JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2003, p. 803–809 Vol. 41, No. 2
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.2.803–809.2003

Evaluation of Four Commercially Available Rapid Serologic Tests for Diagnosis of Leptospirosis

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Received 29 July 2002/Returned for modification 22 October 2002/Accepted 22 November 2002

Four rapid tests for the serologic diagnosis of leptospirosis were evaluated, and the performance of each was compared with that of the current standard, the microscopic agglutination test (MAT). The four rapid tests were a microplate immunoglobulin M (IgM)–enzyme-linked immunosorbent assay (ELISA), an indirect hemagglutination assay (IHA), an IgM dipstick assay (LDS), and an IgM dot-ELISA dipstick test (DST). A panel of 276 sera from 133 cases of leptospirosis from four different geographic locations was tested as well as 642 sera from normal individuals or individuals with other infectious or autoimmune diseases. Acute-phase sera from cases (*n* = 148) were collected ≤14 days (median = 6.0) after the onset of symptoms, and convalescent-phase sera (*n* = 128) were collected ≥15 days after onset (median = 29.1). By a traditional method (two-by-two contingency table), the sensitivities for detection of leptospirosis cases were 93.2% by LDS, 92.5% by DST, 86.5% by ELISA, and 79.0% by IHA. Specificity was 98.8% by DST, 97% by ELISA and MAT, 95.8% by IHA, and 89.6% by LDS. With a latent class analysis (LCA) model that included all the rapid tests and the clinical case definition, sensitivity was 95.5% by DST, 94.5% by LDS, 89.9% by ELISA, and 81.1% by IHA. The sensitivity and specificity estimated by the traditional methods were quite close to the LCA estimates. However, LCA allowed estimation of the sensitivity of the MAT (98.2%), which traditional methods do not allow. For acute-phase sera, sensitivity was 52.7% by LDS, 50.0% by DST, 48.7% by MAT and ELISA, and 38.5% by IHA. The sensitivity for convalescent-phase sera was 93.8% by MAT, 84.4% by DST, 83.6% by LDS, 75.0% by ELISA, and 67.2% by IHA. A good overall correlation with the MAT was obtained for each of the assays, with the highest concordance being with the DST (kappa value, 0.85; 95% confidence interval [CI], 0.8 to 0.90). The best correlation was between ELISA and DST (kappa value, 0.86; 95% CI, 0.81 to 0.91). False-positive LDS results were frequent (≥20%) in sera from individuals with Epstein-Barr virus, human immunodeficiency virus, and periodontal disease and from healthy volunteers. The ease of use and significantly high sensitivity and specificity of DST and ELISA make these good choices for diagnostic testing.

Leptospirosis is a zoonosis caused by spirochetes of the genus *Leptospira*, which has a worldwide distribution (40). Humans become infected through contact with contaminated animal urine, tissues, or water (20). The clinical presentation is difficult to distinguish from dengue, malaria, influenza, and many other diseases characterized by fever, headache, and myalgia (34). Although the patient's exposure history may assist in narrowing the differential diagnosis, a rapid and simple test with high sensitivity and specificity would be useful for early diagnosis and treatment and for public health surveillance (25). Definitive laboratory diagnosis of leptospirosis requires detection of the organism in a clinical specimen or a fourfold or greater rise in microscopic agglutination test (MAT) titer in the setting of an appropriate clinical syndrome.

The most frequently used diagnostic approach for leptospirosis has been that of serology. The MAT is the serological test used in reference laboratories, because of its high degree of sensitivity and specificity (11). However, the MAT is a complex test that requires a large panel of live-cell suspensions to provide adequate coverage of the antigenic diversity represented in a given testing area. Moreover, antibody levels detectable by MAT usually do not appear before day 6 or 7 after development of symptoms; they usually peak by the fourth week, but detectable titers may persist for years (1, 13, 35). Hence, interpretation of the results is difficult without paired specimens collected at the appropriate times; therefore, results are usually not available quickly enough to be useful for patient management.

Several alternatives to the MAT have been developed; those available commercially include an immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) (39), an IgM dipstick assay (LDS) (19), an IgM dot-ELISA dipstick test (DST) (21), and the indirect hemagglutination assay (IHA) (22, 33). Reported evaluations suggest that some of these assays are highly sensitive and specific (5, 10, 21, 22, 27, 30, 31, 39, 41), but they have not been systematically compared to each other and to the MAT. This study was designed to determine the performance of these serologic assays in detecting

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Leptospira-specific antibodies and to compare results obtained with each system to those obtained with the MAT. This information should assist diagnostic laboratories, especially those without the capacity to maintain the MAT, to select a suitable assay for screening serum samples from suspected cases of leptospirosis.

MATERIALS AND METHODS

Case sera. Specimens were selected from serum samples submitted to the Centers for Disease Control and Prevention between 1992 and 1998. These specimens were obtained from individual cases and outbreak investigations. Each case specimen was from a patient who had clinical disease consistent with leptospirosis and at least one of the following test results: a positive *Leptospira* culture, a positive immunohistochemistry test on tissue samples, or a fourfold or greater increase in antibody titers when paired sera were tested by MAT. A total of 276 sera from 133 cases were included in this study; 127 of the cases were confirmed by MAT, 5 were culture confirmed, and 1 was confirmed by immunohistochemistry. Of the 133 cases, 19 (14.3%) were from Thailand, 1 (0.7%) was from Palau, and 113 (85.0%) were from the United States. Among the 113 cases from the United States, 65 (57.5%) were from Hawaii, 19 (16.8%) were from a point source outbreak in Illinois (9), 18 (15.9%) were from Puerto Rico, and 11 (9.7%) were sporadic cases from various other states. The panel of case sera (276 specimens) consisted of 148 acute-phase sera (specimens obtained 0 to 14 days after onset of illness) and 128 convalescent-phase sera (specimens obtained 15 or more days from onset of illness). Paired sera were available for 126 cases, and 7 cases had more than two convalescent-phase specimens. Specimens from cases were collected from 0 to 79 days after the onset of illness.

Control sera. A total of 642 control specimens were obtained; 133 were from healthy donors and 509 were from individuals known to have one of 21 diseases other than leptospirosis. The number of specimens in each disease category ranged from 10 to 44 (see Table 4). Included were specimens from patients with autoimmune diseases (rheumatoid factor, antinuclear antibody, and antineutrophil cytoplasmic antibody), periodontal disease, and viral, parasitic, and bacterial infections (*Treponema pallidum*, *Rickettsia rickettsii*, *Rickettsia typhi*, *Ehrlichia chaffeensis*, *Borrelia burgdorferi*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Neisseria meningitidis*, malaria, human immunodeficiency virus [HIV], Epstein-Barr virus [EBV], dengue virus, viral hepatitis, hantavirus, cytomegalovirus, toxoplasmosis, melioidosis, and brucellosis). Efforts were made to limit the collection of control specimens to individuals residing in areas in which leptospirosis is nonendemic. Information helpful in the interpretation of results, such as travel history, agent- or disease-specific findings, and place of residence, was obtained.

MAT. The MAT was performed using the standard procedure (11), with minor modifications. Live *Leptospira* cell suspensions (representing 22 serovars belonging to 17 serogroups) were added to serially diluted serum specimens in 96-well flat-bottomed microtiter plates and incubated at room temperature for 1.5 h. Agglutination was examined by dark-field microscopy at a magnification of $\times 100$. The reported titer was calculated as the reciprocal of the highest dilution of serum that agglutinated at least 50% of the cells for each serovar used. Serogroups (serovars in parentheses) included in the antigen panel were as follows: Australis (australis and bratislava), Autumnalis (autumnalis), Ballum (ballum), Bataviae (bataviae), Canicola (canicola), Cynopteri (cynopteri), Grippotyphosa (grippotyphosa), Icterohaemorrhagiae (copenhageni, mankarso, and icterohaemorrhagiae), Mini (georgia), Pomona (pomona), Pyrogenes (pyrogenes and alexi), Tarassovi (tarassovi), Sejroe (wolffi), Celledoni (celledoni), Djasiman (djasiman), Javanica (javanica), and Hebdomadis (borincana). Antigen identity and reactivity were confirmed by agglutination with homologous hyperimmune rabbit serum on each test batch. For the purposes of this evaluation, specimen titers of $\geq$200 (areas of nonendemicity) or $\geq$800 (areas of endemicity) against one or more serogroups were considered positive (8, 17).

IgM-ELISA. ELISA kits were obtained from PanBio (Brisbane, Australia). Case and control sera (5 μl) were diluted 1:100 and tested according to the manufacturer's instructions. Optical density at 450 nm was compared with that of a cutoff calibration sample supplied by the manufacturer. Results were interpreted according to the manufacturer's recommendation. Specimens having an absorbance ratio greater than that of the cutoff calibrator were defined as positive.

LDS. LDS kits were obtained from the Royal Tropical Institute (Amsterdam, The Netherlands). Case and control sera (10 μl) were diluted 1:50 and tested according to the manufacturer's instructions. The intensity of staining of the reaction band was read visually and scored from 1 to 4 with a color reference diagram (19). A staining intensity of 2 or more was interpreted as positive.

DST. DST kits were obtained from Integrated Diagnostics Inc. (Baltimore, Md.). Case and control sera (10 μl) were diluted 1:200 in diluent containing goat anti-human IgG and tested according to the manufacturer's instructions. The result was read according to the number of stained test dots; the presence of two or more stained test dots was interpreted as positive.

Indirect hemagglutination. IHA kits were obtained from MRL Diagnostics (Cypress, Calif.). All sera were screened at a 1:50 dilution, and all positive sera were subsequently titrated. Agglutination was scored from +1 to +4, and the titer was recorded as the reciprocal value of the highest dilution of serum showing +2 agglutination. Results were interpreted according to the manufacturer's recommendations. Titers of $\geq$50 were considered positive.

Data analysis. The sensitivity and specificity of each assay were determined, and 95% confidence intervals (CI) were calculated using the standard normal-distribution formula for proportions (14). For each assay, sensitivity and specificity were calculated for case detection, for acute-case sera only, and for convalescent-case sera only. The sensitivity of each assay to detect a case was defined as the percentage of the leptospirosis cases meeting our case definition that were correctly identified by the assay, based on a positive result with either acute- or convalescent-phase sera. Latent class analysis (LCA) was also used to determine the sensitivity and specificity of the assays as well as to estimate sensitivity based on combinations of assays. LCA is useful where a perfect "gold standard" does not exist and thus the disease status remains latent (4). LCA is a mathematical technique that uses a statistical model to relate unobserved (latent) conditions to multiple diagnostic test results. LCA models the probability of each combination of test results conditionally on the latent class (infected or noninfected). From these probabilities, the sensitivity and specificity of all tests included in the model can be estimated. Five variables were included in a two-class LCA: the case or clinical definition and ELISA, DST, LDS, and IHA results. The second model excluded the clinical definition but included the MAT.

The degree of agreement between any two tests in classifying an individual sample correctly was calculated using the kappa statistic (18). A kappa value of more than 0.75 indicates excellent agreement between tests, while a value of less than 0.4 indicates poor agreement. The McNemar test was used to compare the sensitivities of different assays (14). The McNemar value indicates the probability that differences observed between assays are not due to chance. A value of $<$0.01 is significant.

RESULTS

This study evaluated the performance of rapid leptospirosis diagnostic assays on 276 sera from 133 confirmed cases of leptospirosis. The sensitivity of the rapid assays for cases (one or more serum samples testing positive) ranged from 79.0% (IHA) to 93.2% (LDS). The IHA was significantly less sensitive than ELISA, LDS, and DST ($P \leq 0.01$) (Table 1). Using LCA, two models were generated (Table 2); one included the clinical case definition and the second included MAT, the current reference assay. In model 1, the sensitivity ranged from 81.1% for IHA to 95.9% for DST. In model 2, the sensitivity ranged from 81.5% for IHA to 96.4% for DST.

When only acute-phase sera were considered, the sensitivity of the assays ranged from 38.5% (IHA) to 52.7% (LDS) (Table 1). The sensitivity of all of the assays on convalescent-phase sera was higher (Table 1), ranging from 67.2% (IHA) to 84.4% (DST). However, the MAT had the highest sensitivity on convalescent-phase sera (93.8%). The differences in sensitivity of the assays were not statistically significant when acute- and convalescent-phase sera were analyzed separately ($P \geq 0.01$).

Specificities for the rapid assays ranged from 89.6 to 98.8% (Table 1), while the specificity for the MAT was 97.3%. The specificities obtained using LCA for both model 1 and model 2 were very similar (Table 2). The LDS was significantly less specific than the other tests ($P \leq 0.01$), while the DST was significantly more specific than the ELISA ($P \leq 0.01$).

The sensitivity was lowest during the first 3 days of illness

TABLE 1. Sensitivity and specificity of four assays for detection of *Leptospira* antibodies, calculated using two by two contingency tables

Assay	% Sensitivity (95% CI)			% Specificity (95% CI)
	Paired sera[a] (n = 133)	Acute-phase sera[b] (n = 148)	Convalescent-phase sera[c] (n = 128)	
ELISA	86.5 (79.5–91.8)	48.7 (40.4–57.0)	75.0 (66.6–82.2)	97.0 (95.4–98.2)
IHA	79.0 (71.0–85.5)	38.5 (30.6–46.9)	67.2 (58.3–75.2)	95.8 (93.9–97.2)
LDS	93.2 (87.5–96.9)	52.7 (44.3–61.0)	83.6 (76.0–89.6)	89.6 (86.9–91.8)
DST	92.5 (86.6–96.3)	50.0 (41.7–58.3)	84.4 (76.9–90.2)	98.8 (97.6–99.5)
MAT	—[d]	48.7 (40.4–57.0)	93.8 (88.1–97.3)	97.3 (95.8–98.4)

[a] For any assay, a case was considered positive if either the acute- or convalescent-specimen tested positive.
[b] Specimens collected within 14 days of onset of disease.
[c] Specimens collected >14 days after onset of disease.
[d] MAT was part of the case definition and defined most cases.

and increased to a peak by 10 to 12 days after onset for all tests except the LDS, which achieved peak sensitivity on specimens collected 16 to 18 days after onset (Fig. 1). The decrease in the proportion of positive results for sera collected 13 to 15 days after the onset of illness by all the assays was not significant. The differences observed in sensitivity levels between the assays within the first 3 days of illness were not statistically significant ($P \geq 0.01$), while the LDS was significantly more sensitive than the MAT and IHA by 4 to 6 days after onset ($P \leq 0.01$).

The case sensitivity for each of the rapid assays for the 133 cases, grouped according to geographical location, is shown in Table 3. Of the 30 cases from the continental United States, 18 were from a point source outbreak that occurred in Illinois in 1998 among participants in a triathlon event (9). The sensitivity of all of the rapid assays except the LDS was lowest with sera from the triathletes. Specimens from Thailand also had lower sensitivity by ELISA.

The sensitivity of the rapid tests for case sera reactive against one or more of the 22 *Leptospira* serovars was determined by MAT. Excluding serogroups reactive in a single case, the range of the proportion reactive with the serogroups for DST was 82.6 to 100%, that with the serogroups for LDS was 78.3 to 100%, that with the serogroups for IHA was 66.7 to 90%, and that with the serogroups for ELISA was 66.7 to 100%. For all serogroups, the IHA was the least-sensitive assay.

The results obtained with the negative control sera are presented in Table 4. Of the 21 infectious or autoimmune disease groups of controls, false-positive test results were obtained for sera from 11 conditions by IHA, 10 by ELISA, 5 by DST, and 16 by LDS (Table 4). Substantial false-positive results were obtained by IHA with sera from patients with EBV, hantavirus, and HIV infection and by LDS with EBV, HIV, and periodontal disease sera. False-positive IHA, DST, ELISA, and MAT results among healthy volunteers were infrequent; however, 29 (22%) tested positive by LDS. Analysis of the reaction patterns of the rapid assays indicated that only 10.5% (7 of 67) of LDS false-positive specimens had reaction intensities of >2, while the remainder were at the cutoff point of 2. Titers of >100 were obtained for 18.5% (5 of 27) of the sera that gave false-positive results by IHA. Eight control sera positive by MAT reacted with one of the following serogroups at the titers indicated: Ballum (titer, 400 to 800), Canicola (titer, 800 to 3,200), Pyrogenes (titer, 400), Autumnalis (titer, 400), Mini (titer, 400), Djasiman (titer, 400 to 800), and Australis (titer, 200 to 400).

Statistical analysis of agreement between the assays in classifying cases and noncases revealed good agreement for DST and ELISA, with 82% concordance on cases, 96% on controls, and a kappa correlation of 0.86 (95% CI, 0.82 to 0.90) (Table 5). Good agreement was also obtained between the MAT and the DST, with a kappa correlation of 0.85 (95% CI, 0.79 to 0.89), and between ELISA and MAT, with a kappa correlation of 0.78 (95% CI, 0.72 to 0.83). None of the assays had poor agreement (kappa < 0.45).

TABLE 2. Sensitivity and specificity of five assays for detection of *Leptospira* antibodies, calculated using LCA

Assay	Model 1 (includes clinical definition)		Model 2 (includes MAT)	
	% Sensitivity (95% CI), predicted cases[a] (n = 131)	% Specificity (95% CI)	% Sensitivity (95% CI), predicted cases[a] (n = 132)	% Specificity (95% CI)
ELISA	89.9 (84.7–95.1)	97.4 (96.2–98.6)	90.7 (85.6–95.8)	97.4 (96.2–98.6)
IHA	81.1 (74.4–87.9)	95.9 (94.4–97.5)	81.5 (74.8–88.3)	95.9 (94.3–97.4)
LDS	94.5 (90.5–98.4)	89.5 (87.1–91.8)	94.8 (90.8–98.6)	89.4 (87.0–91.7)
DST	95.9 (92.4–99.4)	99.1 (98.3–99.8)	96.4 (93.0–99.7)	98.2 (98.2–99.8)
MAT			98.2 (95.8–100.6)	96.4 (95.0–97.9)
Clinical[b]	98.2 (95.7–100.6)	99.2 (98.6–100.0)		
χ^2 df 20[c]	89.5		84.3	

[a] For all assays, a case was considered positive if either the acute or convalescent specimen tested positive.
[b] MAT was part of the case definition and defined most cases.
[c] Goodness of fit for chi-square test with 20 degrees of freedom.

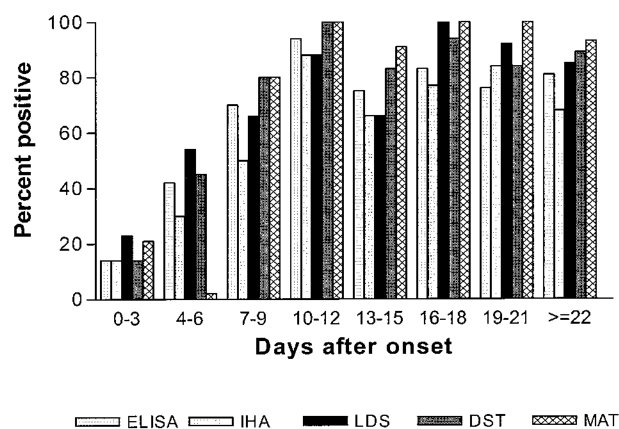

FIG. 1. Effect of specimen timing on the sensitivity of serologic assays for diagnosis of acute leptospirosis.

DISCUSSION

In this study we compared the performance of four rapid assays for the serologic diagnosis of leptospirosis. The overall case sensitivity of the rapid assays was quite good (79 to 93.2%). However, for acute-phase sera, the sensitivity of all the assays (including the MAT) was relatively low (38.5 to 52.7%). The sensitivity for convalescent-phase sera was much higher (67.2 to 93.8%).

The present study included a wide panel of controls from potentially cross-reactive diseases obtained from areas where leptospirosis is not endemic and cases from four different geographic locations representing a wide range of serogroups. In contrast, most previous studies were performed on cases from the same population (16, 21, 22, 41). Reported variations in diagnostic assay performance may reflect population-related differences such as those observed in this and other studies (3, 31, 32). Additionally, one of the main limitations for any evaluation of assays for serologic diagnosis of leptospirosis is the paucity of cases confirmed by culture. As a result, findings from new serologic assays are compared with those from cases that are primarily defined by another serologic assay. Consequently, there are very few reports of sensitivity and specificity of the MAT (12), because it is the gold standard against which other assays are usually compared. The majority of the cases in this study were defined by the MAT. Previous studies indicated 98% sensitivity for the MAT (10, 41), but this figure is much higher than that obtained in studies which defined cases by isolation of leptospires (12). The MAT specificity of 97% in the present study is similar to those previously reported.

The MAT does not have perfect sensitivity and specificity; thus, evaluations based upon cases defined by MAT serology yield results that are inaccurate. To estimate sensitivity and specificity more accurately, we used LCA, which has been shown to be effective in the absence of a true standard (4). The sensitivity of the rapid assays as determined by LCA ranged from 81.1 to 95.9%, and the specificity ranged from 89.5 to 99.1%. In the second model, MAT had a sensitivity of 98.2% but the specificity was 96.4% (Table 2). The MAT sensitivity is similar to that for the clinical case definition, but the specificity of the MAT was lower. The MAT was not included in model 1, because it defined most of our cases and therefore there was insufficient independence from the clinical case definition. Model 1 is believed to give a more accurate estimate, because the sensitivity and specificity for the clinical case definition approached unity and thus paucity of data was not an issue in fitting the model. However, it should be noted that there was a severe lack of fit for the two models described above which can be attributed to two cells, one in which three noncases were positive by ELISA and DST and another in which three cases were positive by LDS only. Without these cells, the models fit very well.

Numerous alternatives to the MAT have been described and evaluated. Of these tests, the most variable in performance is the IHA (33). A wide range of sensitivities (55 to 100%) have been reported for this test (16, 21, 22, 33, 41). In the present study we determined (by LCA [in parentheses]) an overall case sensitivity of 78.6% (81.1%) for the IHA, with sensitivities of 38.5% for acute sera and 68.2% for convalescent-phase sera. Factors that have affected the reported sensitivity of IHA include study design, case definition, and interlaboratory variations in reading the agglutination reaction. The time between the onset of symptoms and the collection of the acute phase specimen greatly affects the sensitivity of the IHA. Only 26% of specimens collected within 7 days of onset were positive by this test, whereas 88% of specimens were positive between days 10 and 12 (Fig. 1). Therefore, laboratories using the IHA need to emphasize the importance of obtaining accurate onset and collection dates for test specimens.

Lower sensitivity of earlier assays compared to that of the newer IgM detection tests has been reported previously (21). Sensitivities ranging from 68 to 100% have been reported for various ELISAs (5, 10, 19, 22, 36, 39, 41). In this study we found a case sensitivity for the IgM ELISA of 86.5% (89.9% by LCA), with sensitivities on acute and convalescent-phase sera of 48.7 and 75.0%, respectively. Geographical location may affect the performance of the ELISA, with sensitivities ranging from 73.7% for patients from Thailand to 100% for patients in the United States. However, the specificities of 96% for IHA and 97% for ELISA are similar to those obtained in earlier studies (19, 22, 33). Sensitivities for specimens from patients from Hawaii in all the assays were >90%. Although these sensitivities were much higher than those previously obtained using the same assays in Hawaii (15), the difference is attributable to the method by which sensitivities were calculated. In the present study, case sensitivity was determined, while in the study by Effler et al. (15), sensitivity by specimen was calculated by using all specimens collected <42 days after onset of

TABLE 3. Sensitivity of each assay among cases from different geographical locations

Location (state[s] or commonwealth or case group)	No. of cases[a]	% Sensitivity by:			
		ELISA	IHA	DST	LDS
United States (Hawaii)	65	90.8	96.5	96.9	90.8
United States (Puerto Rico)	18	94.4	83.3	100	100
United States (other states)	12	100	83.3	83.3	91.7
United States (triathletes[b])	18	66.7	66.7	72.2	94.4
Thailand	19	73.7	79.0	94.7	94.7

[a] One case from Palau was excluded from this analysis.
[b] Point source outbreak among participants of a triathlon event (Springfield, Illinois, 1998).

TABLE 4. Cross-reactivity of each assay with serum specimens from healthy volunteers and those previously diagnosed with other infectious or autoimmune diseases

Specimen category	Total no. of specimens tested	No. (%) of specimens positive by:				
		ELISA	IHA	LDS	DST	MAT
Antinuclear antibody	19	0	0	1 (5.3)	0	0
Autoimmune disease (ANCA)[a]	19	2 (10.5)	0	2 (10.5)	1 (5.3)	0
B. burgdorferi	49	2 (4.1)	1 (2.0)	6 (12.2)	0	0
Brucella spp.	14	0	0	1 (7.1)	0	0
C. pneumoniae	43	1 (2.3)	0	0	0	2 (4.8)
Cytomegalovirus	42	4 (9.5)	1 (2.4)	0	0	1 (2.4)
Dengue virus	29	0	0	4 (13.8)	0	0
EBV	21	1 (4.8)	5 (23.8)	4 (19.5)	2 (9.5)	2 (9.5)
Hantavirus	12	0	2 (16.7)	1 (8.3)	0	0
Viral hepatitis	39	3 (7.7)	4 (10.3)	4 (10.3)	1 (2.3)	4 (10.5)
HIV	20	2 (10.0)	3 (15.0)	6 (30.0)	2 (10.0)	0
L. pneumophila	44	0	2 (4.6)	0	0	1 (2.3)
Mycoplasma spp.	44	1 (2.3)	1 (2.3)	1 (2.3)	0	3 (7.0)
Melioidosis	20	0	2 (10.0)	1 (5.0)	0	0
N. meningitidis group C	10	1 (10.0)	0	0	0	1 (10.0)
Periodontal disease	10	0	0	2 (20.0)	0	0
Plasmodium spp.	10	0	0	1 (10.0)	0	1 (10.0)
Rheumatoid arthritis	19	0	2 (10.5)	2 (10.5)	0	0
Rickettsia spp.-*Ehrlichia* spp.	12	0	0	0	1 (8.3)	0
T. pallidum	13	0	1 (7.7)	1 (7.7)	0	0
Toxoplasma gondii	20	1 (5.0)	0	1 (5.0)	0	0
Healthy volunteers	133	1 (0.8)	2 (2.3)	29 (21.8)	1 (0.8)	2 (1.5)

[a] ANCA, antineutrophil cytoplasmic antibody.

symptoms. However, sensitivities obtained according to the number of days after the onset of illness were similar to those obtained in this study.

The two dipstick assays were found to be more sensitive than the other assays in this study. The LDS was the most sensitive assay, but the specificity was lower than that of the other assays. The sensitivity and specificity in this study were similar to those reported previously (31). According to LCA, the DST had the highest sensitivity (95.9%) and a specificity of 99.1% (Table 2).

Sensitivity of the serodiagnostic assays for acute-phase sera is most important, as it can greatly impact patient management. IgM antibodies have been detected as early as the second day after onset of symptoms, while IgG antibodies were detectable by the seventh day of illness (29). All assays in this study detected antibody in some of the cases within the first 3 days of illness (Fig. 1). In previous studies the IgM-ELISA has been more sensitive than the MAT in tests of early acute-phase sera (2), and in the present study this difference in sensitivity was evident in tests of sera collected as early as 4 to 6 days after the onset of illness (Fig. 1). The highest sensitivities within the first 7 days of illness were obtained with the LDS (40%) and the DST (36%). Although the antibodies involved in *Leptospira* agglutination have been observed to be primarily of the IgM class (1), delayed seroconversion by MAT has been reported, with an estimated occurrence rate of 10% (38). When IgG antibodies are detectable, only low levels are usually obtained (35). This observation favors the use of IgM-specific assays in the diagnosis of leptospirosis. Of the IgM detection assays studied, ELISA had the lowest sensitivity within the first 7 days of illness and LDS had the highest. The sensitivity of the IgM detection assays can be affected as a result of competitive inhibition of IgM binding in the presence of high levels of specific IgG (7); however, this was not the case for DST, in which IgG is removed at the dilution stage. Long-term persistence of IgM, for months or years after recovery, has been reported (2, 13, 29, 35, 39). This may complicate the interpretation of results of IgM-specific assays in areas of endemicity, particularly when a single serum sample is tested.

Given the broad cross-reactivity between *Leptospira* serovars and the possibility of infection with multiple serovars, it is difficult to determine the infecting serovar serologically (20). Most assays use a single serovar as the antigen (2, 35), and this is the case with all of the commercial assays in this study, each of which used serovar patoc. However, all of the assays reacted broadly with the serogroups positive by MAT in this study. The

TABLE 5. Agreement between diagnostic assays for leptospirosis in classifying cases as positive and controls as negative

Assays compared	No. (%) of cases testing positive[a]	No. (%) of controls testing negative[b]	Kappa correlation (95% CI)[c]
ELISA-MAT	115 (86.5)	604 (94.1)	0.78 (0.73–0.84)
ELISA-IHA	95 (71.4)	597 (93.0)	0.66 (0.59–0.73)
ELISA-LDS	110 (82.7)	559 (87.1)	0.62 (0.55–0.69)
ELISA-DST	112 (84.2)	620 (96.6)	0.86 (0.81–0.91)
IHA-MAT	105 (79.0)	595 (92.7)	0.70 (0.63–0.76)
IHA-LDS	101 (75.9)	553 (86.1)	0.57 (0.50–0.64)
IHA-DST	101 (75.9)	608 (94.7)	0.73 (0.67–0.80)
MAT-LDS	124 (93.2)	555 (86.5)	0.66 (0.60–0.72)
MAT-DST	122 (91.7)	614 (95.6)	0.85 (0.80–0.90)
DST/LDS	117 (88.0)	569 (88.6)	0.67 (0.61–0.74)

[a] For each assay, a case was considered positive if the acute- and/or convalescent-phase specimen(s) tested positive. Total number of cases, 133.

[b] Total number of controls, 642.

[c] A kappa correlation of ≥0.75 was considered a good agreement; a correlation of 0.45 to 0.74 was considered a fair agreement, and a correlation of <0.45 was considered a poor agreement.

sera from the triathletes had the lowest sensitivity by all the assays; both culture and serologic investigations suggested that the infecting serovars were of the Grippotyphosa serogroup (23).

All of the assays had fair to good agreement, as evidenced by kappa scores. Previous reports noted high concordance between ELISA and MAT (36). A high agreement (89%) has also been reported between ELISA and LDS (19), but the agreement obtained in this study (80%) was not as high and the kappa value of 0.62 indicates fair agreement only. This decreased concordance between ELISA and LDS may result from the lower specificity of the LDS observed in the present study.

Some of the control sera from cases of other infectious diseases or autoimmune diseases were found to react in all of the five assays (Table 4). A number of these disease agents have been reported by other investigators to cross-react in leptospirosis serologic assays (10, 19, 22, 24, 26, 39). The DST had the least cross-reactivity in this study. The MAT also had low cross-reactivity (Table 4), and this was limited to 7 of the 17 serogroups in the antigen panel. Cross-reactive antibodies associated with syphilis, relapsing fever, Lyme disease, and legionellosis have been detected with the MAT and sometimes even with significant seroconversion (38). Although not all of these conditions yielded cross-reactions in this study, cross-reactions were observed with other agents or conditions not documented previously. The collection of controls was limited to areas of nonendemicity or low prevalence, but prior exposure to *Leptospira* cannot be excluded completely. Cross-reactivity also occurred with sera from some of the healthy controls, possibly as a result of preexisting conditions. Some of the healthy controls testing as positive had a history of travel to an area in which leptospirosis is endemic, but this was shown not to confound our results. The sera from patients with melioidosis were obtained from an area in which leptospirosis is endemic, but cross-reactions to these sera occurred only with the IHA and LDS. Similarly, sera which may be from areas in which leptospirosis is endemic and which tested positive for dengue virus cross-reacted in LDS only. In general, false-positive results obtained in all the assays tended to fall around the cutoff value. Although the LDS showed high sensitivity, it was also the least-specific test, reacting with sera from confirmed cases of 16 of the 21 infectious or autoimmune diseases included as controls.

Two of the assays (DST and ELISA) were based upon EIA technology, and another (LDS) utilized colloidal dye. The remaining assay (IHA) was a biologic assay in which hemagglutination was the endpoint. While the microtiter plate ELISA gave a numerical endpoint, all other assays required interpretation of color intensity or agglutination and thus introduced a degree of subjectivity. The least subjective assay was the DST, in which a positive result was defined by the appearance of two or more discrete dots against a white background.

In recent years, leptospirosis has reemerged as a significant infectious disease in the United States and Central America (6, 23, 28, 37). The second-generation assays included in our study (DST and ELISA) showed significantly higher sensitivity with early acute-phase sera than the reference or first-generation methods (MAT and IHA) while retaining high specificity and should greatly improve the rapid detection of leptospirosis in the field.

ACKNOWLEDGMENTS

We thank the following colleagues from groups within and outside CDC that assisted us with control sera used in this study: Nancy Young, Thomas Ksiazek, Marianna Wilson, Floyd Dewhirst, Wanda Philips, Scott Dowell, Ian Williams, Jim Olson, Janice Lopez, Lorna Pais, William St. Clair, George Carlone, Vance Vorndam, Marty Schriefer, and Thomas Spira.

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