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Simple Latex Agglutination Assay for Rapid Serodiagnosis of Human Leptospirosis

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A newly developed latex agglutination assay for the detection of genus-specific *Leptospira* antibodies in human sera was evaluated. The assay is performed by mixing, on an agglutination card, serum with equal volumes of stabilized antigen-coated, dyed test and control latex beads and is read within 2 min. The latex agglutination test was evaluated with groups of serum samples from patients with leptospirosis and control patients from Hawaii, the Seychelles, Thailand, and The Netherlands. The mean overall sensitivity was 82.3%, and the mean overall specificity was 94.6%. The assay is easy to perform and does not require special skills or equipment. The reagents have a long shelf life, even at tropical temperatures. Together, these factors make the assay suitable for use even at the peripheral level of a health care system as a rapid screening test for leptospirosis.

Leptospirosis, notably Weil's syndrome, is an often severe, acute febrile illness caused by microorganisms of the genus *Leptospira* (4). Currently, more than 200 pathogenic *Leptospira* serovars with different natural hosts and geographic distributions have been described. The clinical diagnosis is ambiguous, and signs and symptoms of leptospirosis may resemble those of other major infectious diseases. The laboratory diagnosis of human leptospirosis relies mainly on serological assays aimed at the detection of *Leptospira*-specific antibodies in serum samples. The microscopic agglutination test (MAT) using live strains is the cornerstone for the serodiagnosis of leptospirosis, as the test has a high sensitivity and can be used for classification (3, 12). Unfortunately, the application of the MAT is laborious and requires expertise. Application of the test also requires detailed knowledge of the locally occurring strains, as the predominant serovars have to be selected for use as antigens. The use of the MAT is thus restricted to specialized and well-equipped laboratories. Other standard assays such as the enzyme-linked immunosorbent assay (ELISA) (12, 13) and the IFAT (14) are less complicated but still require trained personnel and expensive equipment. We have explored latex agglutination for use as a practical and rapid adjunct to the serodiagnosis of human leptospirosis. Latex agglutination tests for the detection of leptospirosis have been described in the past (7, 8), but in these cases the ingredients were not stabilized. Here, we present the results of an evaluation of a latex test which utilizes stabilized dyed latex particles coated with a broadly reactive leptospiral antigen. Serum samples taken from patients with laboratory-confirmed leptospirosis and controls from The Netherlands as well as consecutive samples from patients suspected on clinical grounds of having leptospirosis that were collected in four different countries with different degrees of endemicity and surveillance systems were used.

Heat-stable, broadly reactive antigen prepared from the pathogenic strain Lely 607 (serovar *hardjo*; serogroup Sejroe) was used to coat dyed latex particles. The leptospires were grown in bovine albumin polysorbate medium as described by Ellinghausen and McCullough (2) and modified by Johnson and Harris (6). The antigen was prepared from a well-grown culture (10^9 ml^{-1}) by heating the culture at 95°C and centrifuging it to remove cell debris. A 3% latex suspension was coated and blocked with skim milk as described by Cummins and coworkers (1). The optimal concentration of antigen was determined by agglutination of the coated latex particles (test latex) with positive and negative control sera. A 3% solution of control latex (noncoated) was prepared to screen for nonspecific reactions. Test and control latex was stabilized by freeze-drying. The stability of the latex was determined by testing latex stored at 4, 28, and 45°C with sera showing different degrees of agglutination (negative, 1+, 2+, and 3+ [see below]). The stabilized latex could be stored at these temperatures for at least 12 months without showing loss of activity of both weakly and strongly positive samples. After reconstitution, the latex may be stored at 4°C for 2 months.

The latex agglutination assay was performed by placing two 5-μl drops of a serum sample on a white agglutination card. Subsequently, the serum drops were mixed with equal volumes of control and test latex. Latex and serum were mixed with the disposable tip of a pipette. The card was then shaken gently for 2 min. Samples were considered negative if no agglutination was observed within 2 min and given a score of 3+ when agglutination was observed within 30 s, 2+ when agglutination started between 30 s and 1 min, and 1+ when agglutination became visible between 1 and 2 min. Testing of 100 sera by two investigators gave an agreement of 96.0% between the two series of results (kappa, 0.94). One investigator judged to be negative four serum samples that had been judged to be positive by the other investigator. The agglutination score of all four sera which gave discrepant results was 1+.

The sensitivity and specificity of the latex agglutination assay were first assessed by testing a panel of serum samples consisting of 334 samples from 187 patients with laboratory-confirmed leptospirosis and 352 samples from 352 control patients. The samples were taken from the serum bank of the World Health Organization Leptospirosis Reference Centre of the

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TABLE 1. Sensitivity of the latex agglutination assay according to leptospiral serogroups

Serogroup	No. of positive patients/total no. of patients (%)
Australis	36/41 (87.8)
Autumnalis	12/13 (92.3)
Bataviae	3/4 (75)
Ballum	1/1 (100)
Celledoni	5/5 (100)
Grippotyphosa	21/24 (87.5)
Icterohaemorrhagiae	37/38 (97.4)
Javanica	2/3 (66.6)
Pomona	9/11 (81.8)
Pyrogenes	1/2 (50)
Sejroe	19/22 (86.4)
Unknown	21/23 (91.3)

Royal Tropical Institute and had been submitted for laboratory confirmation of leptospirosis either because of clinical suspicion of leptospirosis or because leptospirosis was an option considered in the differential diagnosis. Patients were considered to have laboratory-confirmed leptospirosis when either leptospires could be cultured from blood or urine or a MAT titer of ≥1:160 was observed for one or more samples. The MAT was performed according to routine procedures (12) with a panel of 16 strains (5). On the basis of the natural course of infection, the samples from the patients with leptospirosis were grouped according to the times collected: during the first 10 days of the disease (59 samples), when antibody production starts; between days 10 and 30 (195 samples), when antibody production usually peaks; and 30 to 100 days (80 samples) after the onset of disease, when antibody levels in convalescent patients start to decline. Two or more samples were tested from 31.9% of the patients with leptospirosis. Only one sample from each of the control patients was included in the study. Half of the samples from the control patients were taken before day 10, and half were taken between days 10 and 30 of the disease. The results revealed an overall sensitivity of 87.7% and an overall specificity of 93.5%. The overall sensitivity and specificity were calculated based on the results of one serum sample from each patient. For those patients with more than one sample, we arbitrarily took the sample collected closest to the 20th day after the onset of the disease. The sensitivity rose from 54.2% for samples collected during the first 10 days of the disease to 93.8% for samples collected 10 to 30 days after the onset of the disease and to 83.7% for samples collected more than 30 days after the onset of the disease. For comparison, the sensitivities of the MAT for samples collected during these three periods were 39.0, 95.4, and 85.0%, respectively. The specificity of the latex agglutination assay was 92.0% for samples collected during the first 10 days and 94.9% for samples collected between days 10 and 30 of the disease. The sensitivity of the latex assay ranged from 81.8 to 97.4% for patients infected with strains of the chiefly represented serogroups, Australis, Autumnalis, Grippotyphosa, Icterohaemorrhagiae, Pomona, and Sejroe (Table 1). These results demonstrate that the latex test has a broad reactivity. The latex agglutination assay was easy to read: of the 228 samples from the patients with leptospirosis that tested positive in the latex agglutination assay, 47 reacted weakly (1+), 66 reacted moderately (2+), and 169 reacted strongly (3+). In contrast, 19 of the 23 positive samples from the control patients were given a score of 1+, only 4 samples were 2+, and none were 3+. The relatively high specificity of the assay was confirmed by testing samples from patients with human immunodeficiency virus ($n = 20$ samples), hepatitis A ($n = 10$), hepatitis B ($n = 9$), syphilis ($n = 20$), malaria ($n = 20$), toxoplasmosis ($n = 11$), meningitis ($n = 10$), meningococcal meningitis ($n = 10$), Lyme borreliosis ($n = 20$), hantavirus infection ($n = 20$), or an autoimmune disease (rheumatoid arthritis [$n = 10$] or systemic lupus erythematosus [$n = 20$]). Only 2 of these 139 samples agglutinated. None of 20 blood bank sera agglutinated.

To further determine the clinical utility of the assay, consecutive samples collected from patients suspected on clinical grounds of having leptospirosis from Hawaii (129 patients with 209 samples), the Seychelles (118 patients with 225 samples), Thailand (125 patients with 211 samples), and The Netherlands (428 patients with 480 samples) were tested. Patients were grouped as those with laboratory-confirmed leptospirosis and control patients according to routine laboratory procedures and criteria. The MAT for the samples from Hawaii was done at the Centers for Disease Control and Prevention in Atlanta, Ga., the MAT for the samples from the Seychelles was done in New Caledonia, and the MAT for the samples from Thailand and The Netherlands was done at the World Health Organization leptospirosis reference laboratory in Amsterdam, The Netherlands. The patient group from Hawaii consisted of 28 patients with laboratory-confirmed leptospirosis (52 samples) and 101 control patients (157 samples), the group from the Seychelles consisted of 75 patients with leptospirosis (151 samples) and 43 control patients (74 samples), the group from Thailand consisted of 17 patients with leptospirosis (33 samples) and 108 control patients (185 samples), and the group from The Netherlands consisted of 17 patients with leptospirosis (40 samples) and 411 control patients (440 samples). From the results, a mean overall sensitivity of 82.3% and a mean overall specificity of 94.6% could be calculated (Table 2). The mean sensitivity for samples collected during the first 10 days of the disease was 37.8%, and the mean sensitivity for samples collected more than 10 days after the onset of the disease was 88.3%. The mean specificities for samples collected during these two periods were 92.5 and 96.7%, respectively. The predictive value of a test depends of the prevalence of the disease among the study population (9, 15). The mean positive predictive value was calculated to be 73.4% and ranged from 40.5% for samples from The Netherlands, where there is a 3.9% prevalence of leptospirosis among patients suspected on clinical grounds of having leptospirosis, to 94.3% for the samples from the Seychelles, where there is a 54.3% prevalence of the disease (Table 2). The mean negative predictive value was 96.7% and ranged from 80.3% for the group of serum samples from the Seychelles to 99.3% for the samples from The Netherlands. In total, 22 (17.7%) patients with leptospirosis from these four patient groups gave a negative result in the latex test. Interestingly, when these samples were tested in an ELISA for the detection of *Leptospira*-specific immunoglobulin M (IgM) antibodies, titers below the cutoff value were observed for the samples of 12 of these 22 patients. Samples from seven patients had borderline titers, and samples from only five patients had a titer above the cutoff value. In contrast, 95.8% of the samples from the patients found to be positive for leptospirosis in the latex agglutination test also were found to be positive in the IgM ELISA. These results indicate that reactivity in the latex assay corresponds with the presence of specific IgM antibodies.

A large number of pathogenic strains belonging to different serovars and serogroups may cause leptospirosis. Different strains may be more prevalent in different areas. As antibodies reactive in the MAT are often serovar specific, a carefully selected panel of strains must be used as antigens in the MAT

TABLE 2. Diagnostic indices calculated for the latex agglutination test for groups of consecutively collected samples from patients suspected on clinical grounds of having leptospirosis from different countries and comparison with the values obtained for the IgM ELISA and the IgM dipstick assay

Assay and study group	% Sensitivity (95% CI)	% Specificity (95% CI)	% Positive predictive value (95% CI)	% Negative predictive value (95% CI)	% Prevalence
Latex agglutination assay					
The Netherlands	83.3	95.0	40.5	99.3	3.9
Thailand	76.5	92.6	61.9	96.2	13.6
The Seychelles	80.6	94.2	94.3	80.3	54.3
Hawaii	82.1	97.0	88.5	95.1	21.7
Mean	82.3 (74–88)	94.6 (93–96)	73.4 (65–80)	96.7 (95–98)	15.3
IgM ELISA					
Mean	85.5 (78–91)	97.9 (96–99)	87.6 (80–93)	97.4 (96–98)	15.3
IgM dipstick assay					
Mean	83.7 (76–90)	95.6 (94–97)	76.9 (69–84)	97.1 (96–98)	15.3

to give a complete coverage of all possible circulating strains causing disease. Therefore, a large number of reactions must be performed to give an accurate diagnosis when the MAT is used. The results of this study show that almost the same efficacy as that of the MAT can be obtained in a single agglutination reaction when the latex agglutination assay is applied. The results of this study demonstrate equally high sensitivities of the latex agglutination assay for detecting infections with strains of the serogroups Australis, Autumnalis, Grippotyphosa, Icteroheamorrhagiae, Pomona, and Sejroe, showing that the assay has a broad reactivity. Further studies will be needed to determine whether the assay is equally sensitive for infections with strains of other serogroups. Information on the serogroup of the infecting strain cannot be obtained from the results of the latex agglutination assay. Knowledge of the serogroup, however, has no clinical implications and is mainly of epidemiological interest.

In leptospirosis, antibodies usually appear within 5 to 7 days after the onset of the symptoms and in a significant proportion of patients, antibodies persist in detectable quantities for many months (3, 4). From a clinical point of view the early recognition of the disease is very important for initiating appropriate treatment to avoid severe complications. The sensitivity of the latex agglutination assay was equal to or slightly higher than that of the MAT for samples collected at an early stage of the disease.

A comparison of the experiments performed on groups of consecutively collected samples from The Netherlands, Hawaii, Thailand, and the Seychelles indicates that the latex agglutination assay may have a wide application. Only minor differences in sensitivity and specificity were noted for the samples from the different countries. The mean sensitivity was 82.3%, and the mean specificity was 94.6%. Together with the relatively high negative predictive value, these results make the assay ideally suited for rapid screening. Expectedly, the predictive values varied with the prevalence of patients with leptospirosis among those suspected of having the disease. With a low prevalence of the disease, the positive predictive value is relatively low and a positive result should be confirmed by another assay such as the MAT. However, when the prevalence is high, as is the case in situations of endemicity or in outbreaks, the positive predictive value is high and confirmation may not be needed.

The results of the latex agglutination assay for the four study groups combined showed 89.4% agreement (kappa, 0.63; 95% confidence interval [CI], 0.57 to 0.69) with the results of the MAT, 95.2% agreement (kappa, 0.81; 95% CI, 0.75 to 0.87) with the results of the IgM ELISA, and 93.4% agreement (kappa, 0.74; 95% CI, 0.68 to 0.80) with the results of a dipstick assay for the detection of *Leptospira*-specific IgM antibodies, which we described previously (5, 10, 11, 16). The sensitivity of the latex agglutination assay was somewhat lower than the sensitivity of the IgM ELISA, the specificity and positive predictive value were significant lower, and the negative predictive value was the same (Table 2). These four diagnostic indices of the latex agglutination assay did not differ from those of the dipstick assay (Table 2). The advantage of the latex agglutination assay is that it gives a result within 2 min, compared with 3 h for the dipstick assay.

Leptospirosis affects mostly people living in tropical countries who cannot rely on hospital facilities with laboratory support. The results of these studies show that the latex agglutination assay will be a valuable tool in the diagnostic armament for leptospirosis. The assay is extremely simple and rapid to perform, uses stabilized components, and can be performed without the need for training or special or expensive equipment. The assay has a good sensitivity and specificity, and acceptable predictive values and results are concordant with those of the MAT, in particular for samples collected early in the disease. Together, these test characteristics make the assay suitable for use in situations were facilities or resources to perform more complicated tests are not available. The assay also gives a quick result, which can be important in the management of patients, especially when attention must be given to a large number of patients.

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