ANTIBODY RESPONSE IN TYPHOID FEVER IN ENDEMIC INDONESIA AND THE RELEVANCE OF SEROLOGY AND CULTURE TO DIAGNOSIS

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Abstract. Culture and serology were performed on blood and serum samples collected at or shortly after admission from 473 patients presented with suspected clinical typhoid. Clinical symptoms at first presentation including confusion, hepatomegaly, splenomegaly, abdominal pain, anemia, and gastrointestinal bleeding were non-specific as they were observed even more often in non-typhoid patients. Culture confirmed the diagnosis in 65.3% of the patients with typhoid fever as the final diagnosis. The sensitivity (58%) and specificity (98.1%) of a rapid dipstick assay for the detection of *S. typhi*-specific immunoglobulin M were somewhat lower than those of culture but higher than those of the Widal test. The dipstick assay thus may well be used in the serodiagnosis of typhoid in situation where culture facilities are not available. Combination of test results of dipstick and culture improved sensitivity to 82.5%. In laboratories that perform blood culture the dipstick assay may be used as a rapid screening tests to facilitate a rapid diagnosis. Sensitivity of the dipstick assay strongly increased with duration of illness and was higher for culture positive than for culture negative patients. Duration of illness, and different pathogen and host factors including dose of infection, pathogenicity and antigenicity, and prior antibiotic use are likely to influence the immune response, therefore the result of the dipstick assay. Duration of illness and presence of *S. typhi* in the blood are major factors that determine severity of disease.

INTRODUCTION

Typhoid fever is one of the most important infectious diseases in South Sulawesi, Indonesia (Velema *et al*, 1997). Typhoid is endemic throughout the region and is the fourth frequently reported infectious disease in the majority of the 24 districts. Typhoid is the most important cause of community-acquired septicemia. Incidence rates exceed 2,500/100,000 and in particular in rural and suburban areas incidence rates of 10,000/100,000 are not unusual. Drug resistance is very rare in Indonesia in contrast to many other countries in Southeast Asia where multi-drug resistance is very common (Mirza *et al*, 1996). In Indonesia chloramphenicol is the first line antibiotic in the treatment of uncomplicated typhoid fever and is still highly effective. Inspite of this, severe disease and complications are common and the case fatality rate is about 5%. Mortality among hospitalized patients is often as high as 50%. Poor hygienic and sanitary conditions, unavailability of clean water supplies, and the presence of carriers and convalescent patients transmitting the pathogen, all contribute to the high incidence.

First time presentation of patients at a late stage of illness and poor compliance contribute to the development of severe disease and complications. Misdiagnosis may play a role. The diagnosis of typhoid fever on clinical grounds is challenging, as symptoms and signs are diverse (Stuart and Pullen, 1946), and are similar to those observed with other common

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febrile illnesses, such as malaria and non-severe dengue fever. Laboratory facilities for blood culture confirmation of typhoid fever are not available in South Sulawesi, Indonesia, and serological testing using the Widal test is done only in few hospitals and primary health care centers. The diagnosis of typhoid fever thus mostly depends on clinical signs and symptoms.

A rapid dipstick assay for the detection of *S. typhi*-specific IgM antibodies in serum and whole blood samples was previously reported and the sensitivity and specificity was evaluated (House *et al*, 2001; Gasem *et al*, 2002; Hatta *et al*, 2002). The results of the studies demonstrated that the assay has a high sensitivity and specificity when testing samples from culture-confirmed typhoid patients collected at the time of hospital admission and that the sensitivity increases with duration of illness. Different host and pathogenic factors that may influence disease severity also may influence the strength and evolution of the immune response. Therefore, a more detailed study comparing the results of culture, disease severity and dipstick test was performed.

MATERIALS AND METHODS

Patients and sample collection

Blood and serum samples were collected from all patients presented with suspected clinical typhoid fever at the Hasanuddin University Hospital of South Sulawesi, three primary health care centers in Makassar, and a district hospital in Gowa district, a suburban area of Makassar, Indonesia. Clinical specimens were collected at the day of hospital admission or in the first few days thereafter from 473 patients. Follow-up serum samples were collected from a subgroup of 175 patients after on average one week and two weeks of first presentation. The duration of hospitalization was less than one week for most patients and in these cases follow-up samples were collected by home visits.

Ethical considerations

The project had been approved by the review boards of the participating institutes and informed consent for participation in the study was obtained from all participants or their parents/guardians.

Laboratory diagnosis

Blood culture and serological testing were performed as described previously (Hatta *et al*, 2002). Briefly, blood culture was performed by inoculation of 15 ml of ox bile broth (Merck) with 5ml of freshly collected blood. Cultures were incubated for 24 hours at 37ºC. The Widal test using the O antigen was performed by incubation of two-fold serial serum dilutions (1/20 - 1/1,280) with an equal volume of the antigen (O) suspension at 50ºC. Tubes were checked for agglutination after 4 hours. According to routine diagnostic criterion, a titer ≥1:320 was considered to be positive. Dipsticks were prepared by KIT Biomedical Research, Amsterdam, The Netherlands. The dipstick assay was performed by incubation of a wetted dipstick in a mixture of 5 µl serum and 250 µl detection reagent for 3 hours at room temperature. At the end of the incubation, the dipsticks were thoroughly rinsed with water and dried. The staining intensity of the antigen band was then graded by comparison with a colored reference strip. The test was scored negative when no staining was observed, 1+ when a weak staining was observed and 2+, 3+ or 4+ when a moderate to strong staining was observed.

Calculations

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using the following formulae: sensitivity is $a/(a + c)100\%$, specificity is $d/(d + b)$ 100%, PPV is $a/(a + b)$ 100%, and NPV is $d/(d + c)100\%$; in these formulae, *a* is test positive and true positive, *b* is test positive and true negative, *c* is test negative and true positive, and *d* is test negative and true negative. 95% confidence intervals (CI) were determined by using the Epi-info computer package.

Chi-square analysis was used to determine statistical significance between groups. Mann-Whitney U test was used to determine significance for continuous variables. A p-value <0.05 was considered to indicate statistical significance.

RESULTS

Patients were stratified as blood culture-proven typhoid patients (n=205), unconfirmed typhoid patients (n=109), and patients with a final diagnosis other than typhoid fever (n=159). The diagnosis of typhoid fever in culture-negative patients was based on the clinical signs and symptoms and response to treatment. Thus, a blood culture positive for *S. typhi* was obtained in 65.3% of the patients with typhoid fever as the final diagnosis.

A positive dipstick result was obtained in 58% of the serum samples collected at first presentation for the combined groups of culture-proven and culture-negative typhoid patients (Table 1). The sensitivity of the dipstick did not statistically differ from the estimated sensitivity (65.3%) of blood culture (p = 0.59). The dipstick tested positive in 72.4% of the samples collected from the culture-proven typhoid patients compared with 49.5% for the samples from the culture-negative typhoid patients. This difference in seropositivity was significant (p = 0.028). Seropositivity and the strength of the staining intensity increased with the duration of illness (Table 1). For the combined groups of culture-proven and culture- negative typhoid patients the sensitivity of the dipstick was 28.9% for samples collected during the first 4 to 6 days of illness, 75% for samples collected between day 7 and 9, and 95% for samples collected at a later stage. Notably, the response in the

Table 1
Dipstick results for serum samples collected at the time of first admission together with results of culture, final diagnosis and duration of fever.

Final diagnosis, result of blood culture and duration of fever (days, range)	Dipstick						Widal test
	No. of patients with a positive result (%)/ No. of patients	Percentage of patients with following staining intensity					No. of patients with a positive result (%)
		Negative	1+	2+	3+	4+	
Typhoid fever (7.4, 4-19)	182 (58)/314	42.0	32.5	5.4	9.2	10.8	157 (50) / 314
S. typhi culture positive (7.2, 4-19)	128 (72.4)/205	37.3	38.5	4.4	10.2	9.3	119 (58.3)
(4-6)	38 (40.0)/95	60.0	35.7	2.1	1.1	1.1	40 (42.6)
(7-9)	62 (77.5)/80	22.5	47.5	7.5	13.8	8.7	53 (66.3)
(>9)	28 (93.3)/30	6.7	2.3	3.3	30.0	36.7	26 (86.7)
Culture negative (7.7, 4-15)	54 (49.5)/109	50.5	21.1	7.3	7.3	13.8	38 (34.9)
(4-6)	3 (6.4)/47	93.6	6.4	-	-	-	6 (12.7)
(7-9)	22 (68.7)/32	40.6	4.1	12.5	9.4	6.3	16 (50)
(>9)	29 (96.7)/30	3.3	23.3	13.3	16.7	43.3	16 (53.3)
Non-typhoid fever (7.1, 4-18)	3 (1.9)/159	98.2	0.6	-	0.6	0.6	31 (19.5)

Table 2
Sensitivity, specificity and positive and negative predictive value of culture and serology, and the clinical diagnosis of typhoid fever.

Test	Assay characteristic % (95% CI)			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Symptoms	35.4 (30-41)	39 (32-47)	53.4 (46-60)	23 (19-29)
Widal O, ≥1:320	50 (44-56)	80.5 (73-86)	83.5 (77-88)	44.9 (39-51)
Culture	65.3 (60-71)	100 (97-100)	100 (98-100)	59.3 (53-65)
Dipstick assay	58 (52-63)	98.1 (94-100)	98.4 (95-100)	54.2 (48-60)
Culture + dipstick assay	82.5 (78-86)	98.1 (94-100)	98.9 (96-100)	73.9 (67-80)

dipstick for sera collected before day 7 of illness was much higher for the culture-proven typhoid patients than for the culture-negative typhoid patients ($p = 0.0003$). However, no major differences in antibody response between these two groups were noted for samples collected at later stages.

The sensitivity of the dipstick (58%) was somewhat lower than that of culture (65.3%), but higher than that of the Widal test (50%), and also higher than diagnosis based on clinical symptoms (35.4%) (Table 2). The combined results of culture and dipstick gave the highest sensitivity (82.5%) together with the highest NPV (73.9%) and acceptable specificity (98.1%) and PPV (98.9%). NPV and PPV were calculated based on the actual 66.4% prevalence of typhoid patients in the study population.

Dipstick assays of the follow-up samples collected on average one and two weeks after the initial samples showed an increase in seropositivity and in strength of the antibody response during treatment and subsequent recovery in convalescent patients (Table 3). Seroconversion as determined by the dipstick test was observed in 29.1% of the culture-proven typhoid patients and in 81.4% of the culture negative-typhoid patients.

Typhoid patients presented with fever and with confusion, hepatomegaly, splenomegaly, abdominal pain, anemia and gastrointestinal bleeding as the most common symptoms at first presentation (Table 4). The same symptoms were observed even more often in non-typhoid patients; 61% of the non-typhoid patients presented with one or more of these symptoms compared with 38.5% for the typhoid patients ($p = 0.002$). The percentage of patients with symptoms was higher in the group of culture-proven typhoid patients (38.5%) than in the group of culture-negative typhoid patients (28.4%) (Table 5). Culture-proven typhoid patients also presented more often with more than one symptom. However, the differences between the two groups were not statistically significant. Culture-proven and culture-negative typhoid patients did not differ in age (22.0 and 22.9 years, respectively), body temperature (38.2 and 38.1ºC), and duration of illness (7.2 and 7.7 days) at first admission (Table 4).

A significant ($p = 0.01$) higher percentage of typhoid patients with a positive dipstick than with a negative dipstick result for the initially collected sample presented with symptoms (Table 5). Typhoid patients with a positive dipstick result also presented significantly ($p = 0.0005$) more often with more than one symptom. In particular culture-proven typhoid patients with a positive dipstick result presented with symptoms; 47.6% of this group of patients had symptoms and 17.2% had more than one symptom. Culture-proven patients with a positive dipstick result differed from the culture-proven patients with a negative dip-

Table 3
Dipstick results for serum samples collected at the time of first admission and after two intervals of one week.

Final diagnosis, samples positive result (%)/ (mean duration of fever, range)	No. of patients with No. of patients	Percentage of patients with following staining intensity					p-value to dipstick positive
		Negative	1+	2+	3+	4+	
Typhoid fever							
S. typhi culture positive							
1st collection (7.7, 4-19)	59 (61.5) / 96	38.5	38.5	3.1	9.3	10.4	
2nd collection (14.7, 9-29)	78 (81.3) / 96	18.8	18.8	32.2	15.6	14.6	p = 0.002
3rd collection (24.7, 15-47)	87 (90.6) / 96	9.4	7.2	28.1	33.3	21.9	p = 0.000002
Culture negative							
1st collection (6.1, 4-12)	4 (8.7) / 46	91.2	4.4	-	2.2	2.2	
2nd collection (13, 9-21)	38 (82.6) / 46	17.4	27.8	19.6	8.7	6.5	p = 0.000
3rd collection (26.6, 16-37)	41 (89.1) / 46	10.9	8.7	17.4	39.1	23.9	p = 0.000
Non-typhoid							
1st collection (8.3, 4-18)	2 (6.1) / 33	94	-	-	3	3	
2nd collection (15.4, 10-26)	2 (6.1) / 33	94	-	-	6	-	p = 1.0
3rd collection (23, 16-41)	2 (6.1) / 33	94	3	-	3	-	p = 1.0

stick result in mean duration of illness at first admission (8.2 *versus* 5.7 days). The two groups did not differ in age (22.1 and 21.9 years, respectively) and body temperature (38.3ºC and 38.2ºC). Culture-proven typhoid patients with a negative dipstick result and culture-negative typhoid patients with either a negative or a positive dipstick result did not differ in the percentage of patients with symptoms and in the percentage of patients with more than one symptom.

DISCUSSION

The diagnosis of typhoid fever often requires laboratory confirmation as clinical symptoms and signs vary and are similar to those of other major infectious diseases. In the present study a variety of clinical symptoms were reported for the patients with typhoid fever. In addition to fever, confusion, hepatomegaly, splenomegaly, abdominal pain, were the main symptoms and these were observed in 38.5% of the culture-confirmed typhoid patients. However these symptoms were neither sensitive nor specific as they were present only in a subset of the culture-proven typhoid patients and were present in an even higher percentage (61%) of the patients with a final clinical diagnosis that was not typhoid fever.

Isolation of *S. typhi* from blood provides definitive proof of the disease. Blood culture, however, has a limited sensitivity in particular after prior antibiotic usage (Gasem *et al*, 1995), at increased duration of illness (Coleman and Buxton, 1907), and when a small blood volume is used (Shanson, 1990). Blood

Table 4
The age, temperature and clinical signs of patients at the time of first admission together with results of culture, final diagnosis and duration of fever.

Final diagnosis, results of blood culture and duration of fever	Age (years, range)	Temperature (degrees, range)	No. of patients with following clinical symptoms[a]									Total number of symptoms
			A	B	C	D	E	F	G	H	I	
Typhoid, culture positive (n = 205)	22.0 (7-56)	38.2 (37.0-40.2)	37	31	23	9	4	1	6	1	1	113
(4-6)	21.6 (7-56)	38.0 (37.0-40.0)	10	4	8	4	1	1	2	-	-	30
(7-9)	23.2 (7-55)	38.4 (37.3-40.2)	20	11	4	4	2	-	1	-	-	42
(>9)	21.2 (9-45)	38.4 (37.2-40.0)	7	16	11	1	1	-	3	1	-	40
Typhoid, culture negative (n = 109)	22.9 (6-52)	38.1 (37.2-40.0)	16	10	7	2	1	1	1	-	-	38
(4-6)	21.5 (7-48)	37.9 (37.2-39.0)	8	1	2	1	-	-	1	-	-	13
(7-9)	22.0 (6-52)	38.0 (37.2-39.2)	2	7	2	-	-	-	-	-	-	11
(>9)	25.7 (12-50)	38.3 (37.4-40.0)	6	3	3	1	1	1	-	-	-	15
Non-typhoid fever (n = 159)	23.5 (7-50)	38.2 (37.0-40.5)	36	48	31	6	3	1	8	1	5	139

[a]Clinical signs: A, confusion; B, hepatomegaly; C, splenomegaly; D, abdominal pain; E, gastrointestinal bleeding ; F, jaundice; G, anemia; H, skin rash; I, conjunctival bleeding.

culture provided definite proof of typhoid fever in 205 patients. Based on the clinical observations and the response to treatment, a final diagnosis of typhoid was made in an additional 109 patients. The sensitivity of blood culture, thus, was estimated to be 65.3%. This sensitivity of blood culture was lower than reported by others and this could be due to various factors including a higher frequency of antibiotic usage, a lower infective dose, and the relatively small blood volume used for culture. Bone marrow culture has a higher sensitivity and is less effected by antibiotic usage (Gasem *et al,* 1995) but is a more invasive and therefore much less commonly used. In this study the use of blood culture was found less practical due to reluctance of patients to donate the required blood volume, the relative long assay time of 3 days, and problems with transportation from the primary health care centers and district hospitals to the central laboratory.

In contrast, the dipstick assay was easy to apply, required only a very small volume of blood, and importantly had acceptable assay characteristics. A sample collected by fingerprick is sufficient to perform the test. The sensitivity (58%) of the dipstick assay was only somewhat lower than that of culture for samples collected at first presentation of patients. The specificity was 98.1%. In addition to sensitivity and specificity, PPV and NPV provided important information for the interpretation of positive and negative assay results. The PPV indicates the chance that a positive assay result is true positive and the NPV indicates the chance that a negative assay result is a true

Table 5
Results of culture and dipstick in patients with typhoid fever and correlation with clinical symptoms.

Final diagnosis, dipstick results	No. of patients with the following number of symptoms				Percentage of patients with symptoms	p-value	Percentage of patients with ≥ 2 symptoms value	p-value
	0	1	2	3				
Culture positive typhoid patients (n=205)	126	53	17	9	38.5		12.7	
Culture negative typhoid patients (n=109)	78	24	7	-	28.4	0.07	6.4	0.08
Typhoid patients								
Dipstick positive (n=182)	108	48	21	5	40.6		14.3	
Dipstick negative (n=132)	96	29	3	4	27.3	0.01	5.3	0.01
Culture positive typhoid patients								
Dipstick positive (n=128)	67	39	17	5	47.6		17.2	
Dipstick negative (n=77)	59	14	-	4	23.4	0.0005	5.2	0.01
Culture negative typhoid patients								
Dipstick positive (n=54)	41	9	4	-	24.0		7.4	
Dipstick negative (n=55)	37	15	3	-	32.7	0.3	5.5	0.7

negative result. Importantly, PPV and NPV did not differ significantly between culture and dipstick for the present study population. The PPV of the dipstick was 98.4% compared with 100% for culture, and the NPV of the dipstick was 54.2% compared with 59.3% for culture. The combined application of culture and dipstick increased sensitivity and NPV to 82.5% and 73.9%, respectively, and the specificity and PPV of the combined test result was 98.1% and 98.9%, respectively. The dipstick assay is a quick and simple test that may well be used in clinical situation where culture facilities are not available. The dipstick also may be used as a rapid screening test in conjunction with culture. The use of the dipstick in combination with culture will improve sensitivity and NPV. Furthermore, the result of the dipstick can be obtained much more quickly than that of culture and thus may facilitate early diagnosis and prompt treatment.

PPV and NPV of a given assay depends not only on assay sensitivity and specificity but also on disease prevalence. In the presence study population, the prevalence of typhoid fever was 66.4%, and the PPV and NPV was calculated to be 98.4% and 54.3%, respectively. At a lower disease prevalence, sensitivity and specificity will remain the same but PPV will decrease and NPV will increase. For instance at an assumed disease prevalence of 25% and assuming that sensitivity and specificity remain the same, PPV can be calculated to be 90.6% and NPV 87.4%. As the PPV is high, a positive result is consistent with typhoid fever and is highly significant even when the prevalence of typhoid fever among the patient population is not that high.

Our results indicated that immune

response in typhoid fever and the result of the dipstick was determined by several factors. These included duration of illness at the time of sample collection, culture positivity, and severity of illness at first presentation. Other factors including dose of infection, strain pathogenicity, previous antibiotic use, and host susceptibility could be important as well. Seropositivity increased with duration of illness and seroconversion was observed in the majority of typhoid patients with a negative dipstick result for the first collected serum sample. Seropositivity in the dipstick increased from less than 30% for samples collected during the first week of illness to over 95% for samples collected from patients ill for more than 9 days. Culture-proven typhoid patients showed a stronger immune response as determined in the dipstick compared with culture-negative typhoid patients, but this was not related to difference in duration of illness at the time of sample collection. A positive result in the dipstick was obtained at the time of first presentation in 72.5% of culture-proven patients compared with 49.5% for the culture-negative patients. The results indicated that the immune response developed much faster in patients that were culture positive than in patients that were culture negative. In culture-proven patients dipstick serology was positive in 40% of the samples collected before day 7 of illness compared with 6.4% for the culture-negative patients. Exposure to a higher dose of infection or to a more pathogenic strain may lead to a higher bacterial load. Previous antibiotic usage and host factors may alter the immune response as well. Prior antibiotic use was not assessed in this study but was likely to be high. Each of the factors that may influence the strength of the immune response at the time of presentation may vary between different areas and clinical settings, and influence the clinical utility of serology. However, the same factors also may have an effect on the result of culture.

Blood culture-proven patients that also were dipstick positive more often presented with symptoms that dipstick-negative culture-proven patients or culture-negative patients. Blood culture-proven, dipstick-positive patients on average presented 2.5 days later for medical care than dipstick-negative patients and this may explain the difference in severity of illness. Time of first presentation thus is an important factor which may contribute to a more severe illness.

In a previous study performed in Vietnam, the sensitivity and specificity of the dipstick for samples collected at hospital admission was determined to be 77% and 95%, respectively (House *et al*, 2001), and in a study performed in Semarang, the sensitivity of the dipstick was calculated to be 86.5% compared with blood culture and 69.8% compared with bone marrow culture (Gasem *et al*, 2002). The specificity (98.1%) calculated for the dipstick in our study was even higher than that reported before, but the sensitivity (58%) was lower. As discussed above, the strength of the immune response depends on various factors. The majority of the serum samples tested in our study were collected at an early stage of illness and were taken from patients with relatively mild disease that responded well to standard chloramphenicol treatment. A higher sensitivity of 72.4% was calculated for culture-positive patients and an even higher sensitivity of 95% was obtained for samples collected at a late stage of illness. The patient population included in the present study may well have been different in a number of these aspects from those included in the previous studies explaining the difference in the calculated sensitivity. For instance, all patients included in the study performed in Vietnam were culture positive with an average duration of illness of 8 days compared with only 65.3% culture-positive patients and an average duration of illness of 7.4 days in our study. Furthermore, as multidrug resistance in Vietnam is widespread, typhoid may lead to more severe illness and stronger immune response in that country.

S. typhi-specific IgM antibodies were not detected in the dipstick assay in the serum samples collected at the first time of presentation of the patients in 27.6% of the blood culture-proven typhoid patients, and in 50.5%

of the culture-negative patients with a final clinical diagnosis of typhoid fever. Testing of follow-up samples showed seroconversion in most patients with an initial negative dipstick result. Seroconversion provides strong evidence of disease but does not contribute to a rapid diagnosis. Seroconversion was seen in 29.1% of the culture-proven typhoid patients and in 81.4% of the culture-negative typhoid patients.

The Widal test is often used in the diagnosis of typhoid fever when culture facilities are not present. The practical use of the Widal test has been reported to vary depending on a number of factors including quality of the reagents and patients population. In a study from Vietnam, the sensitivity and specificity for the O antigen at a cut-off value of (1:100 was determined to be 83% and 89%, respectively (Parry *et al*, 1999), while in a more recent study the same authors reported a sensitivity of 92% and a specificity of 57% (House *et al*, 2001). The sensitivity of the Widal test using the O antigen in our study at the routinely used cut-off of ≥1:320 was 58.3% for the samples collected at the time of hospital admission from the group of culture-proven typhoid patients and was 34.9% for the group of culture-negative patients with a final clinical diagnosis of typhoid fever. The specificity was 80.5%. These results illustrated the variation in Widal test results. Widal titers in serum samples from patients may be as high as 1:160 to 1:640, and in endemic areas antibody levels at or above the cut-off level are not unusual in samples from healthy individuals or non-typhoid patients. The value of the Widal test in the serodiagnosis of typhoid fever thus is limited, as results are difficult to interpret. In this study, the sensitivity and specificity of the dipstick assay were significantly higher than those of the Widal test.

It is well-recognised that patients with confirmed typhoid fever may have a negative Widal test throughout the course of their illness, and this has been contributed to undefined host and pathogen factors (Schroeder, 1968). Also in about 10% of the culture-proven and culture-negative typhoid fever, no specific IgM antibodies could be detected in the dipstick assay during a 2 week follow-up. Any of the suspected factors including strain variation, poor antigenicity, prior antibiotic treatment and host factors could play a role in the absence or weakness of the immune response in the patients.

The dipstick assay is easy to use and does not require specialized training or equipment, and the components are stable without a requirement for refrigeration. The dipstick assay is highly specific and has an acceptable sensitivity. All these factors make the test ideal for developing countries and rural and suburban settings in endemic areas where culture facilities are not readily available and the need for a rapid test that can be applied in district hospitals and primary health care centers is high.

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