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Evaluation of Four Whole-Cell *Leptospira*-Based Serological Tests for Diagnosis of Urban Leptospirosis▽

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Four serologic assays for leptospirosis had sensitivities of 72 to 88% and specificities of 88 to 100% in the setting of highly endemic urban transmission, indicating that assays using enzyme-linked immunosorbency and rapid formats may be used as alternatives to the microscopic agglutination test for diagnosing urban leptospirosis. Testing a second sample will be required in cases with an initial negative result, since sensitivity was low (46 to 68%) during the first week of illness.

Leptospirosis is a major public health problem in developing countries where endemic transmission and outbreaks of this spirochetal disease cause high mortality and morbidity (4, 18, 21). Leptospirosis produces a spectrum of clinical manifestations which range from a mild febrile illness to severe disease forms such as Weil's syndrome and severe pulmonary hemorrhage syndrome (9, 10), and the case fatality rate for severe forms of leptospirosis is 5 to 40%, respectively (4, 9, 10, 21). Prompt identification of leptospirosis is needed, as antibiotic therapy provides the greatest benefit when administered early in the infection stage (9, 38).

Timely diagnosis relies on an effective laboratory test, since the presentation of early-phase leptospirosis is often nonspecific (4, 18, 21). Misdiagnosis is a major problem in regions where other causes of undifferentiated febrile illness and hemorrhagic fever are endemic (12, 14, 17, 23, 37, 39). The microscopic agglutination test (MAT), the standard for diagnosis confirmation, is impractical for clinical decision making since it requires analysis of paired serum samples for proper interpretation and a reference laboratory to perform dark-field microscopy (9, 18). Whole-cell *Leptospira*-based serologic assays using enzyme-linked immunosorbent assay (ELISA) and rapid formats have been developed as more feasibly employed alternatives. Large evaluations, several of which were multicenter trials, found that the sensitivities and specificities of these tests ranged from 28 to 72% and 10 to 99%, respectively (2, 8, 13, 19, 31, 33–35). Performance varies significantly across geographical regions, indicating that these assays need to be validated for distinct epidemiological situations. Furthermore, recent studies have found that their performance was poor in settings of highly endemic rural transmission of leptospirosis (5, 24, 29, 36).

Leptospirosis has become an urban health problem as slum settlements have grown worldwide and outbreaks in these communities have increased (4, 15, 16, 21, 28, 30). To date, a side-by-side comparison of commercially available whole-cell *Leptospira*-based serologic tests has not been performed in defined populations from areas of highly endemic urban transmission. Herein we report the performance of four serologic assays with patient and control subject groups from the city of Salvador, Brazil, where the incidence of severe leptospirosis is 10 people per 100,000 population (16, 30).

A serum panel was created from leptospirosis cases identified during active surveillance and from healthy blood bank donors. Acute-phase samples were evaluated for 96 cases which were randomly selected from among the 296 cases identified between 2000 and 2005 that provided paired serum samples and that had MAT and culture isolation criteria for confirmed leptospirosis (16). We excluded cases that were confirmed on the basis of a single acute-phase sample since these patients presented later in the course of their illness and their inclusion would introduce sample bias. The serum panel included 29 acute-phase samples collected within less than 7 days from the onset of illness. To obtain a statistically solid number of sera to specifically explore the performance of the tests in this clinically important early stage of the disease, an additional group of 43 randomly selected cases was included. Convalescent-phase samples were evaluated from 50 leptospirosis cases randomly selected from the 96 cases. Sera from 80 blood bank donors who were residents of Salvador during the surveillance period were evaluated as control samples. The evaluation of the serum panel ($n = 269$) was conducted in a double-blinded manner. The *Leptospira* immunoglobulin M (IgM) ELISA and Dip-S-Tick (PanBio, Inc.), LeptoTek Dri-Dot (Biomérieux), and EIE-IgM-Leptospirose (Bio-Manguinhos, Oswaldo Cruz Foundation) assays were performed according to the manufacturers' instructions. All assays detected IgM antibodies except the Dri-Dot test, which detected *Leptospira*-specific antibodies. Sensitivity and specificity were defined, respectively, as the proportion of samples from leptospirosis patients which were positive and the proportion of samples from healthy individuals which were negative according to the criteria stipulated for each kit. EpiInfo version 3.3 (Centers for Disease Control and Prevention) was used to

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TABLE 1. Performance of four serologic assays for leptospirosis in subject groups from an urban center with highly endemic transmission in Brazil

Assay[a]	% Sensitivity (95% CI) for group in:			% Specificity (95% CI) (n = 80)
	Acute phase (n = 96)	Acute phase (n = 72)[b]	Convalescent phase (n = 50)[c]	
MAT	68.8 (58.4–77.6)	45.8 (34.2–57.9)	100.0 (91.1–100.0)	100.0 (94.3–100.0)
ELISA BM	79.2 (69.4–86.5)	54.2 (42.1–65.8)	96.0 (85.1–99.3)	95.0 (87.0–98.4)
ELISA PB	87.5 (78.8–93.1)	66.7 (54.5–77.1)	92.0 (79.9–97.4)	87.5 (77.8–93.5)
DD	80.0 (70.3–87.2)	50.0 (37.9–62.1)	84.0 (70.3–92.4)	95.0 (87.0–98.4)
DS	72.3 (62.0–80.8)	32.9 (22.4–45.2)	80.0 (65.9–89.5)	100.0 (94.2–100.0)

[a] ELISA BM, EIE-IgM-Leptospirose (Bio-Manguinhos); ELISA PB, *Leptospira* IgM ELISA (PanBio); DS, Dip-S-Tick (PanBio); DD, LeptoTek Dri-Dot (bioMérieux); MAT, using a screening titer criterion of ≥1:100 in the microagglutination test.

[b] The acute-phase group included twenty-nine acute-phase patients and an additional 43 randomly selected patients; sera were collected <7 days from the onset of illness. CI, confidence interval.

[c] Convalescent-phase cases were randomly selected from the 96-acute-phase-patient group.

calculate 95% confidence intervals for sensitivity and specificity estimates and determine significant differences ($P < 0.05$) according to the chi-square test. The study protocol was approved by the institutional review board committees of the Oswaldo Cruz Foundation-Brazilian Ministry of Health and the Weill Medical College of Cornell University.

The sensitivities of the assays for identifying acute-phase leptospirosis ranged from 72 to 88% (Table 1). The mean interval between the onset of illness and the time of collection of acute-phase samples was 8.5 ± 3.8 days. There were no significant differences among the sensitivities of the four assays. The assays had greater sensitivities than that obtained with the MAT titer criterion of ≥1:100, which is recommended as a criterion for acute-phase illness screening (38). The sensitivities of the assays increased to 80 to 96% for convalescent-phase samples, which were collected 23.3 ± 7.4 days after the onset of illness (Table 1). Specificities of the assays were, in general, high, although the PanBio ELISA had a lower specificity (88%) than that of the other assays (95 to 100%) (Table 1).

The overall concordance among the four serologic assays was good (kappa, >0.67) in tests of acute- and convalescent-phase samples from leptospirosis cases and healthy subject samples. Concordance among the *Leptospira* IgM ELISA and the LeptoTek Dri-Dot and EIE-IgM-Leptospirose assays with the MAT screening criteria was moderate to good (kappa, 0.41 to 0.90) for all three sample groups. However, agreement between the Dip-S-Tick and the MAT screening was poor (kappa, 0.34) when sera collected less than 7 days after onset of illness were tested.

The sensitivities of the four assays were 33 to 67% in diagnosing leptospirosis with acute-phase samples that were obtained less than 7 days from the onset of symptoms (Table 1). When results were stratified further, the assays had sensitivities of 33 to 52% and 45 to 78% in detecting leptospirosis on the second to fourth and the fifth to seventh days of illness, respectively (Fig. 1). The PanBio ELISA had the highest sensitivity during these intervals.

The study findings indicate that the available whole-cell *Leptospira*-based serologic assays are useful methods for the laboratory diagnosis of urban leptospirosis. We found overall performance characteristics in the urban setting of endemicity were similar to those reported previously for large multicenter evaluations (33, 34). Despite limitations with respect to the sensitivity during early-phase illness, the assays have high specificities and will be more feasibly implemented than the MAT. Our findings differ with those of recent evaluations which showed that whole-cell *Leptospira* assays had low specificity in rural regions with highly endemic transmission (5, 7, 35, 36). These discrepancies most likely relate to differences in the timing of the collection of serum samples and/or the proportions of patients who experienced primary and secondary *Leptospira* infections among the studies. Alternatively, the differences may relate to the etiologic serovar(s) associated with rural and urban settings. Copenhageni is the serovar most frequently reported for urban leptospirosis (16, 25, 27, 28), whereas rural leptospirosis is often due to concurrent transmission of several serovars (9, 18). Our evaluation was conducted from a single site and therefore may not necessarily apply to other urban settings of endemicity. However, the study's findings are consistent with those of evaluations of in-house assays or individual commercial kits which were performed with predominantly urban-based subjects from other sites (3, 22, 25, 28, 32).

Although the assays used different formats and antigen preparations, they demonstrated similar sensitivities and specificities. This finding is not unexpected, since whole-cell *Lep-*

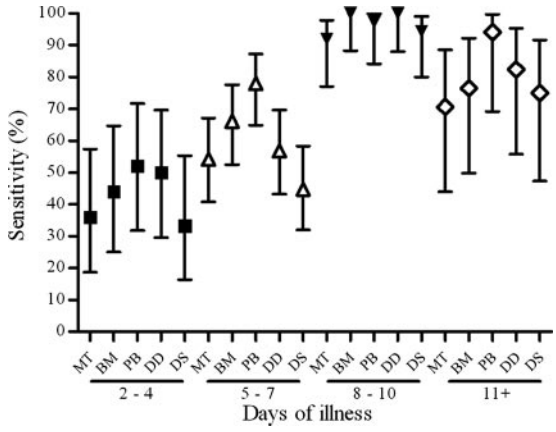

FIG. 1. Sensitivities of the whole-cell *Leptospira* assays according to the number of days of illness. Mean sensitivity and 95% confidence intervals are shown for the MAT screening titer criterion of ≥1:100 (MT) and the EIE-IgM-Leptospirose (BM), *Leptospira* IgM ELISA (PB), Dri-Dot (DD), and Dip-S-Tick (DS) assays.

tospira-based assays appear to detect antibodies against immunodominant carbohydrate epitopes, such as broad reactive antigen (1, 9, 20). The type of assay format, whether ELISA or rapid detection based, was not associated with a significant increase in performance. The selection of an assay will therefore rely more on availability, cost, and the feasibility of implementing the test for point-of-care diagnosis. It is important to note that screening assays do not discriminate among infections due to different infecting serogroups. Additional diagnostic methods, such as culture isolation and the MAT, will continue to be required in order to monitor changes in circulating serogroups that may occur during surveillance.

The major limitation of whole-cell *Leptospira*-based serologic assays is the low sensitivity (<67%) to samples obtained from patients in the first week of illness. This finding appears to be a phenomenon widely observed across geographical regions (2, 7, 13). In our study, the assays' sensitivities increased to >90% for leptospirosis patients who presented after 8 to 10 days of illness (Fig. 1). Therefore testing of a second sample, as in the case of dengue, is recommended for suspected cases with initial negative or doubtful results. Nevertheless, there is a continued need to develop new diagnostic approaches, such as recombinant protein-based serologic tests and antigen capture assays, which detect leptospirosis early in the course of illness and can be feasibly applied in point-of-care settings (6, 11, 26). Such assays may form the basis of public health responses that aim to initiate timely therapeutic interventions and reduce high mortality due to severe disease forms.

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