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Evaluation of Dipstick Serologic Tests for Diagnosis of Brucellosis and Typhoid Fever in Egypt

Tharwat F. Ismail,[1]* Henk Smits,[2] Momtaz O. Wasfy,[1] Joseph L. Malone,[1] Moustafa A. Fadeel,[1] and Frank Mahoney[1,3]

U.S. Medical Research Unit No. 3, Cairo, Egypt[1]; Department of Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands[2]; and Centers for Disease Control and Prevention, Atlanta, Georgia[3]

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Two dipstick assays for the detection of *Brucella*- and typhoid-specific immunoglobulin M, recently developed by the Royal Tropical Institute of The Netherlands, were evaluated by use of 85 plasma samples from Egyptian patients. Both dipsticks were simple and accurate rapid diagnostic assays, and they can be useful adjuncts for the diagnosis of typhoid fever and brucellosis.

Typhoid fever and brucellosis are frequent causes of bloodstream infections in many countries (2, 5). The diagnosis of these infections is challenging because they can have diverse clinical manifestations with symptoms that overlap with a wide spectrum of other diseases (1). Cultures of blood or bone marrow are the most definitive diagnostic methods. In many developing countries, both diseases are diagnosed on clinical grounds and treated empirically (3, 15). Serology assays are often utilized as a diagnostic tool (8, 16); however, neither the Widal nor the *Brucella* agglutination assay is sufficiently sensitive, specific, or practical in areas of endemicity (6, 11).

In this study, we evaluated the sensitivity and specificity of the Royal Tropical Institute of The Netherlands (RTI) dipstick assay. Representative plasma samples were originally collected from patients admitted to five infectious disease hospitals in Egypt according to certain case definitions and laboratory tests using conventional blood culture and serological techniques (12, 15). Eighty-five plasma samples were screened by Widal and *Brucella* agglutination assays (7, 14). The remaining portions were preserved at −70°C for evaluation with the dipstick assay, which was performed within 2 to 3 months postadmission. Results were related to the other findings for the purpose of this study.

Among those patients, 25 had blood culture-proven typhoid fever, 25 had blood culture-proven brucellosis, 25 had acute fever but negative blood cultures and serology, and 10 had negative blood cultures but were seroreactive to Widal ($n = 5$) or *Brucella* ($n = 5$) agglutination.

The RTI dipstick assay is based on the binding of human *Brucella*- or *Salmonella enterica* serovar Typhi-specific immunoglobulin M (IgM) antibodies with the respective antigen, which is detected with an anti-human IgM dye conjugate (13). IgM has been reported to appear a few days after infection, reaching a peak within 3 to 4 weeks, and then it declines gradually over a similar period of time (6).

The test sample was incubated for 2 h at 35°C. The built-in internal control band provided a check on the integrity of the detection reagent and the presence of serum. To avoid bias, investigators were blinded to the blood culture results until the dipstick assays were completed. Blood culture results were considered the "gold standard" and were used to determine the positive and negative results by dipstick assays.

Of the 30 patients with laboratory-confirmed brucellosis (25 culture positive and 5 positive by tube agglutination test), 28 tested positive by the *Brucella* dipstick (93% sensitivity). Additionally, the *Brucella* dipstick was positive for 4 of 55 serum samples that were negative by culture and *Brucella* agglutination testing (specificity = 93%) (Table 1). Of the 30 patients with a laboratory diagnosis of typhoid fever (25 culture positive plus 5 Widal positive), 27 had a positive dipstick test (sensitiv-

TABLE 1. Sensitivity and specificity of *Brucella* dipstick assay

Brucella assay and result (n)	*Brucella* dipstick assay result (no. of specimens)		% Sensitivity[a]	% Specificity[b]
	Positive	Negative		
Culture positive (25)	23	2	92	
Agglutination positive (5)	5	0		100
Culture or agglutination test positive (30)	28	2	93	
Culture and agglutination test negative (55)	4	51		93

[a] Calculated by dividing the number of true positives by the total number of positives (true positives and false negatives).
[b] Determined by dividing the number of true negatives by the total negatives (true negatives plus false positives).

* Corresponding author. Mailing address: U.S. Naval Medical Research Unit #3, (Cairo) PSC 452, Box 5000, FPO AE09835-0007. Phone: (202) 684-1375, ext. 372. Fax: (202) 684-0186. E-mail: IsmailT@namru3.med.navy.mil.

TABLE 2. Sensitivity and specificity of typhoid dipstick assay

Typhoid assay and result (*n*)	Typhoid dipstick assay result (no. of specimens)		% Sensitivity	% Specificity
	Positive	Negative		
Culture positive (25)	22	3	88	
Widal test positive (5)	5	0		100
Culture or agglutination test positive (30)	27	3	90	
Culture and Widal test negative (55)	2	53		96

ity = 90%). The typhoid dipstick was positive in only 2 of 55 samples derived from patients without laboratory evidence of typhoid fever (specificity = 96%) (Table 2). Table 3 shows that readings of the *Brucella* agglutination test and dipstick assay were the same, recognizing titers ≥160 in 92% of samples collected from culture-positive patients. However, in the case of typhoid fever, the Widal test was negative for 8 of 22 (32%) dipstick-positive specimens, suggesting a lower sensitivity or titer (<160).

Six patients with blood culture-confirmed infections (three typhoid and three *Brucella* infections) had an initial negative dipstick test. Upon testing with a twofold dilution to rule out a prozone phenomenon, one patient with brucellosis became positive.

Of the 25 patients with culture-proven typhoid fever, only 14 (56%) had Widal titers ≥1:160 (Table 3). Ten samples (100%) from patients with a negative blood culture but serologic evidence by Widal or *Brucella* agglutination reacted with the respective dipsticks. Since the typhoid dipstick assay was based on IgM detection in acute infections, antibodies peculiar to chronic carriers (anti-Vi-specific IgG) could not be detected. Passive hemagglutination or enzyme-linked immunosorbent assays have been advocated for such discrimination in epidemiological surveys (8).

The RTI dipstick test is a simple, fast, and reliable method for the diagnosis of typhoid fever and brucellosis and confirmed results from previous studies (4, 6, 7, 9, 10, 13).

With sensitivity and specificity of both dipsticks in excess of 90%, the kits performed well in settings where both brucellosis and typhoid fever are endemic. Published studies suggest that blood cultures are positive in only approximately 60% of patients with brucellosis or typhoid fever (12, 15). The findings that culture-negative patients were positive by dipsticks may be useful in settings where antibiotic use is high and no cultures are available.

One concern noted with dipstick kits was that 16% of the patients with culture-proven brucellosis reacted with the typhoid dipstick and 8% of the samples from patients with culture-proven typhoid fever reacted with the *Brucella* dipstick. The reason for both dipstick tests having a positive reaction from a single patient is not clear, but in addition to simple cross-reactivity, potential options could include recent past infection with one organism and current infection with the second organism or persistence of IgM antibodies in some patients.

Another interesting finding was the high correlation (100%) between serological results and the dipstick assay results from patients with clinically suspected brucellosis or typhoid fever among patients who had negative blood cultures. However, for patients with positive cultures, the sensitivity of the typhoid dipstick was found to be significantly higher than that of the corresponding serological results. The relatively low sensitivity of the Widal test in this study may have resulted from an undetectable level of IgM antibody, which was probably due to the young age of patients and/or a relatively short length of illness of the typhoid patients (7).

For the purpose of communicable disease surveillance, the Ministry of Health in Egypt indicated that tube agglutination titers ≥1/160 are to be regarded as positive for brucellosis and typhoid fever. This is based on extensive clinical and laboratory findings collected over a number of years. Consequently, the dipstick assay would not be expected to show false-positive results, given the results of positive, negative, and control samples of this study. However, the *Brucella* dipstick assay showed 8% cross-reactivity with typhoid patients who had no evidence

TABLE 3. Comparison of typhoid and *Brucella* dipstick results with serologic assay results

Plasma samples	No. tested	No. of samples (%) with indicated dipstick assay result for:				No. of samples (%) positive by indicated serologic assay	
		Serovar Typhi		*Brucella*		Widal ≥1:160	*Brucella* agglutination ≥1:160
		Positive	Negative	Positive	Negative		
Typhoid culture positive	25	22 (88)	3 (12)	2 (8)	23 (92)	14 (56)	0
Brucella culture positive	25	4 (16)	21 (84)	23 (92)	2 (8)	0	23 (92)
Culture negative and Widal test positive	5	5 (100)	0	0	0	5 (100)	0
Culture negative and *Brucella* agglutination positive	5	0	0	5 (100)	0	0	5 (100)
Culture negative and Widal- and agglutination-negative	25	0	25 (100)	0	25 (100)	0	0

of *Brucella* infection, but the corresponding agglutination titers were considerably lower than the cutoff.

Our preliminary results are quite promising and lead us to recommend the use of the dipstick assays in the primary characterization of infection. Being specific, faster, and easier than conventional methods, they could be of value in areas where diagnostic capabilities for culture and serology are poor.

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