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International Multicenter Evaluation of the Clinical Utility of a Dipstick Assay for Detection of *Leptospira*-Specific Immunoglobulin M Antibodies in Human Serum Specimens

HENK L. SMITS,[1]* YULIA V. ANANYINA,[2] ANNETTE CHERESHSKY,[3] LOUELLA DANCEL,[4] RUDY F. M. LAI-A-FAT,[5] HOWARD D. CHEE,[6] PAUL N. LEVETT,[7] TOSHIYUKI MASUZAWA,[8] YASUTAKE YANAGIHARA,[8] M. A. MUTHUSETHUPATHI,[9] EDUARD J. SANDERS,[10,11] DAVID M. SASAKI,[12] HARRY DOMEN,[13] CLAUDE YERSIN,[14] TIN AYE,[15] SANDRA L. BRAGG,[15] GEORGE C. GUSSENHOVEN,[1] MARGA G. A. GORIS,[1] WIEPKO J. TERPSTRA,[1] AND RUDY A. HARTSKEERL[1]

Department for Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands[1]; Gamaleya Research Institute for Epidemiology and Microbiology, Russian Academy of Medical Science, Moscow, Russia[2]; ESR: Health Communicable Disease Centre, Wellington, New Zealand[3]; Department of Medical Microbiology, College of Public Health, University of Philippines Manila, Manila, Philippines[4]; Department of Dermatology and Department of Internal Medicine,[6] Academic Hospital, Paramaribo, Surinam; Leptospira Laboratory, Ministry of Health and the Environment, St. Michael, Barbados[7]; Department of Microbiology, School of Pharmaceutical Sciences, dUniversity of Shizuoka, Shizuoka, Japan[8]; Department of Nephrology, Chennai Medical College of Tamil Nadu, Chennai, India[9]; Dengue Branch, Division of Vector-Born Infectious Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico[10]; Epidemiology Program Office[11] and Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases,[15] Centers for Disease Control and Prevention, Atlanta, Georgia; Epidemiology Branch, Department of Health, Hawaii, Department of Health, Honolulu, Hawaii[12]; Medical Microbiology Branch, Department of Health, Pearl City, Hawaii[13]; and Department of Medicine, Victoria Hospital, Victoria, Seychelles[14]

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We performed a multicenter evaluation of a robust and easily performed dipstick assay for the serodiagnosis of human leptospirosis. The assay is aimed at the detection of *Leptospira*-specific immunoglobulin M (IgM) antibodies. The study involved 2,665 serum samples collected from 2,057 patients with suspected leptospirosis in 12 countries on five continents with different levels of endemicity and different surveillance systems. The patients were grouped as laboratory-confirmed leptospirosis case patients and noncase patients based on the results of culturing and the microscopic agglutination test. Paired samples from 27.7% of the subjects were tested. Of the 485 case patients, 87.4% had a positive dipstick result for one or more samples. Of the 1,513 noncase patients, only 7.2% had a positive result. Whereas most (88.4%) of the positive samples from the case patients showed moderate to strong (2+ to 4+) staining in the dipstick assay, most (68.1%) of the positive samples from the noncase patients showed weak (1+) staining. The sensitivity of the dipstick assay increased from 60.1% for acute-phase serum samples to 87.4% for convalescent-phase samples. The specificities for these two groups of samples were 94.1 and 92.7%, respectively. The dipstick assay detected a broad variety of serogroups. The results of the dipstick assay were concordant (observed agreement, 93.2%; kappa value, 0.76) with the results of an enzyme-linked immunosorbent assay for the detection of specific IgM antibodies, a test which is often used in the laboratory diagnosis of current or recent leptospirosis. This study demonstrated that this easily performed dipstick assay is a valuable and useful test for the quick screening for leptospirosis; has a wide applicability in different countries with different degrees of endemicity; can be used at all levels of the health care system, including the field; and will be useful for detecting and monitoring outbreaks of leptospirosis.

Leptospirosis is a worldwide zoonosis caused by pathogenic spirochetes of the genus *Leptospira* (2, 4). Leptospirosis is a fairly common disease in humid and warm climates (1, 3). The disease varies from a mild flu-like form to severe forms, such as Weil's syndrome, which are characterized by renal failure, liver impairments, and hemorrhages and have a high mortality rate. Due to the complexity of the clinical symptoms and signs, leptospirosis is often misdiagnosed. Thus, laboratory support of the clinical diagnosis is essential (10).

Laboratory confirmation of leptospirosis is obtained when either the pathogen is isolated or a positive serological result is obtained. The microscopic agglutination test (MAT) is considered the reference test for leptospirosis. The result of the MAT is considered consistent with leptospirosis when either a ≥4-fold increase in titer is observed between paired serum samples or a significantly increased titer is observed for a single serum

* Corresponding author. Mailing address: Department of Biomedical Research, Royal Tropical Institute, Meibergdreef 39, 1105 AZ Amsterdam, The Netherlands. Phone: 31-20-5665470. Fax: 31-20-6971841. E-mail: H.Smits@kit.nl.

TABLE 1. Criteria used to define leptospirosis case patients for the different study groups

Country or state	Case definition[a]
Barbados	Culture positive, a ≥4-fold rise in the MAT titer, or an MAT titer of ≥1:800 (8)
India	MAT titer of ≥1:80
Kenya[b]	MAT titer of ≥1:320
New Zealand	Culture positive, a ≥4-fold rise in the MAT titer, or an MAT titer of ≥1:400
Philippines[c]	MAT titer of ≥1:400
Puerto Rico[d]	≥4-fold rise in the MAT titer or an MAT titer of ≥1:320
Surinam[b]	MAT titer of ≥1:320
The Netherlands	Culture positive, a ≥4-fold rise in the MAT titer with a minimum titer of 1:160 for the second sample, or an MAT titer of ≥1:160 (5)
Thailand[d]	≥4-fold rise in the MAT titer with a minimum titer of 1:320 for the second sample or an MAT titer of ≥1:320
Hawaii[e]	Culture positive or a ≥4-fold rise in the MAT titer and a minimum titer of 1:200 for the second sample[f]
Russia	MAT titer of ≥1:100 or a positive slide agglutination test (9)
Seychelles[g]	≥4-fold rise in the MAT titer and a minimum titer of 1:100 for the second sample[h]

[a] Patients with a clinical suspicion of leptospirosis but who did not meet the criteria as defined in Materials and Methods were considered noncase patients.
[b] The MAT was performed at the Royal Tropical Institute, Amsterdam, The Netherlands.
[c] The MAT and dipstick assay were performed at the University of Shizuoke, Shizuoke, Japan.
[d] The MAT and dipstick assay were performed at the Royal Tropical Institute.
[e] The MAT was performed at the Centers for Disease Control and Prevention, Atlanta, Ga.
[f] Patients with a minimum MAT titer of 1:200 but without a ≥4-fold rise between paired samples or with a minimum titer of 1:200 for single samples were considered probable leptospirosis patients.
[g] The MAT was performed at Institute Pasteur, Noumea, New Caledonia.
[h] Suspected leptospirosis patients with MAT titers of ≥1:400 but who did not show a ≥4-fold rise were considered probable leptospirosis patients.

sample (13). Recently, we developed a quick and easily performed dipstick assay, the LEPTO dipstick (5), for the serodiagnosis of leptospirosis. This assay, like the immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) (11, 12), detects *Leptospira*-specific IgM antibodies in human sera and is aimed at the serodiagnosis of current or recent leptospirosis. The test needs no special equipment, and its ingredients are highly stable and do not need refrigeration.

A previous evaluation of the dipstick assay with selected serum samples collected mainly in The Netherlands demonstrated high sensitivity and specificity and showed that the results correlated well with those of the ELISA (5). We argued that the dipstick assay could be a useful diagnostic tool for health facilities with few resources and in countries with different levels of endemicity. The detection of specific IgM antibodies could be useful in particular in countries with a high degree of endemicity to help distinguish between acute or recent leptospirosis and past leptospirosis. The present study was conducted to evaluate the performance of the dipstick assay in different countries, including countries with low and countries with high prevalences of the disease. In this study, the performance of the dipstick assay was evaluated with serum samples from patients with suspected leptospiral infection and grouped as laboratory-confirmed leptospirosis case patients or noncase patients based on the results of culturing and the MAT. The results of the dipstick assay also were compared with those of the IgM ELISA.

MATERIALS AND METHODS

Participating laboratories and study groups. Serum samples collected during a certain period from all patients with a clinical suspicion of leptospirosis at nine laboratories in Barbados, Hawaii, India, New Zealand, the Philippines, Russia, the Seychelles, Surinam, and The Netherlands were included in the study. In Surinam, samples were collected during two different periods (studies I and II). In addition, the dipstick assay was tested with samples collected from patients with fever in rural hospitals in Kenya and Thailand. In Puerto Rico, the dipstick assay was tested with samples collected from patients who had a dengue-like illness, who failed to demonstrate anti-dengue virus IgM antibodies (6, 7), and who were thus considered negative for dengue.

Leptospirosis case definition and composition of study groups. Patients with a clinical suspicion of leptospirosis were grouped as laboratory-confirmed leptospirosis case patients and noncase patients based on the results of laboratory procedures (culturing and MAT) performed and interpreted according to criteria routinely used in each of the laboratories performing the tests (Table 1). Paired serum samples were available from 27.7% of the patients, so confirmation of leptospirosis by demonstration of seroconversion or a ≥4-fold rise in the MAT titer was possible for only a portion of the patients. Hence, a single raised MAT titer was accepted as the confirmation of leptospirosis for single serum samples (13). Patients in the study groups from Hawaii and the Seychelles and with MAT titers above the cutoff value but not showing seroconversion or a ≥4-fold rise in titer were considered probable leptospirosis patients. These patients were excluded from the analysis. The numbers of case patients and noncase patients, the number of serum samples for each of these groups, and the percentage of patients with more than one sample are shown in Table 2.

The MAT was performed at the Royal Tropical Institute, Amsterdam, The Netherlands, for the study groups from The Netherlands, Surinam, Thailand, and Kenya. The MAT was performed in New Caledonia for samples from the Seychelles, in Japan for samples from the Philippines, and in the United States for samples from Puerto Rico and Hawaii (Table 1). For all other study groups, the MAT was performed at the collaborating laboratories contributing the samples. A detailed description of the panels of strains used in the MAT and the experimental procedures used for performing the MAT can be obtained from the authors.

The IgM ELISA was performed for seven of the study groups. The results of the IgM ELISA, although often used in the serodiagnosis of leptospirosis, were not taken into consideration for the definition of leptospirosis case patients, as doing so would cause bias for the presence of specific IgM antibodies.

LEPTO dipstick assay. Participants in the study were provided with sealed vials containing (i) 5 ml of lyophilized detection reagent, (ii) 5 ml of reconstitution fluid, and (iii) 5 ml of dipstick fluid; a tightly closed container containing dipsticks and a desiccant; test tubes; and a test tube rack. Participants were asked to perform the test according to the instructions given in an accompanying protocol (5). Briefly, the dipstick assay is performed by incubation of a wet dipstick in a mixture of 250 μl of reconstituted detection reagent and 5 μl of serum at an ambient temperature for 3 h. At the end of the incubation period, the dipstick is rinsed with tap water and air dried, and the staining intensity of the antigen band is compared with that of a colored reference strip showing bands colored at different (1+ to 4+) intensities. In the evaluation of the dipstick assay, a staining intensity of ≥+1 is considered positive. Dipsticks are coated with a heat-stable antigen prepared from a culture of *Leptospira biflexa*.

ELISA. An ELISA for the detection of *Leptospira*-specific IgM antibodies (IgM ELISA) was performed at the Department for Biomedical Research of the Royal Tropical Institute with the serum samples from The Netherlands, Thailand, Puerto Rico, Hawaii, Surinam (study II), and the Seychelles. The ELISA was performed with antigen prepared from strain Wijnberg as described elsewhere (11, 12). The results of the ELISA were considered positive when a titer of ≥1:40 was obtained. For the Barbados samples, the ELISA was performed with antigen prepared from strain Patoc I as described previously (8).

Statistical evaluation. To calculate the sensitivity and specificity of the dipstick assay, the serum samples from leptospirosis case patients and noncase patients were stratified in stage I and stage II. For patients with paired samples, the samples collected first were considered stage I and the samples collected second were considered stage II. For patients from whom more than two samples were obtained, only the first two samples were considered. Single samples were considered stage I when collected during the first 10 days of the disease and stage II

TABLE 2. Composition of study groups and number and percentage of patients with a positive result in the dipstick assay

Country or state	Case patients			Noncase patients		
	No. of patients (% of patients with paired sera)	No. of sera	No. (%) of patients with a positive result	No. of patients (% of patients with paired sera)	No. of sera	No. (%) of patients with a positive result
Barbados	44 (100)	88	43 (97.7)	90 (46.7)	132	2 (2.2)
Hawaii	34 (91.2)	79	26 (88.2)	167 (65.3)	287	20 (12.0)
India	63 (0)	63	49 (77.8)	100 (0)	100	1 (1.0)
Kenya				165 (0)	165	1 (0.6)
New Zealand	34 (82.3)	61	31 (91.2)	110 (32.7)	149	15 (13.6)
Philippines	53 (0)	53	49 (92.5)	18 (0)	18	2 (11.1)
Puerto Rico	7 (42.9)	10	7 (100)	97 (17.5)	114	5 (5.2)
Russia	46 (8.6)	50	42 (91.3)	41 (12.2)	46	7 (17.1)
Seychelles	75 (100)	151	59 (78.7)	43 (72.1)	74	4 (9.3)
Surinam						
Study I	51 (0)	51	45 (88.2)	135 (0)	135	27 (20.0)
Study II	44 (13.6)	51	44 (100)	26 (11.5)	29	4 (14.8)
The Netherlands	17 (76.5)	40	16 (94.1)	411 (11.9)	440	17 (4.1)
Thailand	17 (76.5)	32	13 (76.5)	110 (40.9)	161	5 (4.6)

when collected more than 10 days after the onset of the disease, as IgM antibodies usually develop during the first 10 days of the disease (2). Single samples for which the duration of the disease was not reported were excluded from the analysis. Also, samples from patients with reported past leptospirosis were excluded from the analysis.

To compare the performance of the dipstick assay with that of the IgM ELISA, the intermethod agreement between the results of the two tests was determined. Kappa statistics were applied, as they offer a measure of agreement which is not attributable to chance. In general, a kappa value of >0.80 represents almost perfect agreement beyond chance. Values below 0.40 represent slight agreement, and values between 0.40 and 0.80 represent fair to good agreement.

RESULTS

Study population. In 12 countries on five continents, 2,665 serum samples from 2,057 patients with a clinical suspicion of leptospirosis were tested with the dipstick assay. According to the results of the MAT and culturing, 485 patients with 729 samples were considered leptospirosis case patients, 1,513 patients with 1,841 samples were considered noncase patients, and 59 patients with 95 samples were considered patients with probable leptospirosis. Paired samples were tested from 27.7% of the patients, including 217 case patients and 317 noncase patients (Table 2).

Percentage of leptospirosis case patients and noncase patients with a positive dipstick assay result. The mean percentage of case patients with one or more serum samples that showed staining of the antigen band of the dipstick was 87.4%; the percentages ranged from 76.5 to 88.2% for five studies and to over 90% for seven other studies (Table 2). For the studies performed in India, the Seychelles, and Thailand, the percentages of case patients with a positive dipstick result were relatively low, namely, 77.8, 78.7, and 76.5%, respectively. The samples from two of the four case patients in the study group from Thailand with negative results in the dipstick test showed borderline titers in the MAT. These samples also were negative in the IgM ELISA. The samples from 13 of the 17 case patients from the Seychelles with negative dipstick test results showed seroconversion in the MAT to a titer at or just above the cutoff value. The samples from 12 of these 13 case patients also were negative in the IgM ELISA. The agglutinating titers were not specified for the study performed in India. Also, an ELISA was not performed on the samples from India.

Samples from 7.2% (on average) of the noncase patients tested positive in the dipstick assay. The percentage of noncase patients with a positive score in the dipstick assay ranged from 0.6 to 20% for the different study groups (Table 2).

The percentage of probable leptospirosis patients from Hawaii and the Seychelles that tested positive in the dipstick assay did not differ from the percentage of case patients that tested positive.

Staining intensity of the antigen band. Semiquantitation of the dipstick results was used for all study groups, with the exception of some of the samples from Hawaii. The staining intensity of the antigen band on the dipstick was moderate to strong (≥2+) for most (88.4%) of the samples from the case patients with a positive score (Table 3). In contrast to the findings for the other study groups, a large proportion of the sera from the case patients in the Philippines stained weakly (1+) (data not shown).

As expected, a higher percentage of the stage II serum samples from the case patients showed staining, and the stain-

TABLE 3. Staining intensity of positive serum samples and stratification according to the duration of the disease

Patients and stage[a]	No. of patients	No. (%) of serum samples with the following staining intensity:				
		0	1+	2+	3+	4+
Case	650	149 (22.9)	58 (8.9)	112 (17.2)	185 (28.5)	146 (22.5)
I	219	81 (37.0)	21 (9.6)	32 (14.0)	46 (21.0)	39 (17.8)
II	220	24 (10.9)	13 (5.9)	52 (23.6)	73 (33.2)	58 (26.4)
Noncase	1,554	1,463 (94.1)	62 (4.1)	16 (1.0)	10 (0.6)	3 (0.2)
I	595	559 (93.9)	23 (3.9)	8 (1.3)	3 (0.4)	2 (0.3)
II	305	288 (94.1)	8 (2.6)	4 (1.3)	4 (1.3)	1 (0.3)

[a] See the text for explanations of stages.

TABLE 4. Sensitivity and specificity of the LEPTO dipstick assay in the acute phase of disease for groups of serum samples stratified according to the duration of the disease

Country	% Sensitivity (95% confidence interval) for stage[a]:		% Specificity (95% confidence interval) for stage[a]:	
	I	II	I	II
Barbados	81.0 (65–91)	97.6 (86–100)	98.9 (93–100)	95.3 (83–99)
Hawaii	50.0 (34–66)	77.1 (59–89)	92.3 (87–96)	89.0 (81–94)
India	81.1 (64–91)	69.2 (38–90)	99.0 (94–100)	99.9 (94–100)
New Zealand	40.9 (21–63)	90.0 (67–92)	88.1 (80–93)	84.5 (74–79)
Puerto Rico	85.7 (42–99)	100.0 (31–100)	97.1 (91–99)	92.3 (62–100)
Russia	65.2 (43–83)	100.0 (85–100)	87.0 (65–97)	84.0 (63–95)
Seychelles	35.1 (25–47)	77.8 (66–86)	93.0 (80–98)	96.8 (82–100)
Thailand	40.4 (18–67)	80.0 (51–95)	96.4 (91–99)	97.7 (87–100)
The Netherlands	62.5 (26–90)	95.2 (74–99)	93.2 (83–98)	97.1 (92–95)
Mean (SD)	60.1 (17.0)	87.4 (10.9)	94.1 (4.4)	92.7 (5.8)

[a] See the text for explanations of stages.

ing intensity was, on average, stronger than that of stage I serum samples (Table 3). Analysis of the results for study groups for which the time point of sample collection in relation to the duration of the disease was well documented (Barbados, The Netherlands, the Seychelles, and Hawaii) indicated that the majority of the positive samples showed moderate to strong staining intensity by day 6 or 7 after the onset of the disease (data not shown).

The staining intensity for 68.1% of the samples from the noncase patients with a positive result in the dipstick assay was rated 1+ (Table 3). Staining intensities of ≥2+ for samples from the noncase patients were notably found in the study groups from New Zealand and Surinam (study I).

Sensitivity and specificity of the dipstick assay. The mean sensitivity of the dipstick assay for stage I serum samples was calculated to be 60.1% (standard deviation [SD], 17.0) and ranged from just over 35% for the samples studied in the Seychelles, Thailand, and New Zealand to about 80% for the samples studied in Barbados, India, and Puerto Rico (Table 4). The mean sensitivity for stage II samples was 87.4% (SD, 10.9) and ranged from 69.2% for the samples studied in India to over 95% for the samples studied in Barbados, New Zealand, Puerto Rico, Russia, and The Netherlands.

The mean specificities of the dipstick assay for stage I and stage II samples were calculated to be 94.1% (SD, 4.4) and 92.7% (SD, 5.8), respectively (Table 4).

The sensitivity and specificity for the study groups for which information on the duration of the disease was lacking are presented in Table 5. The mean sensitivity was 91.6% (SD, 2.5), and the mean specificity was 88.6% (SD, 7.0).

Comparison of the dipstick assay and the IgM ELISA. The IgM ELISA was performed for the samples from Barbados, Hawaii, The Netherlands, Puerto Rico, Surinam (study II), the Seychelles, and Thailand. A comparison of the test results of the dipstick assay and the IgM ELISA yielded kappa values (agreement beyond chance) ranging from 0.69 for the group of samples from Puerto Rico to 0.83 for the group of samples from the Seychelles (Table 6). The mean observed agreement was 93.2%, and the mean kappa value was 0.76. These results show that the dipstick assay and the IgM ELISA yield concordant results. Discrepant results were obtained mainly for sera either with a borderline titer in the ELISA or showing weak staining in the dipstick assay.

DISCUSSION

The present study was initiated to evaluate the clinical utility of a dipstick assay for the detection of *Leptospira*-specific IgM antibodies in different areas likely to have different degrees of endemicity and where strains of different serovars cause leptospirosis. The results show that the dipstick assay combines a high specificity with a high sensitivity, in particular for samples collected in the convalescent phase of the disease. The mean sensitivity increased from 60.1% (SD, 17.0) for samples collected early in the acute phase of the disease to 87.4% (SD, 10.9) for samples collected later in the disease. The mean specificities for these two groups of samples were 94.1% (SD, 4.4) and 92.7% (SD, 5.8), respectively. Furthermore, most (88.4%) of the positive samples from the case patients gave a moderate to strong (2+ to 4+) staining intensity, and most (68.1%) of the positive samples from the noncase patients gave a weak (1+) staining intensity only.

We previously evaluated the dipstick assay with selected serum samples from leptospirosis case patients and noncase

TABLE 5. Sensitivity and specificity of the LEPTO dipstick assay in the acute phase of disease for groups of serum samples for which the exact duration of the disease was not reported

Country	% Sensitivity (95% confidence interval)	% Specificity (95% confidence interval)
Kenya		99.4 (96–100)
Philippines	92.5 (81–98)	88.9 (64–98)
Surinam		
Study I	88.2 (74–95)	80.0 (72–86)
Study II	94.1 (83–99)	86.2 (76–96)
Mean (SD)	91.6 (2.5)	88.6 (7.0)

TABLE 6. Comparison of the results of the IgM ELISA and the LEPTO dipstick assay

Country	Observed agreement (%)	Kappa value (SE)
Barbados	90.7	0.80 (0.07)
Hawaii	95.6	0.81 (0.07)
Puerto Rico	94.5	0.69 (0.09)
Seychelles	92.8	0.83 (0.05)
Surinam (study II)	88.8	0.77 (0.11)
Thailand	94.4	0.71 (0.07)
The Netherlands	95.4	0.74 (0.05)
Mean (SD)	93.2 (2.4)	0.76 (0.05)

patients from The Netherlands (5). In that study, a sensitivity of 63.0% for acute-phase serum samples, a sensitivity of 85.7% for convalescent-phase serum samples, and a specificity of 92.7% were calculated. The present multicenter study included samples collected prospectively in The Netherlands and sent to the Royal Tropical Institute in 1996 because of a suspicion of leptospirosis or because leptospirosis was in the differential diagnosis. The results of both studies show good agreement. In the present study, a higher sensitivity (95.2%) was observed for convalescent-phase samples, the sensitivity (60.1%) for acute-phase samples was similar, and the specificity was slightly higher.

The incidence of leptospirosis is relatively low in The Netherlands. In the present study, we demonstrated that the dipstick assay can be applied successfully in different parts of the world, including areas with a high endemicity of leptospirosis. The variation in test performance noted for the different study groups likely is attributable to differences in the interpretation of the results of the reference test (MAT) by the different centers involved in the study. Differences in clinical practices and in the collection of clinical data may have contributed to some variation as well. Differences in methodology presumably had a stronger effect on the results of the dipstick assay than did possible epidemiological differences. Differences in the sensitivity calculated for the dipstick assay for samples collected in the acute phase of the disease most likely were due to differences in the accuracy of the reported duration of the disease at the time of sampling. For instance, the relatively high sensitivity calculated for the acute-phase samples in the study performed in India could have been caused by the fact that the patients in this study group first sought medical attention at a late stage of the disease and underreported the duration of the disease. Also, the high sensitivity calculated for the study groups in the Philippines and Surinam (Table 5) suggested that the samples from these groups were collected at a relatively late stage of the disease. The relatively low sensitivity observed for the convalescent-phase samples from the study groups in Hawaii, India, the Seychelles, and Thailand could have been a result of false-positive results obtained in the reference test. The majority of the case patients with serum samples for which a negative result was observed in the dipstick assay had low or borderline titers in the MAT as well as borderline or negative results in the IgM ELISA. The absence of detectable *Leptospira*-specific IgM levels in these patients suggests that the final diagnosis of laboratory-confirmed leptospirosis could be disputed for these patients. It is possible that some of these patients had residual antibody levels agglutinating in the MAT from a previous *Leptospira* infection.

We previously demonstrated that the dipstick assay reacted equally well with sera from patients infected with strains of the serogroups Australis, Autumnalis, Icterohaemorrhagiae, Grippothyphosa, Sejroe, and Pomona (5). In the present study, reactivity with a total of 22 serogroups was demonstrated (data not shown). The lack of reactivity with sera from some case patients was not related to agglutination by strains belonging to a specific serogroup. The results of this multicenter study indicate that the dipstick assay has a broad reactivity and is widely applicable. As the dipstick assay yields results quickly and is simple to perform and the assay components are highly stable and do not need refrigeration, the test in particular may fulfill needs in situations where facilities or resources needed to perform more complicated standard laboratory tests such as the MAT or the ELISA are lacking. The observed high degree of concordance between the results of the dipstick assay and the ELISA (mean observed agreement, 93.2%; kappa value, 0.76) shows that similar results will be obtained when either of the tests is used. The sensitivity of the IgM ELISA is lower than that of the dipstick assay for acute-phase samples but is slightly higher for convalescent-phase samples (5). The specificity of the IgM ELISA is higher (5). Assays aimed at the detection of *Leptospira*-specific IgM antibodies have somewhat lower sensitivity and specificity than the MAT. In our study, serum samples from about 10% of the case patients did not react in the dipstick assay, and samples from about 9% of the noncase patients showed weak to moderate staining. Although it is possible that some of the patients were misdiagnosed by the reference test, one should consider the possibility of false-positive and false-negative results when applying the dipstick assay.

When the dipstick assay is used as a screening test, a high negative predictive value is important. The predictive value of a test varies with the prevalence of the disease in the target population. The prevalence of leptospirosis among patients with a clinical suspicion of leptospirosis in the study group tested in The Netherlands was 4.1%. The negative predictive value for this study group was 98.0% (95% confidence interval, 96 to 99). From the results of Table 3 it can be calculated that a mean prevalence of leptospirosis of 29.5% among patients with a clinical suspicion of leptospirosis, the negative predictive value would be 90.8% (95% confidence interval, 89 to 92). The dipstick assay primarily has been developed as a rapid screening assay. It is envisaged that due to a lack of facilities or resources needed to perform more complicated confirmatory tests, the dipstick assay may be used as a diagnostic assay. A high positive predictive value will be important for use of the dipstick assay as a diagnostic test. As the majority of the dipstick assay positive results obtained for the noncase patients showed weak (1+) staining, the positive predictive value was calculated separately for results with a weak staining intensity and for results with a moderate to strong (≥2+) staining intensity. For a test result with a moderate to strong staining intensity, a positive predictive value of 91.2% (95% confidence interval, 75 to 98) was calculated for the study performed in The Netherlands. The positive predictive value at a prevalence of 29.5% would be 93.8% (95% confidence interval, 91 to 96). The positive predictive value for a test result with a weak staining intensity was 47.1%.

The results of the dipstick assay, like the results of the MAT and the ELISA, should be interpreted with respect to clinical findings. The results obtained in this study show that a test result with a moderate to strong staining intensity is highly consistent with leptospirosis. Seroconversion usually takes place 6 to 7 days after the onset of the disease, and a negative result or a result with a weak staining intensity may be obtained when samples are collected early in the disease. The present study shows that a moderate to strong reaction in the dipstick assay is obtained by day 6 or 7 for most case patients (data not shown). Demonstration of seroconversion provides strong evidence of leptospirosis. Therefore, it is advised that the assay be repeated with a sample collected a few days later when a negative result is obtained for a sample collected early in the disease and when the suspicion of leptospirosis remains.

The use of the dipstick assay may well lead to improved diagnosis and treatment of patients with leptospirosis and to better knowledge and understanding of the prevalence and epidemiology of the disease. As the dipstick assay is genus specific, it does not provide information regarding the serovar involved in the infection. Knowledge of the serovar is not essential for treatment, but it might be important for identifying possible sources of infection and for developing control programs. Application of culturing and/or the MAT will be required to obtain this information.

The dipstick assay may be universally applicable as a quick screening test for leptospirosis. During this study, the value of the dipstick test was demonstrated by showing a high proportion of strongly positive serum samples among samples from patients with a clinical suspicion of leptospirosis but for whom the resources to perform a reference test were not locally available. Furthermore, the dipstick assay clearly demonstrated the presence of leptospirosis among patients for whom the disease was not considered, as was the case for the group of dengue-negative patients from Puerto Rico. In the study performed in Kenya, the dipstick assay excluded leptospirosis for patients with pyrexia of unknown origin but proven to suffer from several infectious diseases at a later stage.

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REFERENCES

1. **Alexander, A. D.** 1980. *Leptospira*, p. 367–382. *In* E. H. Lennette, A. Barlows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
2. **Faine, S.** 1987. Guidelines for the control of leptospirosis. Bull. W. H. O. **67:**1–171.
3. **Faine, S.** 1994. Leptospira and leptospirosis. CRC Press, Inc., Boca Raton, Fla.
4. **Farr, R. W.** 1995. Leptospirosis. Clin. Infect. Dis. **21:**1–8.
5. **Gussenhoven, G. C., M. A. W. G. van der Hoorn, M. G. A. Goris, W. J. Terpstra, R. A. Hartskeerl, B. W. Mol, C. W. van Ingen, and H. L. Smits.** 1997. LEPTO dipstick, a dipstick assay for the detection of *Leptospira*-specific immunoglobulin M antibodies in human sera. J. Clin. Microbiol. **35:**92–97.
6. **Innis, B. L., A. Nisilak, and S. Nimmannitya.** 1989. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am. J. Trop. Med. Hyg. **40:**418–427.
7. **Kuno, G., I. Gómez, and D. J. Gubler.** 1987. Detecting artificial anti-dengue IgM immune complexes using an enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg. **63:**3153–3159.
8. **Levett, P. N., and C. U. Whittington.** 1998. Evaluation of the indirect hemagglutination assay for diagnosis of acute leptospirosis. J. Clin. Microbiol. **36:**11–14.
9. **Mazzolini, J., G. Dorta de Mazzolini, and M. Mailloux.** 1974. Possibilité de diagnostic serologique des leptospires à l'aide d'un antigène unique. Ned. Mal. Infect. **4:**253.
10. **Palmer, M. F.** 1988. Laboratory diagnosis of leptospirosis. Med. Lab. Sci. **45:**174–178.
11. **Terpstra, W. J., G. S. Ligthart, and G. J. Schoone.** 1980. Serodiagnosis of human leptospirosis by enzyme-linked-immunosorbent-assay (ELISA). Zentbl. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. Reihe A **247:**400–405.
12. **Terpstra, W. J., G. S. Ligthart, and G. J. Schoone.** 1985. ELISA for the detection of specific IgM and IgG in human leptospirosis. J. Gen. Microbiol. **131:**377–385.
13. **World Health Organization.** 1998. Communicable disease surveillance kit. World Health Organization, Geneva, Switzerland.