

## ORIGINAL ARTICLE

## EFFICACY OF DIAZO URINE TEST IN THE DIAGNOSIS OF TYPHOID FEVER IN CHILDREN

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**Abstract**

**Aim:** To compare the diagnostic efficacy of Diazo test vis-a-vis blood culture and Widal test in enteric fever in children.

**Design:** A randomized, open, controlled clinical trial in a teaching hospital in Moradabad.

**Methods:** One hundred twelve children suffering from enteric fever (based on suggestive clinical picture and either positive blood culture, Typhidot M or Widal in study group and 60 children with fever of confirmed aetiology other than enteric fever in control group were included in the study. Results of diazo test were compared with blood culture positive, Typhidot M positive or Widal.

**Results:** Of the 112 blood cultures, only 18 (16.1%) were positive for Salmonella typhi. In the first week of illness, Typhidot M was positive in 35 (89.7%) out of 39 cases whereas it was positive in all the 112 cases in the second week of illness. In the first week, only 4 (10.3%) out of 39 patients showed a positive Widal test. In the second week, 71 out of 73 new cases as well as all the 39 old cases showed positive Widal test. In the third week, 110 out of the total 112 cases showed positive result. In the first week of illness, 27 (69.2%) out of 39 patients in the study group showed a positive diazo test. In the second week, 99 (88.4%) out of 112 patients in the study group showed a positive Diazo test. In the third week of illness, 81 patients out of the total 112 cases had a positive Diazo test. Diazo test showed sensitivity, specificity, positive and negative predictive value, and likelihood ratio of positive and negative test of 88.4%, 93.3%, 96.1%, 81.2%, 13.2% and 0.12% respectively as compared to Typhidot M and 90%, 93.5%, 96.1%, 81.2%, 13.9% and 0.11% as compared to Widal and 88.9%, 11.7%, 16.1%, 84.6%, 1% and 0.95% as compared to blood culture.

**Conclusion:** Diazo test is a simple bedside, quick and cheap test with significant amount of reliability for the diagnosis of enteric fever in children.

**Key Words:** enteric fever, Diazo test, sensitivity, efficacy.

**Introduction**

Typhoid fever is a potentially fatal multi-systemic infection caused by the bacterium Salmonella enterica serovar Typhi. Salmonella enterica serovars Paratyphi A, B (*S. schottmuelleri*), and C (*S. hirschfeldii*) cause the clinically similar condition, paratyphoid fever. (1) Typhoid and paratyphoid fevers are collectively referred to as enteric fevers. In most endemic areas, approximately 90% of enteric fever is typhoid. (1) Humans are the natural host and reservoir. Salmonella bacteria can survive for days in groundwater or seawater and for months in contaminated eggs and frozen oysters. (2-5) Transmission of infection occurs by ingestion of food or water contaminated with faeces. Other established risk factors include recent contact

with a typhoid patient or carrier, eating ice cream, flavoured iced drinks or food from street vendors, and raw fruit and vegetables grown in fields fertilized with sewage. (4) The disease remains a serious public health problem in developing countries due to poor sanitation and low socioeconomic status. (7,8) The disease is endemic in South-east Asia and Africa and persists in the Middle East, few southern and eastern European countries and central and South America. (1,9) The disease carries a significant morbidity and mortality in both pediatric and adult populations. Despite being a global health problem, very little progress has been made in the diagnosis of enteric fever. Diagnosis is still made mostly clinically supported by Widal test, Typhidot M test and very rarely by blood culture. Blood culture is not only the gold standard for diagnosis but also suggests the sensitivity pattern of the organism. Widal test has only moderate sensitivity and specificity. (10,11) Despite these limitations the test is useful, particularly in areas that cannot afford the more expensive diagnostic methods. Typhidot M, an Enzyme Immuno-Assay test, offers simplicity, speed, early diagnosis and high negative and positive predictive values. (12)

Diazo test is a simple non-invasive, inexpensive bedside test to diagnose typhoid fever. Diazo test, originally described by Huckstep in 1962, is a diagnostic tool in the diagnosis of Typhoid fever. (13-18) It is known that the putrefaction of a protein in the intestine of patients with typhoid fever leads to a breakdown product being excreted in the urine as a phenol-ring compound. This is detected by Diazo test. This study analyses the value of diazo test vis-a-vis Widal test, Typhidot M and Blood culture.

**Materials and Methods**

The present study was conducted during the year 2011-12 in the Departments of Pediatrics and Microbiology, Teerthankar Mahaveer Medical College, Moradabad from January 2011 to August 2012. These cases were selected from outdoor and indoor wards. Patient selection criterion was any child from 6 months to 12 years with fever more than four days and a clinical suspicion of typhoid fever. Diagnosis was made on the basis of clinical history, examination, investigations including complete blood counts and positive blood culture, Widal (O titer  $\geq 1/160$ ) or Typhidot test. All the cases had received antibiotics prior to admission. Simultaneously, control cases were selected from similar age group having acute febrile illness with confirmed diagnosis other than enteric fever. Cases with fever with unconfirmed etiology were excluded.

Diazo test was performed on early morning urine samples of all cases daily from their time of inclusion in the study to the time of discharge.

**Diazo test:** Diazo reagent is prepared from two stock solutions 'A' and 'B'. Their compositions were as below: a) Stock solution A: Sulphanilic acid- 0.5 gm,

Concentrated HCL- 5 mL, Distilled water- 100 mL. b) Stock solution B: Sodium nitrite- 0.5 gm, Distilled water- 100 mL. This test solution is made by mixing forty parts of stock solution 'A' with one part of stock solution 'B', and must be made freshly once a day. The stock solution 'B' must be made freshly once every three weeks, and must be kept in a refrigerator at about 4-8°C. Solution 'A' is stable.

The test was performed by pouring a small quantity of early morning urine sample into a test tube, and adding an equal amount of diazo solution. This was mixed gently and then 5 drops of 30% ammonium hydroxide were added. The mixture was shaken well and the color of the froth noted. A red or pink coloration of the froth, on two consecutive days or more was considered as a positive reaction. Any colour except red or pink was considered negative. The test was done daily to find out how long it remained positive and what was the effect of antibiotic on the test.

The results of diazo test were compared with the results of blood culture, Typhidot M and Widal test. Statistical analyses of the results were carried out to determine: sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio of positive test and likelihood ratio of negative test.

**Results**

A total of 112 cases were included in the study group and 60 in control group. Male: female ratio was 1.6:1 in study group and 1.5:1 in control group. Mean age in boys was 7.0±2.8 years in cases and 6.1±2.5 in controls and mean age for girls was 7.2±2.7 years in cases and 5.8±2.5 in controls. Overall mean age was 7.1±2.1 years with a range of 9 months to 12 years in study group and 6.0±2.5 years in control group with a range of 1.5 years to 12 years. Age and gender distribution is depicted in table 1.

Thirty-nine patients (34.8%) had fever for less than 7 days, while the remaining 73 (65.2%) had history of fever of more than 7 days at the time of inclusion in the study. Of the 112 blood cultures, only 18 (16.1%) were positive for Salmonella typhi of which 16 (41%) out of 39 were positive in the first week of illness, while only 2 out of 73 (2.7%) had a positive blood culture in the second week of fever. None of the cases in the control group was culture positive for S. typhi. Blood

culture was performed only once in each patient in both the study and control group. In the first week of illness, Typhidot M was positive in 35 (89.7%) out of 39 cases whereas it was positive in all the 112 cases in the second week of illness. The test was not repeated in the third week due to cost factor. The test was negative in all the control cases. Widal test was done in all the cases in the study group and control group at the time of admission as well as in the 2nd and 3rd week of illness. In the first week, only 4 (10.3%) out of 39 patients showed a positive Widal test. In the second week, 71 out of 73 new cases as well as all the 39 old cases showed positive Widal test. In the third week, 110 out the total 112 cases showed positive result. In the control group, none of the patient had a positive Widal test. In the first week of illness, 27 (69.2%) out of 39 patients in the study group showed a positive diazo test. In the second week, 99 (88.4%) out of 112 patients in the study group showed a positive Diazo test. In the third week of illness, 81 patients out of the total 112 cases had a positive Diazo test. In the control group, Diazo test was positive in only four (6.7%) patients. Table 2 depicts sensitivity and specificity of diazo test as compared to blood culture, widal and Typhidot test.

**Discussion**

Typhoid fever is endemic in India but reliable data to estimate the burden of disease is difficult to obtain. Blood culture provides unequivocal evidence of the disease. Population-based estimates of blood culture confirmed typhoid cases are sparse. Most hospitals lack facilities for blood culture and up to 90% of patients with fever are treated as outpatients. In Asia, disease burden estimates rely on clinically diagnosed cases of typhoid fever compiled by governments and hospitals, usually with uncertain denominators. (8,9) In our study, Diazo test showed a sensitivity of 88.4%, 90% and 88.9% respectively when validated against Typhidot, Widal and blood culture whereas, it showed a specificity of 93.3%, 93.5% and 11.7% respectively, against the three test. Low specificity compared to blood culture can be understood by the fact that blood culture had an overall sensitivity of 16.1% in the current study. Similar results were found by other researchers as shown in Table 3.

**Table 1: Age & Sex Distribution of patients**

Age group (years)	Study group Male (%)	Study group Female (%)	Total (%)	Control group Male (%)	Control group Female (%)	Total (%)
1-3	7 (6.3)	3 (2.7)	10 (8.9)	7 (11.7)	4 (6.7)	11 (18.3)
4-6	26 (23.2)	15 (13.4)	41 (36.6)	10 (16.7)	12 (20)	22 (36.7)
7-9	20 (17.9)	15 (13.4)	35 (31.3)	18 (30)	6 (10)	24 (40)
10-12	16 (14.3)	10 (8.9)	26 (23.2)	1 (1.7)	2 (3.3)	3 (5)
Total	69 (61.6)	43 (38.4)	112 (100)	36 (60)	24 (40)	60 (100)

**Table 2: Comparison of Typhidot M test, Widal and Blood culture with Diazo test**

Diazo test	Typhidot M test		Widal test		Blood culture	
	Positive (n=112)	Negative (n=60)	Positive (n=110)	Negative (n=62)	Positive (n=18)	Negative (n=94)
Positive	99 (88.4%)	4 (6.7%)	99 (90%)	4 (6.5%)	16(88.9%)	83(88.3%)
Negative	13(11.6%)	56(93.3%)	11 (10%)	58 (93.5%)	2(11.1%)	11(11.7%)
False positive (%)	6.7		6.5		88.3	
False negative (%)	11.6		10.0		11.1	
Sensitivity (%)	88.4		90.0		88.9	
Specificity (%)	93.3		93.5		11.7	
Positive predictive value (%)	96.1		96.1		16.1	
Negative predictive value (%)	81.2		81.2		84.6	
Likelihood ratio positive	13.2		13.9		1.0	
Likelihood ratio negative	0.12		0.11		0.95	

**Table 3: Comparison of our results with previous studies**

Study	Standard for diagnosis	Sensitivity of diazo test	Specificity of diazo test
Raman et al, 1994 (15)	Blood culture	92	83
Shivpuri et al, 2003 (16)	Blood culture & Widal test	81	90
Tanyigna et al, 2008 (19)	Widal test	27.3	-
Beig et al, 2010 (17)	Blood culture, Typhidot & Widal	86.7	85.7
Present study	Blood culture, Typhidot & Widal	88.4	93.3

Blood culture is generally regarded as 'Gold standard' for diagnosis of enteric fever. Recovery of the organism from blood cultures depends on several factors including the volume of blood cultured, the ratio of the volume of blood to the volume of culture broth in which it is inoculated (the ratio should be at least 1:8) and inclusion of anti-complementary substances in the medium (such as sodium polyetholsulfonate or bile). (1) But the delay involved in getting the culture report and the feasibility of submitting blood for culture in the first week of illness and before antibiotics have been started, along with its poor sensitivity, (13) makes blood culture not a very ideal choice in developing countries. The Widal test has suboptimal sensitivity and specificity. (20,21) It can be negative in up to 30% of culture proven cases of typhoid fever. Suboptimal sensitivity results from negativity in early infection, prior antibiotic therapy and failure to mount an immune response by certain individuals. (10,21,22) Poor specificity is an even greater problem and is a consequence of pre-existing baseline antibodies in endemic areas, cross reactivity with other gram-negative infections and non-typhoidal salmonella, anamnestic reactions in unrelated infections and prior typhoid vaccination. (11) Notwithstanding these problems, the Widal test may be the only test available in certain resource poor set ups for diagnosis of enteric fever. Hence, it is important to realize the

limitations of the Widal test and interpret the results with care in accordance with appropriate local cut-off values for the determination of positivity. (10,20-22) Evaluation studies have shown that Typhidot-M is superior to the culture method. (12) Although culture remains the gold standard it cannot match Typhidot-M in sensitivity (>93%), negative predictive value and speed. (21) The high negative predictive value of the test suggests that Typhidot-M would be useful in areas of high endemicity. (12,21)

This study shows that diazo test has a high degree of sensitivity and specificity. The test has a positive predictive value, negative predictive value, likelihood ratio positive and negative of 96.1%, 81.2%, 13.2% and 0.12% respectively as compared to Typhidot M test. This simple and quick test is a useful diagnostic tool in the early diagnosis of typhoid fever especially in areas with limited resources. More importantly, prior administration of antibiotics does not interfere with the test.

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- 
- E-published:** 1st October 2013 Art # 55
- 
- DOI: 10.7199/ped.oncall.2013.55**



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