmer *et al.* [16] and Zochowski *et al.* [17]. The titre was

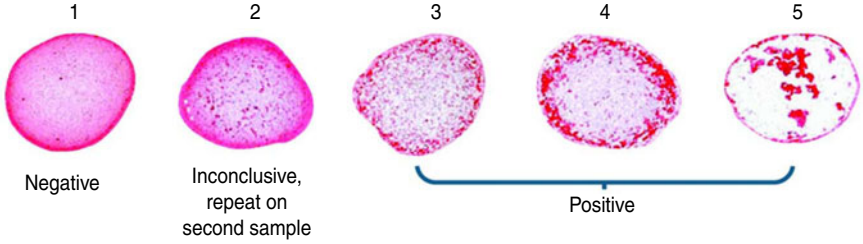

Fig. 1. [*colour online*]. Scoring of the Linnodee latex agglutination test. Test serum is mixed with an equal volume (5 μl) of antigen-bound latex beads on a 12-well glass slide. The bead/sera mix is placed on a Gyro rocker to promote drying. After 5 min the extent of agglutination was scored on a scale of 1–5 (1, seronegative; 2, inconclusive; 3–5, seropositive).

taken as the highest dilution giving 50% agglutination of leptospires.

The serovar ranges of antigens used within each MAT analysis and cut-off values are given in Supplementary Table S1 (available online).

Linnodee Leptorapide

The Leptorapide assay was performed as described in the kit instructions. Antigen-bound beads were re-suspended by gentle swirling or rolling the bottle immediately before use. Using a new pipette for each sample, 5 μl of test sera/positive control sera was mixed with 5 μl of the antigen-bound latex beads on a 12-well glass slide. The bead/serum mix was placed on a Gyro rocker (Sigma-Aldrich, USA) with gentle rotation to promote drying. The extent of agglutination was scored on a scale of 1–5 (according to a score card included with the kit) ranging from 1 (no agglutination) to 5 (strong positive agglutination) (see Fig. 1). Four separate tests with Leptorapide were used for the study in Hereford, UK and three separate tests with Leptorapide were used to evaluate the samples in KIT, The Netherlands. An average of the results was obtained by determining the majority overall result within the tests performed.

Statistical methods

The sensitivity and specificity of Leptorapide for the detection of the of anti-leptospiral antibodies was determined in comparison with the MAT. The following calculations were used:

$$\text{Sensitivity} = a/(a+c) \times 100,$$

where a is the number of samples testing positive by Leptorapide and MAT and c is the number of samples testing positive by MAT, but negative for Leptorapide.

$$\text{Specificity} = d/(b+d) \times 100,$$

where d is the number of samples testing negative by Leptorapide and MAT and b is the number of samples testing negative by MAT, but positive by Leptorapide.

$$\text{Accuracy} = (a+d)/(a+b+c+d) \times 100.$$

$$\text{Kappa index} = (a+d-P)/(1-P),$$

where $P = (a+c)(a+b) + (b+d)(c+d)$.

RESULTS

A total of 107 sera samples (34 MAT positive, 73 MAT negative) collected from Hereford, UK and KIT, The Netherlands were tested with Leptorapide and used to determine its sensitivity and specificity relative to the MAT. Sera was considered positive for the presence of leptospiral antibody if the MAT titre >1:40 (Hereford, UK) or $\geqslant$1:160 (KIT, The Netherlands) and if the Leptorapide score was 3–5. Supplementary Table S2 outlines the results obtained and shows Leptorapide compares well to the MAT; with a sensitivity of 97·1% [95% confidence interval (CI) 82·9–99·8] and specificity of 94·0% (95% CI 80·7–95·7). In this analysis, there was very good agreement between Leptorapide and MAT, with a Kappa index of 83·5%.

Ninety-six MAT-confirmed negative samples were obtained from the reference centre in Poland and used to determine the specificity of Leptorapide in relation to the MAT. Supplementary Table S6 outlines the results obtained and shows Leptorapide compares well to the MAT; with a specificity of 95·8% (95% CI 89·1–98·7).

To determine the efficacy of Leptorapide as a screening test for diagnosis of leptospirosis, validation on diagnostic submissions was performed by the CDC and the ISS. The MAT was used to diagnose

leptospirosis in patient samples, with a MAT titre >1:100 indicative of disease. In total, 172/472 clinically suspected patient samples from CDC were confirmed positive for leptospirosis. Of the 172 samples, 11 were single sera samples obtained from individual patients and 77 samples were paired sera (71×2, 5×3, 1×4). Overall specificity for Leptorapide was 93% and overall sensitivity 71%. Leptorapide performed better with samples obtained during the convalescent phase of the disease; with a sensitivity of 81·4% *vs.* 61·6% sensitivity during the acute phase of the disease. Similar results were obtained by the ISS; overall specificity for Leptorapide was 90·9% and overall sensitivity was 78·8%. Comparable results were seen with samples obtained from patients after 1 week and 2 or 3 weeks from onset of symptoms, with both sets of sera producing a sensitivity of ~80% in relation to the MAT and a high specificity of ~90% (93·0% in week 1 and 86·4% in weeks 2/3). Weeks 1–2 are considered the acute phase of this disease and it can sometimes be difficult regardless of the assay being used to identify the antibodies at this stage of the disease. To determine a correct diagnoses any negative results in patients experiencing acute symptoms should be interrupted with caution and should be again tested within the 2- to 3-week time period to give a more accurate diagnoses and confirm the initial negative result.

In total, 168 samples (MAT titre >1:100) obtained from patients with suspected leptospirosis in Brazil, were used to validate the efficacy of Leptorapide in an endemic region. Three different tests with Leptorapide were performed (tests 1, 2 and 3). The average of the three tests was determined and used as a representative result. Supplementary Table S3 outlines the results from testing the samples with both Leptorapide and the commercially available Panbio ELISA. Both Leptorapide and the IgM ELISA detected a similar number of positive samples; 66 testing positive with IgM ELISA and 87 of the samples testing positive with Leptorapide. When tested with Leptorapide, 36 samples were deemed inconclusive requiring a repeat test and 45 samples tested negative.

An independent study sponsored by the WHO was performed by the ICMR. Sera samples ($n = 406$) from the endemic region of South Andaman were collected from clinical submissions and the indices of accuracy were estimated for four rapid tests available for the diagnosis of leptospirosis: latex agglutination test prepared at the Regional Medical Research Centre, Port Blair, Leptorapide (Linnodee Ltd, Northern Ireland), SD Leptospira IgM ELISA (Standard Diagnostics, Korea) and Leptocheck (Zephyr Biomedicals, India). Results showed Leptorapide was the most accurate of the four tests evaluated (Supplementary Tables S4 and S5). In particular, Leptorapide was extremely efficient at diagnosis of leptospirosis >1 week after onset of illness, with sensitivity and specificity values of 90·0% and 87·3%, respectively (Supplementary Table S5).

DISCUSSION

The purpose of this paper is to describe the validation of the commercially available latex agglutination test, Leptorapide, for the rapid serological diagnosis of human leptospirosis. The sensitivity and specificity of Leptorapide in relation to the MAT was determined using 107 sera samples; 55 obtained from the Leptospira Reference Unit, Hereford, UK and 52 from KIT, The Netherlands. All samples had *Leptospira* antibody titres pre-determined by the MAT in the respective laboratory of origin. Of the 34 MAT-positive samples, Leptorapide correctly identified 33 (97·1%) of these. Leptorapide identified 66 (94·0%) of the 73 MAT-negative samples. To further support the specificity of Leptorapide as a rapid screening test, 96 MAT-confirmed negative sera were obtained from the reference centre in Poland, all samples from this reference centre were negative samples as it was a random selection from the region and leptospirosis is not a common disease in developed countries such as Poland, unfortunately no positive samples were found. Leptorapide correctly identified 92 (95·8%) of the 96 sera samples as negative, with the remaining four samples testing positive. Leptorapide testing with these MAT-confirmed samples has shown the test to be in agreement with the current gold-standard test for the detection of leptospirosis. Therefore, the results support the suitability of Leptorapide as an accurate, rapid screening test for the diagnosis of leptospirosis.

To further support the use of Leptorapide as a rapid screening test for the detection of leptospirosis, the CDC and ISS tested the performance of Leptorapide on serum samples obtained from patients presenting with symptoms indicative of leptospirosis. Although all of these patients were displaying symptoms of leptospirosis not all of the samples tested positive with the gold-standard MAT, this could have been due to the wide and varied range of symptoms that occur with leptospiral infections. Leptospirosis

symptoms such as migraine, flu-like symptoms and jaundice are common to other diseases such as dengue fever and meningitis. MAT testing was used for clinical diagnosis of each sample. Results from CDC show a high (93%) specificity for Leptorapide in relation to the MAT, with increased sensitivity of the test during the convalescent phase of the disease (81·4% *vs.* 61·6% during the acute phase). Similar findings were observed from the validation performed by the ISS. Again, a high overall specificity (90·9%) of Leptorapide was detected in relation to the MAT. Sensitivity of the test was comparable from samples obtained within 1 week and 2 or 3 weeks after the onset of symptoms; 80·3% and 78·4%, respectively. Taken together, these findings suggest the suitability of Leptorapide as a rapid screening test for use on diagnostic submissions. However, as mentioned in the Results section of this publication, negative samples regardless of the diagnosing assay should be treated with caution and a retest should be performed within 2–3 weeks of onset of the disease to confirm any results obtained at this time. Similarly, results obtained after antibiotic treatment were not considered in this work which could cause false negatives or antibody titres that were too low to be identified by MAT for comparison.

To determine the efficacy of Leptorapide as a rapid screening test in an endemic region, sera was obtained from patients with pyrexia of unknown origin submitted to the Centre for Zoonoses, in the southern Brazilian state of Rio Grande de Sul. A panel ($n = 168$) of MAT seropositive samples (titre $\geqslant 1/100$) was acquired. Although the MAT is the current gold-standard test for leptospirosis, it cannot differentiate between acute, ongoing infection and previous convalescent infection [18, 19]. Ribeiro *et al.* [20] reported that IgM-detecting ELISAs were more sensitive than MAT in detection of the acute phase of the disease in humans. Concomitant with this finding, results show that the IgM ELISA and Leptorapide detected a similar proportion of positive samples in the panel; 66/168 samples testing positive with IgM ELISA and 87/168 samples testing positive with Leptorapide. With both the IgM ELISA and Leptorapide detecting a similar degree of positivity in the samples, this suggests that the MAT cannot be used to correctly diagnose acute disease. The ability of Leptorapide to detect a greater proportion of positive samples than the IgM ELISA may indicate a greater sensitivity inherent in Leptorapide, which could in turn be due to its dependence on IgM and high-affinity IgG to work. Forty-five of 168 of the MAT-positive sera were deemed negative by Leptorapide and 36/168 samples produced an inconclusive result. These samples may represent individuals with a previous convalescent infection containing significant levels of antibody isotypes other than IgM, in accord with Brandao *et al.* [21].

An independent study funded by the WHO and performed by the ICMR further supports the use of Leptorapide as a screening test in an endemic region. Leptorapide was deemed the most accurate of four rapid tests validated (Vijayachari *et al.*, unpublished data, 2008). In particular, Leptorapide was extremely efficient at diagnosis after 1 week of onset of symptoms. Taken together, results from validation on clinical submissions suggest the suitability of Leptorapide as a rapid and accurate screening test during an epidemic. When considering the reduced sensitivity of the Leptorapide latex agglutination test, especially in these sub-tropical regions of the world where seronegative samples were confirmed on suspect cases, it should be noted that leptospirosis symptoms mimic those of many other infectious disease which would be common to sub-tropical regions of the world such as hantavirus, dengue and malaria. The lower sensitivity of Leptorapide in these areas could be explained by hantavirus infections where all symptoms of leptospirosis are present except haemoptysis, thereby resulting in a misdiagnosis of *Leptospira* infection and failure of antibiotic treatment in these patients.

We have described the suitability of Leptorapide as a rapid screening test for diagnosis of leptospirosis. The assay has been validated with a large sample size obtained from individuals originating from various parts of the world; demonstrating the ability of Leptorapide to detect a wide range of globally distributed serovars. The main advantages of the assay are that it can be performed without the use of specialized equipment by untrained personnel, results are obtained within minutes and it corresponds well in comparison to the MAT in terms of its sensitivity and specificity. Thus, Leptorapide is a valuable tool for rapid diagnosis of the disease, particularly in endemic regions that frequently cannot rely on adequate medical support.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268813002112.

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DECLARATION OF INTEREST

None.

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