The rapid diagnosis of leptospirosis: A prospective comparison of the dot enzyme-linked immunosorbent assay and the genus-specific microscopic agglutination test at different stages of illness

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THE RAPID DIAGNOSIS OF LEPTOSPIROSIS: A PROSPECTIVE COMPARISON OF THE DOT ENZYME-LINKED IMMUNOSORBENT ASSAY AND THE GENUS-SPECIFIC MICROSCOPIC AGGLUTINATION TEST AT DIFFERENT STAGES OF ILLNESS

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The Rapid Diagnosis of Leptospirosis: A Prospective Comparison of the Dot Enzyme-Linked Immunosorbent Assay and the Genus-Specific Microscopic Agglutination Test at Different Stages of Illness

The microscopic agglutination (MA) test is considered the serodiagnostic test of choice for leptospirosis [1, 2], but its complexity limits its use to reference laboratories. Simpler techniques have been described, but information is lacking on their relative merits and adaptability to endemic areas where sophisticated laboratories may be absent. The IgM-specific dot ELISA was recently shown to be comparable to the classic MA test in its ability to detect recent exposure to leptospires; it is also rapid and simpler to use [3]. The genus-specific MA test uses a single broadly reactive, nonpathogenic antigen [4] - *Leptospira biflexa* serovar Patoc 1 - to replace a battery of antigens. We prospectively compared the sensitivity and specificity of these two tests in patients with proven leptospirosis that was usually severe and often late in its course.

Patients and Methods

**Patients.** The study was conducted at the San Lazaro Hospital, Manila, the national infectious disease hospital of the Philippines, during the rainy-season months of September through November 1985 and July through October 1986. Sera were collected from patients > 16 y old. Cases were classified as severe if renal dysfunction, jaundice, or signs of bleeding were present.

**Diagnosis of leptospirosis.** Leptospirosis was diagnosed on the basis of a fourfold or greater rise in antibody titer, as determined by the MA test or by the isolation of leptospires from blood or urine. Only serological results from patients having confirmed disease were analyzed.

**IgM-specific dot ELISA.** The dot ELISA procedure followed was that of Pappas et al [3, 5], except that seven to 10-d-old cultures of *L. biflexa* serovar Patoc 1 were used as antigen. Briefly, antigen disks were prepared by "dutting" 1 μL of leptospiral antigen (770 ng of protein, Lowry method) on the dull side of 5-mm nitrocellulose filter disks. Antigen disks were then placed in wells of flat-bottomed microtiter plates and blocked with 75 μL of triethanolamine-buffered saline (TBS) containing 5% bovine serum albumin for 1 min with shaking on a plane rotator. After aspiration of blocking solution, 50 μL of patients’ sera at a dilution of 1:8 was added to wells, agitated for 1 min, and incubated for 30 min at room temperature (≤23 C). Wells were washed three times with 100 μL of TBS containing a 0.05% concentration of the surfactant nonidet P-40. Fifty microliters of horseradish peroxidase-conjugated antibody to human IgM at a dilution of 1:200 was then incubated with antigen disks for 30 min at room temperature. After washing as above, 50 μL of peroxidase-chromogenic, precipitable substrate (4-chloro-l-naphtol [Kirkegaard & Perry Laboratories, Gaithersburg, Md]), diluted in TBS, was added to test wells, shaken, and incubated for 30 min. Antigen disks were then washed three times with TBS and read. Both positive and negative controls were incorporated into each assay. Positive reactions appeared as clearly defined blue-purple dots.

**Genus-specific MA test.** The procedure followed was that of the classic MA test, except that *L. biflexa* serovar Patoc 1 was used as the sole antigen instead of the usual battery of 24 pathogenic serovars. Ten- to 14-d-old cultures were centrifuged for 5 min at 500 g, and 0.1 mL of supernatant was used as antigen. This was added to 0.1 mL of sera, and both test mixtures and controls were incubated for 3 h at 28-30 C. Each well was then examined for agglutination by using a dark-field microscope. Sera showing positive reactions at 1:100 were then read at 1:1600; further dilutions were read if there was a positive reaction at 1:1600. The endpoint agglutination titer was the highest dilution giving >50% agglutination of leptospires.

**Study design and statistical analysis.** In all cases, sera were assayed for leptospiral antibody when the patients were admitted to the hospital. Additional serum specimens were subsequently taken from most patients, either to establish a diagnosis or to detect changes in antibody titer. Only when sera were assayed by both dot ELISA and MA simultaneously were the results of a test included. The dot ELISA was positive if the characteristic blue-purple dots were present at serum dilutions of 1:8. The MA test was positive if there was >50% agglutination of leptospires at a serum dilution 2:1600. The number of patients testing positive by each method were compared by χ² with Yates's correction.
The specificity of the two assays was determined by testing sera from patients with the three diseases most commonly confused with leptospirosis at the study hospital: viral hepatitis, falciparum malaria, and typhoid fever.

Results

Patients. There were 27 men and five women, ranging in age from 19 to 44 y (median age, 30 y). Most patients had received antibiotics before hospital admission, and 75% had severe disease. One hundred three sera were assayed. In five patients only an admission specimen was obtained; the other 27 patients were tested on more than one occasion.

Diagnosis of leptospirosis. A positive diagnosis was made on the basis of a fourfold rise in antibody titer alone in 19 patients and on isolation of leptospires alone in four patients. The remaining nine patients had both positive cultures and fourfold rises in titers.

Sensitivity. Overall, 31 (50%) of 103 dot ELISA tests and 55 (53%) of 103 genus-specific MA tests were positive. There was a trend for the dot ELISA to be more sensitive in the first week of illness (47% vs. 29% positive) and for the MA test to be more sensitive during the third week of illness (70% vs. 45% positive; figure 1). These differences, however, were not statistically significant (P > .1).

All 32 patients had at least one positive assay on subsequent testing (figure 2), and in 22 patients (69%), either the admission dot ELISA or the MA test was positive. The dot ELISA was slightly more sensitive as an initial screening test than was the MA (56% vs. 41% positive), but with repeat testing ~80% of patients would have been diagnosed by either test alone (dot ELISA, 78%; MA, 84%). On initial testing, nine patients (28%) were only positive by dot ELISA and four (13%) only by the MA test. On repeat testing, five patients were positive only by dot ELISA and seven only by the MA test.

Specificity. Sera from 24 patients with viral hepatitis, 24 patients with falciparum malaria, and 24 patients with typhoid fever were assayed by both dot ELISA and the genus-specific MA test. All tests were negative.

Discussion

An accurate, rapid method for diagnosing leptospirosis has vital importance for both clinician and patient. The results of this study are therefore encouraging. Using both tests, we diagnosed 22 (69%) of the 32 patients on an initial serum assay, and upon repeat testing, the diagnosis was made in the remaining 10 (figure 2). The sensitivities of each test method could have been improved by lowering the threshold of positivity. Accepting lower cutoff values, however, would have risked detecting low levels of antibody from previous exposure rather than a serological response to acute infection. The specificity was excellent. There were no false-positive results in 72 sera from patients having diseases that are often clinically confused with leptospirosis.

No clear-cut advantages of either method emerged from this study. There was a trend for the dot ELISA to be the more sensitive of the two tests during the first week of illness, although differences were not statistically significant (figure 1). Other authors have found that leptospiral antibodies detected by ELISA appear early [6]. There was also a nonsignificant trend toward the MA test being more sensitive during the third week of illness (figure 1). These
trends require further evaluation before recommendations
can be made concerning advantages of one method over
another at a particular stage of disease. The dot ELISA
results were available within 5 h after blood specimens were
received and MA test results were available within 4 h. The
dot ELISA was more difficult to perform than the MA
test, but the interpretation of results was easier. The dot
ELISA required no electrical equipment and only one di-
lution, whereas a dark-field microscope and several dilu-
tions were needed for the MA test.

In conclusion, both tests proved suitable for use in the
general diagnostic laboratory and both effectively diag-
nosed acute leptospirosis. We recommend their use in areas
where this potentially fatal disease remains a public health
problem. Recently the dot ELISA has been further refined
into single- and multiple-antigen “dipsticks” [7]. Improve-
ments such as these offer the hope that in the future the
diagnosis of leptospirosis can be made even simpler and
perhaps even be made available to rural areas where
standard laboratory facilities are not.

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References

1. Wolff JW. The laboratory diagnosis of leptospirosis. Pub-
ication no. 183, American Lecture Series. Springfield, Ill:
Charles C. Thomas, 1954

2. Galton MM, Sulzer CR, Santa Rosa CA, Fields MJ. Appli-
cation of a microtechnique to the agglutination test for lep-

3. Pappas MG, Ballou WR, Gray MR, Takafuji ET, Miller RN,
Hockmeyer WT. Rapid serodiagnosis of leptospirosis using
the IgM-specific dot-ELISA: comparison with the micro-
scopic agglutination test. Am J Trop Med Hyg 1985;34:
346–54

4. Cox CD, Alexander AD, Murphy LC. Evaluation of the hem-
olytic test in the serodiagnosis of human leptospirosis. J In-
fect Dis 1957;101:210–8

5. Pappas MG, Hajkowski R, Hockmeyer WT. Dot enzyme-linked
immunosorbent assay (dot-ELISA): a micro technique for
the rapid diagnosis of visceral leishmaniasis. J Immunol
Methods 1983;64:205–14

6. Thiermann AB, Garrett LA. Enzyme-linked immunosorbent
assay for the detection of antibodies to Leptospira inter-
rogans serovars hardjo and pomona in cattle. Am J Vet Res
1983;44:884–7

7. Pappas MG. Rapid serodiagnosis of parasitic infections by dot-
1986;80:1006
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16. SUPPLEMENTARY NOTATION 

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) 
The IgM-specific dot ELISA and Genus-specific MA tests are suitable for use in the general diagnostic laboratory and both effectively diagnosed acute leptospirosis. We recommend their use in areas where this potentially fatal disease remains a public health problem. There is hope that in the future the diagnosis of leptospirosis can be made even simpler and perhaps can even be made available to rural areas where standard laboratory facilities are absent.