SHORT COMMUNICATION

# Comparative Study of Dot Enzyme Immunoassay (Typhidot-M) and Widal Test in the Diagnosis of Typhoid Fever

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We compared the sensitivity and specificity of Typhidot-M and Widal test with blood culture (gold standard) for diagnosing typhoid fever in 105 children aged 1-15 years admitted with clinical suspicion of typhoid fever. Of the 105 cases, blood culture was positive for *S.typhi* in 41 (39%) children, Widal test was positive in 48 (45.7%) and Typhidot-M was positive in 78 (74.3%) cases. Sensitivity and specificity of Typhidot-M was 92.6% and 37.5% while sensitivity and specificity of Widal test was 34.1% and 42.8%, respectively. In children with fever of less than 7 days duration, Typhidot-M was positive in 97%, compared to 24.2% by Widal test. Typhidot-M is a simple and sensitive test for early diagnosis of typhoid fever in children.

**Key words:** *Blood culture, Salmonella typhi, Typhoid fever, Typhidot-M, Widal test.*

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Typhoid fever continues to be a global health problem, especially in tropics and subtropics(1-3). Early and accurate diagnosis is necessary for prompt and effective treatment. One has to rely on serological diagnosis since many diagnostic laboratories in developing countries do not have facilities for blood culture(4,5).Widal test is the mainstay in the diagnosis of typhoid fever in most laboratories but it has drawbacks(6-8).

We evaluated the sensitivity and specificity of Typhidot-M, a dot enzyme immunoassay, for diagnosis of typhoid fever in children.

## METHODS

We enrolled 105 children in the age group of 1-15 years, who presented with fever of 5 days or more with clinical symptoms and signs suggestive of typhoid fever. Children with documented typhoid fever within past 8 weeks and those who were immunized against typhoid fever were excluded. Clearance from the ethical committee and informed consent from the parents of all the children enrolled was obtained.

All cases were subjected to a detailed history and thorough clinical examination. Following investigations were done on the day of admission: hemoglobin, total and differential leucocyte count, platelet count, peripheral smear, Typhidot-M test, blood culture and widal test. Standard procedures were used for isolation of organism from brain heart infusion/bile broth. For Widal test, a titer of 1 in 160 or more was considered as positive.

Typhidot-M is a dot enzyme immunoassay for the detection of specific IgM to *Salmonella typhi*. In this test, IgG is inactivated before carrying out the assay as for the Typhidot. The test uses a nitrocellulose membrane strip dotted with the 50 KDa specific protein and a control antigen. 2.5 $\mu$L of patient serum and controls are pre-absorbed for at least one minute with 90 $\mu$L of IgG inactivation reagent. 250 $\mu$L of sample diluent was then added into the reaction wells

and the mixture incubated at room temperature on a rocker platform for 20 min. The strips were washed thrice for a total of five minutes, and 250 $\mu$L of anti-human IgM conjugate was added and incubated for 15 min. The strips were washed as before, and 250 $\mu$L of color development solution was added and incubated for 15 minutes. The reaction was stopped, by washing the strips in distilled water and the results were read. When both the dots on the test strip were as dark or darker than their corresponding dots on the positive control strip, they were reported as positive.

All the statistical operations were done through SPSS for windows, version 10.0 (SPSS Inc, 1999, New York) and by using Epi Info 6 (CDC, Atlanta). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

## RESULTS

Of 105 cases, *S.typhi* was isolated from blood culture in 41 cases (39%), and the remaining 64 cases were blood culture negative, among which 13 cases had final alternative diagnosis and treated accordingly. Widal test was positive in 48 cases (45.7%) and Typhidot-M test was positive in 78 cases (74.3%). The sensitivity, specificity, and positive and negative predictive value of Typhidot-M and widal test is compared in ***Table* I**.

In children having fever of less than 1 week duration ($n$=33), Typhidot-M was positive in 32 (96.9%) cases, compared to 8 cases positive by Widal test (24.2%) ($P$<0.001). Typhidot-M was positive in 100% of cases who came with fever of 5 days and 6 days duration. This was significantly higher than Widal, which was positive in 20% and 11.1%, respectively in fever of 5 days and 6 days duration. In children presenting with fever of 7 days, there was no statistically significant difference between Typhidot-M and Widal test positivity.

**TABLE I** COMPARISON OF TYPHIDOT M AND WIDAL TEST WITH BLOOD CULTURE

| Blood culture | Typhidot-M | | Widal test | |
|---|---|---|---|---|
| | Positive | Negative | Positive | Negative |
| Positive ($n$=41) | 38 (92.7) | 3 (7.3) | 14 (34.1) | 27 (65.9) |
| Negative ($n$=64) | 40 (62.5) | 24 (37.5) | 37 (57.8) | 27 (42.2) |
| Total ($n$=105) | 78 | 27 | 51 | 54 |

*Figures in parenthesis indicate percentages. Typhidot-M: Sensitivity 92.6%, specificity 37.5%, positive predictive value 48.7% and negative predictive value 88.8%; widal test: sensitivity 34.1%, specificity 42.8%, positive predictive value 27.4% and negative predictive value 50.0%.*

## DISCUSSION

In the present study, the sensitivity of Typhidot-M is 92.6% which is comparable to most of the other studies(4,9-14). Thus Typhidot-M meets one of the criteria of an ideal diagnostic test as it doesn't usually miss the diagnosis when compared to blood culture. Only 3 cases, which were blood culture positive, were negative by Typhidot-M. All these 3 cases presented with duration of fever more than 7 days. Probably decreasing levels of IgM against outer membrane protein of the bacterial cell and masking of IgM by IgG in the second week may be the reason for negativity by Typhidot-M. This problem could have been obviated if Typhidot (IgG) was used as well(9,11).

There were 40 cases, which were apparently false positive by Typhidot-M out of 64 blood culture negative cases. However, 22 out of these 40 cases presented with fever of >7 days and blood culture might not have shown positive results in them. Since Typhidot-M also detects IgM antibodies in the second week, more number of cases has been picked up by this test compared to blood culture.

Typhidot-M was positive in 97% of cases who presented with fever of <7 days among blood culture positives as compared to Widal, which was positive in 24.2%. This indicates that Typhidot-M can also be effectively used for early diagnosis of typhoid fever, as also reported earlier(10).

Typhidot-M meets many of the criteria for an ideal diagnostic test - is simple, sensitive, early, rapid (only 3 hours compared to 48 hours for blood culture and 24 hours for Widal test) and requires minimal, operator training. Limitations include higher cost and cold storage requirement for test reagents.

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**WHAT THIS STUDY ADDS?**

- Typhidot-M (Dot enzyme immunoassay) is a sensitive (92.6%) test for early diagnosis of typhoid fever in children.

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