

Two Methods for Rapid Serological Diagnosis of Acute Leptospirosis

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Leptospirosis is a common and underdiagnosed zoonosis. Two rapid assays for serological diagnosis of acute leptospirosis in diagnostic laboratories, the immunoglobulin M (IgM)-dipstick assay and the indirect hemagglutination assay (IHA), were evaluated and compared with standard assays. Sera were examined from 104 patients admitted to a hospital for investigation in a leptospirosis diagnostic protocol. Specimens for serology were taken on days 1 and 4 of the patients' hospital stay. Antibodies were detected using an IgM-enzyme-linked immunosorbent assay (ELISA), microscopic agglutination test (MAT), an IgM-dipstick assay, and an IHA. Fifty-one patients were found to have leptospirosis. The sensitivity of the IgM-dipstick assay was 98%, its specificity was 90.6%, its positive predictive value was 90.9%, and its negative predictive value was 98%. The sensitivity of the IHA was 92.2%, its specificity was 94.4%, its positive predictive value was 95.9%, and its negative predictive value was 92.7%. The standard IgM-ELISA and MAT, were positive in the first samples tested from 67 and 55% of the cases, respectively, and the rapid IgM-dipstick assay and IHA were positive in 71 and 49%, respectively, in the first sample tested. Both rapid assays are highly sensitive and specific. Neither requires specialized equipment, and both are suitable for use in diagnostic laboratories.

Leptospirosis is an acute febrile disease, widely recognized as being emergent or reemergent (5, 13). In tropical and subtropical regions, the disease is endemic, and exposure to infection is widespread (8, 12, 20). In temperate climates the disease is primarily one of occupational or recreational exposure, as evidenced by a recent large outbreak in the United States that was associated with swimming during a triathlon (6).

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). The mortality rate in severe leptospirosis can be as high as 15% (12); early diagnosis is essential if antibiotic treatment is to be effective.

Detection of immunoglobulin M (IgM) antibodies by enzyme-linked immunosorbent assay (ELISA) has been used widely (1, 19) and is more sensitive than MAT (7). Several rapid methods for antibody detection are now available commercially, which detect genus-specific antibodies, either IgM (17, 21) or both IgG and IgM (2, 4, 14). The reported sensitivities of these assays have ranged from 87 to 100%. In this study we evaluated two of those rapid assays, which can be used in laboratories with little specialized equipment, the IgM-dipstick assay and the indirect hemagglutination assay (IHA).

MATERIALS AND METHODS

Specimens. Samples were obtained from patients admitted to the Queen Elizabeth Hospital, Bridgetown, Barbados, who had a history and clinical manifestations suggestive of leptospirosis. The diagnostic protocol used in this study has been described previously (14). Blood samples for serology were collected

from patients on the day of admission and on the fourth day after admission, and for some patients a convalescent sample was also taken before discharge from the hospital or during a follow-up visit to the outpatient clinic. Blood cultures were made on the day of admission by inoculating three drops of blood into 10 ml of polysorbate medium at the patient's bedside (EMJH; Difco Laboratories). Urine from patients who were not anuric on the fourth day of their admission was inoculated into the same medium within 1 h of collection.

ELISA. IgG and IgM titers were determined by ELISA (19), using *Leptospira biflexa* Patoc 1 (serovar patoc) as antigen. An IgM titer of ≥ 160 was regarded as positive.

MAT. Sera were examined by the MAT, using a battery of 22 serovars to establish seroconversion or a rise in titer (7). The antigens used included both reference strains and locally prevalent serovars of the following serogroups (serovars in parentheses): Australis (bajan, barbadensis, and bratislava), Autumnalis (bim and fortbragg), Ballum (arborea and ballum), Bataviae (bataviae and brasiliensis), Canicola (canicola), Cynopteri (cynopteri), Grippityphosa (grippityphosa), Icterohaemorrhagiae (copenhageni), Mini (georgia), Panama (mangus and panama), Pomona (pomona), Pyrogenes (pyrogenes), Tarassovi (tarassovi), Sejroe (hardjo and sejroe), and *L. biflexa* Semarang (patoc).

The diagnosis of leptospirosis was confirmed by a fourfold rise in titer between two sera tested by the same method, an initial titer of ≥ 800 in the MAT, an IgM titer of ≥ 160 in the ELISA, a positive culture from blood or urine, or any combination of these results.

IHA. The IHA (MRL Diagnostics, Cypress, Calif.) was performed as described previously (14). Fifty microliters of a 1:50 dilution of each serum specimen was mixed with 25 μ l of either antigen-coated test cells or uncoated control cells, in the wells of a U-bottomed microtiter tray. Plates were incubated at 25°C for 1 h. Hemagglutination was read on a scale of 0 to +++++. Positive and negative control sera were tested each time the test was performed.

IgM-dipstick assay. IgM antibodies were detected using a semiquantitative dot-ELISA dipstick assay (Integrated Diagnostics, Baltimore, Md.). All steps were carried out at 50°C. In this assay, 10 μ l of serum and 40 μ l of goat antihuman IgG absorbent (proSorb G) were diluted in 2 ml of sample diluent and incubated at 50°C for 10 min prior to addition of the assay strip. Alkaline phosphatase-conjugated goat anti-human IgM (μ chain) was used as described previously (3). The assay required approximately 2 h for completion. After assay strips were allowed to dry, positive dots were gray to blue with distinct borders against a white background. Each assay strip contained positive and negative control dots. The test was scored on a scale of 1 to 4 dots; only strips with two or more positive dots were recorded as positive tests. Each positive dot represented an approximately fourfold difference in MAT titer.

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TABLE 1. Distribution of test results in A1 samples^a

No. of samples	Results for serological test			
	IgM-ELISA titer	MAT titer	IHA	IgM-dipstick
10	≤80	Neg	Neg	Neg
4	≤80	Neg	Neg	Pos
1	≤80	100–400	Neg	Neg
1	≤80	100–400	Pos	Neg
1	≤80	100–400	Neg	Pos
2	≥160	Neg	Neg	Neg
1	≥160	Neg	Neg	Pos
6	≥160	Neg	Pos	Pos
1	≥160	100–400	Neg	Neg
2	≥160	100–400	Neg	Pos
4	≥160	100–400	Pos	Pos
4	≥160	≥800	Neg	Pos
14	≥160	≥800	Pos	Pos

^a For description of scoring, see Materials and Methods.

RESULTS

Diagnostic samples. Two-hundred nine specimens were examined from 104 patients investigated using a protocol for the diagnosis of leptospirosis. Fifty-one patients were found to have leptospirosis, and 53 did not. Diagnoses in the 53 patients without leptospirosis included dengue fever (18 cases), acute hepatitis B (3 cases), typhoid (1 case), alcoholic hepatitis (5 cases), and obstructive jaundice (4 cases). A variety of other noncommunicable conditions was also represented in individual cases. Leptospire were isolated from 24 of 50 cases (48%), while the remaining cases were confirmed by serology. The isolates were identified as *Leptospira kirschneri* serovar bim, *L. interrogans* serovar copenhageni, and *L. borgpetersenii* serovar arborea.

The IgM-dipstick assay detected 50 cases of leptospirosis; there were five false-positive results. The sensitivity of the IgM-dipstick for detection of acute leptospirosis cases was 98%, its specificity was 90.6%, its positive predictive value was 90.9%, and its negative predictive value was 98%. The IHA detected 47 cases of leptospirosis; there were two false-positive IHA results. The sensitivity of IHA for detection of acute leptospirosis was 92.2%, its specificity was 94.4%, its positive predictive value was 95.9%, and its negative predictive value was 92.7%.

One-hundred three serum specimens were examined from 51 patients diagnosed with leptospirosis. First acute (A1) samples were taken on the day of admission to the hospital. The mean time from onset of symptoms to the collection of the A1 sample was 6.1 days (range, 1 to 17 days). The date of onset of symptoms was unknown for six cases. The second acute (A2) samples were taken 4 days after admission to the hospital, and a convalescent sample was available from one patient, taken 10 days after admission. One-hundred six specimens were examined from 53 patients whose diagnosis was not leptospirosis.

Results on A1 samples. The distribution of test results on A1 samples from leptospirosis patients is shown in Table 1. Seroconversion occurred as indicated by the MAT in all patients, and the A1 sample was negative in 23 of 51 patients (45%), whose samples were taken a mean of 5.1 days after onset. Eighteen (35%) of the A1 samples gave a MAT titer of ≥800; these samples were taken a mean of 8.1 days after onset of symptoms. In one patient, seroconversion did not occur until the convalescent sample, which was taken 10 days after admis-

sion to the hospital. In the remaining patients there was a fourfold rise in titer between the A1 and A2 samples, taken 4 days apart.

The IgM-dipstick assay was positive in 34 of the A1 samples (67%) taken a mean of 6.6 days after onset and negative in 17 (33%), of which 5 were IgM-dipstick positive and one was IHA positive. The IgM-dipstick was positive in A1 samples from 36 of 51 patients (71%) with leptospirosis, whereas IHA was positive in 25 of 51 A1 samples (49%). The intervals between onset of symptoms and positive and negative results in the respective tests are shown in Table 2. The sensitivity of diagnosis based on a positive A1 sample was 35% for MAT, 67% for IgM-ELISA, 71% for the IgM-dipstick assay, and 49% for the IHA.

DISCUSSION

The diagnosis of leptospirosis is often unconfirmed, because of a lack of clinical suspicion, inappropriate sample collection, the unavailability of testing facilities, or a combination of these factors. Several rapid assays have been developed recently (2, 11, 15, 16, 18), which can be used for screening of acutely ill patients.

Both of the rapid tests evaluated in this study exhibited high sensitivities (>92%) and specificities (>90%) for detection of cases of severe, acute leptospirosis. The IgM-dipstick (dot-ELISA) assay was more sensitive than the IHA, and it became positive earlier (Table 2). Assays which detect IgM are more sensitive than the MAT and give positive results earlier in the acute phase of the disease (7). This is important because if treatment decisions are to be based on laboratory results, they must be made as early as possible, often without having available the results from paired sera. When only samples from acutely ill patients were considered, the IgM-dipstick assay was of comparable sensitivity to the IgM-ELISA, whereas the sensitivity of the IHA was closer to that of the MAT.

The results of this study confirm our previous findings on the utility of the IHA in a population in which severe leptospirosis occurs at a high incidence (14). In other populations the sensitivity of the IHA has not been as high (9, 22). However, differences in case definition and case ascertainment may account for some of the observed difference in sensitivity. Both the present evaluation and our previous study (14) were performed in a population of hospitalized patients admitted for investigation and management of severe, acute leptospirosis. In one recent study, the sensitivity of IHA was considerably higher in leptospirosis cases that were hospitalized and in those from whom leptospire were isolated than in the overall population studied (9). Moreover, similar differences in test per-

TABLE 2. Time between onset of symptoms and positive and negative tests in 51 patients with acute leptospirosis

Method	Negative tests (days after onset)	Positive tests (days after onset)
MAT (titer ≥800)	5.1	8.1
IgM-ELISA (titer ≥160)	5.0	6.6
IgM-dipstick	4.8	6.5
IHA	5.0	7.3

formance were noted between these two populations in a recent multicenter evaluation of another IgM-dipstick assay (17).

The IgM-dipstick assay was simple to perform, and the only equipment required was a water bath or heating block. The assay can therefore be performed in peripheral laboratories with only rudimentary equipment. Each assay strip contains positive and negative control dots, making the IgM-dipstick economical for single-sample testing. In our experience the only limitation to its use has been the relatively small number of samples (8 to 10) that can be tested in one batch. However, each assay was completed in approximately 2 h. If large numbers of tests must be performed, then a conventional microtiter plate ELISA assay can be used.

The IgM-dipstick is a semiquantitative assay, with each extra dot representing an approximately fourfold increase in titer. Thus it is possible to detect both seroconversion and a rising titer of IgM antibodies. In contrast, another recently developed dipstick assay allows only approximate estimation of staining intensity (11, 17). For assays which are intended to be used as screening tests, this lack of quantitation is relatively unimportant.

Both of the rapid assays detected antibodies in patients infected with all three leptospiral serovars isolated from patients in this study. The range of serovars isolated from patients in Barbados over the past 20 years has been limited to four, including bim, copenhageni, arborea, and *L. noguchii* serovar bajan/barbadensis (10). The latter serovar has been isolated only rarely and was not recovered in this study.

In this study we evaluated two rapid assays for early diagnosis of acute leptospirosis in a hospital-based population. Both assays were highly sensitive and specific. Neither required specialized equipment, and could be performed in peripheral laboratories with relatively little expertise. The selection of a serodiagnostic assay is dependent on several factors, including the clinical likelihood of disease, the anticipated workload, and the availability of confirmatory testing in more specialized laboratories. Either of the tests studied may be suitable for use in diagnostic laboratories for screening sera from acutely ill patients.

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