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Evaluation of a Commercial Latex Agglutination Assay for Serological Diagnosis of Leptospirosis

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Leptospirosis is a febrile zoonosis of worldwide distribution. A latex agglutination assay was evaluated in two studies, the first using a panel of well-characterized sera from patients with leptospirosis and from patients with other disease states and the second, a prospective hospital-based study, evaluating sera from 186 consecutive patients admitted to hospital with acute febrile illness. The confirmed leptospirosis serum panel included paired acute- and convalescent-phase specimens from 40 cases, of which 34 gave positive latex tests (case sensitivity, 85%; 95% confidence interval [95% CI], 70 to 94%). The other diseases represented in the panel of 112 specimens from nonleptospirosis patients included autoimmune diseases, brucellosis, dengue, melioidosis, malaria, syphilis, toxoplasmosis, viral hepatitis, and a number of other viral infections. The specificity of latex agglutination using this panel was 81% (95% CI, 73 to 87%). Among the patients with acute febrile illness, there were 25 cases of leptospirosis and 161 patients with other diagnoses. The sensitivity and specificity of latex agglutination in this group were 88% (95% CI, 72 to 97%) and 98% (95% CI, 95 to 100%), respectively. In this evaluation, the two distinct groups of specimens gave similar results for sensitivity, but specificity was different in each study. The sensitivity and specificity observed for the hospital study were similar to those obtained in evaluations of other rapid tests in the same population. The results of this study suggest that multiple evaluations of new diagnostic assays should be performed, because performance characteristics may vary in different populations.

Leptospirosis is an acute febrile disease, widely recognized as being emerging or reemerged (5, 13). In tropical and subtropical regions, the disease is endemic and exposure is widespread (8, 11, 22). In temperate climates, the disease is primarily one of occupational or recreational exposure (12), as evidenced by a large outbreak in the United States associated with swimming during a triathlon (17). The mortality rate in severe leptospirosis can be as high as 15% (11); early diagnosis is essential if antibiotic treatment is to be effective.

Leptospirosis is frequently underdiagnosed because of the nonspecific symptoms early in the disease and the difficulty of performing both culture and the reference serological test, the microscopic agglutination test (MAT). Detection of immunoglobulin M (IgM) antibodies by enzyme-linked immunosorbent assay (ELISA) has been used widely (1, 21) and is more sensitive than the MAT (6). However, the MAT remains the gold standard due to difficulties in interpretation of IgM results, especially in areas where leptospirosis is endemic. Several rapid methods for antibody detection, which detect genus-specific antibodies, either IgM (3, 19, 23) or both IgG and IgM (2–4, 16), are now available commercially. The reported sensitivities of these assays range from 87 to 100%. In this study, we evaluated a latex agglutination assay for the detection of anti-*Leptospira* IgM antibodies which can be used in laboratories with little specialized equipment.

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Two groups of specimens were studied. The first was comprised of banked serum specimens collected from patients with leptospirosis and other diseases, used in a previous study (3). The serum panel included paired acute- and convalescent-phase specimens from 40 cases of leptospirosis and 112 specimens from nonleptospirosis patients with a variety of diagnoses, including autoimmune diseases, brucellosis, dengue, melioidosis, malaria, syphilis, toxoplasmosis, viral hepatitis, and a number of other viral infections. Each case specimen was from a patient who had clinical disease consistent with leptospirosis and at least one of the following results: a positive leptospiral culture, a positive immunohistochemistry test on tissue samples, or a fourfold or greater antibody titer between paired sera by MAT.

The second group of samples was obtained from consecutive patients admitted to the Queen Elizabeth Hospital, Bridgetown, Barbados, between June 2001 and October 2003 with a history and clinical manifestations suggestive of leptospirosis. On the day of admission, blood samples for serology were collected and blood cultures were made by inoculating three drops of blood into 10 ml polysorbate medium at the patient's bedside (PLM-5; Intergen Co., Purchase, NY). Urine from patients who were not anuric on the fourth day of their admission was inoculated into the same medium within 1 hour of collection. On the fourth day after admission, and for some patients before discharge from the hospital or at a follow-up visit to the outpatient clinic, a convalescent-phase sample was taken. All testing of the Barbados patient specimens was done at the Barbados Leptospira Laboratory. The diagnosis of leptospirosis was confirmed by a fourfold rise in titer between two

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TABLE 1. Sensitivity and specificity of the latex agglutination assay

Group of specimens	Latex assay result (95% CI)			MAT % sensitivity for acute-phase specimens (95% CI)
	% Case sensitivity	% Specificity	% Sensitivity for acute-phase specimens	
Banked specimen panel	85 (70–94)	81 (73–87)	40 (25–57)	23 (11–38)
Barbados patients	88 (72–97)	98 (95–100)	52 (31–72)	40 (21–61)

sera tested by the same method, an initial titer of ≥800 in the MAT, an IgM titer of ≥160 in the ELISA, or a positive culture from blood or urine (9). Diagnoses for the nonleptospirosis cases were obtained from patients' records following discharge from the hospital.

IgG and IgM titers were determined by ELISA as previously described (21), using the strain Patoc I (serovar Patoc) as the antigen. An IgM titer of ≥160 was regarded as positive. Sera were examined by the MAT, using a panel of 22 serovars to establish seroconversion or a rise in titer (10). The antigens used included both reference strains and locally prevalent serovars of the following serogroups (serovars are in parentheses): Australis (Bajan, Barbadensis, Bratislava), Autumnalis (Bim, Fortbragg), Ballum (Arborea, Ballum), Bataviae (Bataviae, Brasiliensis), Canicola (Canicola), Cynopteri (Cynopteri), Grippotyphosa (Grippotyphosa), Icterohaemorrhagiae (Copenhageni), Mini (Georgia), Panama (Mangus, Panama), Pomona (Pomona), Pyrogenes (Pyrogenes), Tarassovi (Tarassovi), Sejroe (Hardjo, Sejroe), and Semaranga (Patoc).

All specimens from leptospirosis cases and nonleptospirosis cases described above from both the banked panel and the Barbados hospital studies were tested by latex agglutination test. The latex agglutination tests were manufactured using a broadly reactive, heat-stable antigen composed of serovar Hardjo strain Lely 607 (20). Latex tests were obtained from the manufacturer (Organon Teknika B.V., Durham, NC) and were used according to the manufacturer's recommendations. Briefly, 10 μl of serum was added to the dried reagent spot on a card and mixed with a plastic stirrer. The card was rocked gently for a total of 30 s, and the results were read. Any specimen that showed agglutination was recorded as positive, and no agglutination was recorded as negative. Positive and negative controls were tested on each day.

The sensitivity and specificity of the latex assay were determined and 95% confidence intervals (95% CI) calculated using the standard normal distribution formula for proportions (18). Sensitivity was calculated for paired-case sera and acute-case sera only. The sensitivity of the latex assay to detect a case was defined as the percentage of the leptospirosis cases that were correctly identified by the assay based on a positive result from one or more specimens. Predictive value of positive and negative results was calculated for the Barbados patients only, since samples were from the same population.

Paired acute- and convalescent-phase banked serum panel specimens from 34 of the 40 cases of leptospirosis were positive (case sensitivity, 85%; 95% CI, 70 to 94%), and 21 of the 112 specimens from nonleptospirosis cases were also positive, resulting in a specificity of 81% (95% CI, 73 to 87%) (Table 1). In the prospective hospital study in Barbados, positive latex agglutination results were obtained in 22/25 cases. Case sensitivity was 88% (95% CI, 72 to 97%), and specificity was 98% (95% CI, 95 to 100%). There were 25 confirmed cases of leptospirosis and 161 patients with other diagnoses in the Barbados hospital studies. The discharge diagnosis was available for 108 of these 161 patients (Table 2) and included acute viral hepatitis, undifferentiated viral illness, bacterial sepsis, dengue and dengue hemorrhagic fever, gastroenteritis, hematologic conditions, and a number of other medical and surgical conditions. Three false-positive latex agglutination results were from patients with viral encephalitis, jaundice of unknown origin, and pyrexia of unknown origin. The positive predictive value of the latex agglutination test in this population was 88%, and the negative predictive value was 98%.

TABLE 2. Latex assay reactivity with nonleptospirosis case specimens

Group of specimens	Condition or discharge diagnosis of patient[a]	No. of specimens from patients with condition or diagnosis	No. (%) of specimens positive by latex assay
Banked specimen panel	Meningococcal disease	1	0
	HIV	6	0
	Toxoplasmosis	2	0
	Legionellosis	1	0
	Brucellosis	6	1 (16.7)
	Syphilis	8	0
	Viral hepatitis	16	9 (56.3)
	Malaria	8	4 (50.0)
	Melioidosis	5	1 (20.0)
	Dengue	24	2 (8.3)
	ANCA	8	1 (12.5)
	ANA	9	3 (33.3)
	Hantaviral infection	5	0
	Lyme disease	5	1 (20.0)
	Cytomegalovirus	4	2 (50.0)
	Epstein-Barr virus	4	1 (25.0)
Barbados prospective hospital-based study	Aseptic meningitis	1	0
	Bacterial sepsis	13	0
	Toxoplasmosis	2	0
	Atypical pneumonia	4	0
	Obstructive jaundice	4	0
	Alcoholic hepatitis	3	0
	Viral hepatitis	17	0
	Rhabdomyolysis	2	0
	Gastroenteritis	6	0
	Dengue	11	0
	Hematologic conditions	6	0
	Guillain-Barré syndrome	1	0
	Undifferentiated viral illness	15	0
	Toxic shock syndrome	1	0
	Bacterial endocarditis	1	0
	Others (medical/surgical)	21	3 (14.3)

[a] HIV; human immunodeficiency virus; ANCA, antinuclear antibodies; ANA, antineutrophil cytoplasmic antibodies.

When acute-phase serum specimens alone were tested, positive latex agglutination results were obtained from 16/40 (40%; 95% CI, 25 to 57%) specimens in the serum panel and from 13/25 (52%; 95% CI, 31 to 72%) specimens in the prospective hospital study (Table 1). The latex agglutination assay was more sensitive for acute-phase samples than the MAT, which was positive in 23% (9/40; 95% CI, 11 to 38%) of the serum panel and 40% (10/25; 95% CI, 21 to 61%) of the Barbados patient specimens.

In this evaluation, the two distinct groups of specimens gave similar results for sensitivity, but specificity was different in each study. The specificity of the latex assay for the serum

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panel was significantly lower than that for the clinical study in Barbados. Three of the false-negative cases from the banked serum panel were from a point source outbreak among triathletes (17). Specimens from this outbreak also showed lower sensitivity in a larger study of several other rapid diagnostic assays (3). Isolates from this outbreak have been characterized as serovar Grippotyphosa (17), which may indicate a lack of sensitivity of rapid tests for antibodies against this serovar. Among other disease states, false-positive results occurred most frequently with samples from patients with viral hepatitis, malaria, and autoimmune diseases.

The performance of the latex assay in the Barbados cases was similar to that of other rapid tests in this population (15, 16, 19). As we have observed previously, sensitivity was considerably reduced when acute-phase serum specimens (Table 1), collected 4 to 5 days after onset of symptoms, were analyzed (14–16). These specimens are collected at or about the time of seroconversion, and the MAT is also invariably negative in most cases at this time (3, 6).

The results of this study emphasize the need to evaluate assays for use with different populations. The construction of test panels using archived sera facilitates the testing of new assays against a wide range of disease states, which may not be possible in a single population. However, the specificity obtained in such studies may not reflect the performance of the assay in clinical studies conducted in diverse locations. This is particularly true for a disease with a wide spectrum of symptoms like leptospirosis, because the differential diagnosis is extremely broad and geographically variable.

Moreover, the incidence of diseases with similar symptoms is also subject to geographical variation. In addition to variation in the differential diagnosis, geographic variation in infecting serovars is significant, as serological tests may show variable sensitivities with different serovars, further emphasizing the need for multicenter evaluations (19). Studies conducted with consecutive clinical cases are also valuable in determining the usefulness of an assay for a given population based on predictive value calculations. The persistence of the antibody response detectable by the latex agglutination assay is unknown. However, other IgM assays have been shown to detect antibodies for long periods after infection (7).

Overall, the latex agglutination test for serologic diagnosis of leptospirosis performed well. This assay has several potential advantages, which include its simplicity and portability, limited amount of generated biomedical waste, and prolonged shelf life at ambient temperatures ranging from 2°C to 45°C. The test can be used in resource-poor settings, by investigators with only limited training. Given the high specificity of the latex assay, its ability to detect more cases using acute-phase sera only, and its ease of use, this would be an appropriate test for use in field studies, particularly in outbreak investigations.

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