

## Comparison of the performance of Tubex® TF, widal immunodiagnostic assay and blood culture in diagnosis of enteric fever in a private tertiary care hospital Peshawar, Pakistan

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### Abstract

**Objective:** To determine and compare the performance of TUBEX®TF, Widal test and blood culture in the diagnosis of enteric fever.

**Method:** The retrospective study was conducted at the Northwest General Hospital and Research Centre, Hayatabad, Peshawar, Pakistan, and comprised medical record from January to December 2018 related to patients who presented with fever. Typhidot, Widal test and blood culture had been performed as part of evaluation. Data was analysed using SPSS 16.

**Results:** Of the 241 patients, 68(28.21%) tested positive for salmonella in blood culture. Among them, TUBEX®TF was positive in 29(42.64%) and Widal was positive in 25(36.76%). TUBEX®TF had positive predictive value 33.33%, negative predictive value 71.77%, sensitivity 42.65% and specificity 62.34%. The corresponding values for Widal were 24.51%, 69.06%, 36.76% and 55.49%.

**Conclusion:** Sensitivity, specificity, positive predictive value and negative predictive value of TUBEX®TF and Widal test were very low compared to blood culture.

**Keywords:** Enteric fever, TUBEX®TF, Widal, Blood culture. (JPMA 71: 909; 2021) DOI: <https://doi.org/10.47391/JPMA.455>

### Introduction

Enteric fever (EF) is a common infectious disease worldwide caused by *Salmonella* (*S.*) *typhi* and *S. paratyphi*.<sup>1</sup> According to the best global estimates, approximately 600,000 EF deaths occur annually, and majority of them occur in developing countries, including Pakistan.<sup>1</sup> A study showed Pakistan and India as having the highest EF prevalence.<sup>1</sup>

Clinical manifestations are non-specific and diagnosis is confirmed by isolating the organism for culture. Unfortunately, in Pakistan, the diagnosis of typhoid fever has been challenging. Diagnostic approaches used for detection include microbiological culturing of blood, urine, bone marrow and serologic assays, such as the Typhidot and Widal. Blood culture and bone marrow culture, being the gold standard, are not practised due to the lack of availability of these tests in peripheral areas, and clinicians routinely depend on antigen testing methods, such as Typhidot and Widal, to confirm EF diagnosis. These tests have wide variability in sensitivity and specificity, and, hence, are not recommended across the world.<sup>2,3</sup>

The current study was planned to determine and compare the performance of TUBEX®TF, Widal test and blood culture in EF diagnosis.

### Materials and Methods

The retrospective study was conducted at the Northwest General Hospital and Research Centre, Hayatabad, Peshawar, Pakistan, and comprised medical records from January to December 2018 of patients who presented with fever. After approval from the institutional ethics committee the sample size was calculated using the formula:<sup>4,5</sup>  $n = z^2 p(1-p) / e^2$ ; where *p* is the estimate of sensitivity/specificity; *z*<sup>2</sup> is the value for 95% confidence level which was 1.96; *e* is margin of error; *p* is sensitivity assumed as 0.83.<sup>6</sup> Margin of error was set as 0.05.

The sample was raised using convenience sampling, and data related to all patients who presented with fever to the hospital and had undergone the three tests was included, while that of patients who did not have all the three tests performed as part their initial evaluation was excluded. To exclude contaminations, standard protocols were followed to collect 3-5mL of venous blood for the cultures and 3-5ml for serological essays, including Widal and TUBEX®TF. The samples were further assigned in the automatic incubator for the growth using B12 medium. Cultures having positive growth of bacteria were further evaluated for species. Antibiotic sensitivities were checked accordingly. TUBEX®TF kit was used to detect immunoglobulin M (IgM) antibodies to *S. typhi* O9 antigen. Widal assay was performed using 2-fold diluted plasma and the agglutination titres to detect the presence of antibodies reacting to *S. flagellar* (H) and / or lipopolysaccharide (O) antigens. The values were considered positive with a 4-fold increase in the titre from

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acute to convalescent phase of illness, or a single titre at either time point of 320. Taking blood culture as the gold standard, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of TUBEX®TF and Widal were calculated. Sensitivity was calculated as: sensitivity = True Positive (TP) / [TP + False Negative (FN)] × 100%. Specificity was calculated as: specificity = True Negative (TN) / [TN + False Positive (FP)] × 100%. PPV was calculated as: PPV = TP / [TP + FP] × 100%, and NPV was calculated as: NPV = TN / [TN + FN] × 100%.<sup>7</sup>

Data was analysed using SPSS 16.

## Results

Of the 241 patients, 68(28.21%) tested positive for salmonella in blood culture. Among them, TUBEX®TF was positive in 29(42.64%) (Table 1) and Widal was positive in 25(36.76%) (Table 2).

Compared to the gold standard, TUBEX®TF had PPV 33.33%, NPV 71.77%, sensitivity 42.65% and specificity 62.34% (Table 3). The corresponding values for Widal were 24.51%, 69.06%, 36.76% and 55.49% (Table 4).

**Table-1:** Blood culture versus TUBEX®TF.

Test/result	Blood Culture		Total
	Positive	Negative	
TUBEX®TF Positive	29	58	87
TUBEX®TF Negative	39	96	135
Total	68	154	225

**Table-2:** Blood culture versus Widal.

Test/result	Blood Culture		Total
	Positive	Negative	
Widal TO > 80	25	77	102
Widal TO < 80	43	96	139
Total	68	173	241

TO is Widal Antigen Titer

**Table-3:** The performance of the TUBEX®TF test for the diagnosis of enteric fever taking blood culture as the gold standard.

	Percentage	Confidence Interval
Sensitivity	42.65%	30.72%--55.23%
Specificity	62.34%	54.18%--70.01%
Positive predictive value	33.33%	26.20%--41.32%
Negative predictive value	71.11%	65.97%--75.76%

**Table-4:** The performance of the TUBEX®TF test for the diagnosis of enteric fever taking blood culture as the gold standard.

	Percentage	Confidence Interval
Sensitivity	42.65%	30.72%--55.23%
Specificity	62.34%	54.18%--70.01%
Positive predictive value	33.33%	26.20%--41.32%
Negative predictive value	71.11%	65.97%--75.76%

## Discussion

There is an ongoing outbreak of extensively drug resistant (XDR) typhoid fever in Pakistan that began in Hyderabad in November 2016. Most *S. typhi* strains are multi drug resistant (MDR). The outbreak has spread to Karachi and to multiple cities.<sup>8</sup> In 2018, 3 XDR cases diagnosed with EF were reported in people who had travelled from the United Kingdom and the United States.<sup>9</sup>

The current study was conducted to evaluate the performance of TUBEX®TF and Widal compared to blood culture in EF diagnosis. Both TUBEX®TF and Widal had low sensitivity and specificity, and, thus, should not be practised. TUBEX®TF sensitivity was lower compared to 60% reported from Bangladesh and 76% from India.<sup>10,11</sup> TUBEX®TF PPV was 33.33% which was very low compared to 90% in Bangladesh<sup>10</sup> and 89.2% in Egypt.<sup>12</sup>

TUBEX®TF specificity was 62.34% which was low compared to 96-99% in India<sup>11</sup> and 75% in Egypt.<sup>12</sup> A study in Tanzania reported 56-95% sensitivity of TUBEX®TF with an average of 69%, and specificity of 72-95% with an average of 88%.<sup>13</sup>

In the current study, TUBEX®TF had an NPV of 71.77% , which was significantly high compared to studies conducted in Bangladesh and Egypt.<sup>10,12</sup>

The sensitivity, specificity, PPV and NPV of Widal in the current study were significantly lower compared to values reported from Tanzania.<sup>13</sup>

The limitations of the current study include the time duration from the onset of clinical symptoms and investigations which affected quite significantly the result of the immunoassays and culture. Also, the impact of previous usage of antibiotics and the result of immunoassays were not considered by the current study. This is critical since it can give a negative blood culture while the immunoassays may remain positive in patients who had taken antibiotics before presenting to hospital.

## Conclusion

Sensitivity, specificity, PPV and NPV of TUBEX®TF and Widal test were found to be very low compared to blood culture, and thus, should be avoided.

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**Conflict of interest:** None.

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