



## CROSS-REACTIVITY AND SPECIFICITY: A COMPARATIVE ANALYSIS OF WIDAL AND TYPHI DOT IN ENTERIC FEVER DIAGNOSIS

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### Abstract:

This study aimed to conduct a comparative analysis of the Widal and Typhidot tests in the diagnosis of enteric fever, focusing on cross-reactivity and specificity. A prospective, cross-sectional study was conducted over 12 months, involving 216 patients with suspected enteric fever. Blood culture served as the gold standard for comparison. The Typhidot test demonstrated superior performance with higher sensitivity (86.2% vs 72.4%) and specificity (88.0% vs 83.5%) compared to the Widal test. ROC curve analysis confirmed the Typhidot's better overall diagnostic accuracy (AUC 0.87 vs 0.78). Cross-reactivity analysis revealed higher false-positive rates for the Widal test, particularly with non-typhoidal *Salmonella* (40%) and other Enterobacteriaceae (25%). The Typhidot test showed improved specificity with lower cross-reactivity. Multivariate analysis identified age <15 years and prior antibiotic use as significant factors influencing Widal test outcomes, while fever duration >7 days affected Typhidot results. Despite substantial agreement between the tests (Cohen's Kappa 0.68), discordant results highlighted the potential for misclassification when relying on a single test. While the Typhidot test demonstrated better overall performance, both tests showed limitations in cross-reactivity and specificity. These findings underscore the need for careful interpretation of serological test results in the context of clinical and epidemiological data. Future research should focus on developing more specific antigens, exploring combination approaches, and integrating host biomarkers to enhance enteric fever diagnosis in endemic settings.

**Keywords:** Enteric fever, Typhoid fever, Widal test, Typhidot test, Cross-reactivity, Specificity, Serological diagnosis, *Salmonella* Typhi, Diagnostic accuracy

### Introduction

Enteric fever, a systemic infection primarily caused by *Salmonella enterica* serovar Typhi (S. Typhi) and *Salmonella enterica* serovar Paratyphi A, B, and C, remains a significant public health concern in many parts of the world, particularly in developing countries (Crump & Mintz, 2010). The World Health Organization (WHO) estimates that enteric fever affects 11-20 million people annually, resulting in approximately 128,000-161,000 deaths (Mogasale et al., 2014). The disease is characterized by prolonged fever, abdominal pain, and various systemic manifestations, making early and accurate diagnosis crucial for effective management and prevention of complications. The gold standard for diagnosing enteric fever has traditionally been blood culture, which offers high specificity

but suffers from limited sensitivity, especially in areas where antibiotic use prior to seeking medical attention is common (Parry et al., 2011). Moreover, blood culture requires specialized laboratory facilities, skilled personnel, and takes several days to yield results, which can delay appropriate treatment. These limitations have led to the development and widespread use of serological tests for the diagnosis of enteric fever, with the Widal test being one of the most commonly employed methods in resource-limited settings.

The Widal test, first described by Georges Fernand Widal in 1896, is based on the agglutination of patient serum with O and H antigens of *S. Typhi* (Olopoenia & King, 2000). Despite its long history and widespread use, the Widal test has been criticized for its lack of standardization, variable performance across different geographic regions, and potential for cross-reactivity with other Enterobacteriaceae (Anduaem et al., 2014). The test's interpretation is further complicated by the need for paired sera to demonstrate a rise in antibody titers, which is often impractical in clinical settings where prompt diagnosis is required. In response to these challenges, newer serological tests have been developed, including the Typhidot test, which detects IgM and IgG antibodies against the 50 kDa outer membrane protein of *S. Typhi* using an enzyme-linked immunosorbent assay (ELISA) format (Choo et al., 1994). The Typhidot test has shown promise in terms of improved specificity and earlier detection of antibodies compared to the Widal test (Keddy et al., 2011). However, like all serological tests, it is not without limitations, including the potential for cross-reactivity with other *Salmonella* species and the persistence of antibodies from previous infections or vaccinations.

The comparative analysis of Widal and Typhidot tests in the diagnosis of enteric fever is of particular interest due to their widespread use and the ongoing debate regarding their relative merits and limitations. Cross-reactivity, defined as the reaction of an antibody with an antigen other than the one that induced its formation, is a critical factor affecting the specificity of these tests (Levinson, 2014). In the context of enteric fever diagnosis, cross-reactivity can lead to false-positive results, potentially resulting in unnecessary antibiotic treatment and contributing to the growing problem of antimicrobial resistance. Specificity, on the other hand, refers to the ability of a test to correctly identify individuals who do not have the disease (Maxim et al., 2014). High specificity is crucial in the diagnosis of enteric fever to avoid misdiagnosis and inappropriate treatment, especially in endemic areas where the prevalence of other febrile illnesses may be high. The balance between sensitivity and specificity is a key consideration in the evaluation of diagnostic tests, with the ideal test offering both high sensitivity for early detection and high specificity to minimize false-positive results.

The comparative analysis of Widal and Typhidot tests must consider several factors that can influence their performance. These include:

1. Antigenic variability: *S. Typhi* and *S. Paratyphi* exhibit antigenic variations that can affect the performance of serological tests. The O and H antigens used in the Widal test may not fully capture this variability, potentially leading to false-negative results (Baker et al., 2010). The Typhidot test, targeting a specific outer membrane protein, may offer improved specificity but could miss infections caused by strains with variations in this protein.
2. Timing of antibody response: The kinetics of antibody production in enteric fever can vary among individuals and may be influenced by factors such as prior exposure, vaccination status, and immune competence (Ismail, 2020). Understanding the temporal dynamics of IgM and IgG antibody responses is crucial for interpreting the results of both Widal and Typhidot tests.
3. Endemic setting: In areas where enteric fever is endemic, the background prevalence of antibodies in the population can affect the positive predictive value of serological tests. This is particularly relevant for the Widal test, where determining appropriate cut-off titers for diagnosis can be challenging (Adhikari et al., 2015).
4. Cross-reactivity with other pathogens: Both Widal and Typhidot tests may cross-react with antibodies produced in response to other infections, including non-typhoidal *Salmonella* species, other Enterobacteriaceae, and even non-enteric pathogens such as malaria parasites (Maude et al., 2015). Understanding the extent and nature of these cross-reactions is essential for accurate interpretation of test results.

5. Impact of prior antibiotic use: The widespread use of antibiotics, often without prescription in many endemic areas, can affect the performance of both blood culture and serological tests. Antibiotics may suppress bacterial growth in blood culture and alter the antibody response detected by serological tests (Andrews & Ryan, 2015).

6. Technical factors: The performance of both Widal and Typhidot tests can be influenced by technical factors such as antigen quality, test kit storage conditions, and operator expertise. Standardization of these factors is crucial for ensuring consistent and comparable results across different settings (Adhikari et al., 2015).

The comparative analysis of Widal and Typhidot tests in terms of cross-reactivity and specificity has important implications for clinical practice and public health. In clinical settings, understanding the strengths and limitations of these tests can guide their appropriate use and interpretation, potentially leading to more accurate diagnoses and targeted treatment. From a public health perspective, improved diagnostic accuracy can contribute to better surveillance of enteric fever, informed decision-making regarding vaccination strategies, and more effective control measures.

Recent advances in molecular diagnostics, including polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) assays, offer promising alternatives for the rapid and specific detection of *S. Typhi* and *S. Paratyphi* (Prakash et al., 2019). However, these methods often require specialized equipment and trained personnel, limiting their widespread adoption in resource-constrained settings where enteric fever is most prevalent. As such, serological tests like Widal and Typhidot are likely to remain important diagnostic tools in many parts of the world for the foreseeable future. The ongoing evolution of antimicrobial resistance in *S. Typhi* and *S. Paratyphi* further underscores the importance of accurate and timely diagnosis. The emergence of extensively drug-resistant (XDR) strains of *S. Typhi*, particularly in South Asia, poses a significant threat to effective treatment and control of enteric fever (Klemm et al., 2018). In this context, diagnostic tests that can rapidly and accurately identify cases of enteric fever are crucial for guiding appropriate antibiotic therapy and preventing the further spread of resistant strains.

The aim of this study was to conduct a comparative analysis of the Widal test and Typhidot test in the diagnosis of enteric fever, with a specific focus on cross-reactivity and specificity, to inform their appropriate use in clinical practice and public health interventions.

## **Methodology**

### **Study Design:**

A prospective, cross-sectional study was conducted to evaluate and compare the performance of the Widal test and Typhidot test in the diagnosis of enteric fever. The study employed a comprehensive approach, incorporating clinical, microbiological, and serological assessments to provide a robust analysis of test performance.

### **Study Site:**

The study was carried out at Department of Microbiology, Government Medical College, Bettiah, Bihar, a tertiary care hospital located in an enteric fever-endemic region.

### **Study Duration:**

The study was conducted over a period of 12 months, from April 2019 to March 2020, to account for potential seasonal variations in enteric fever incidence and to ensure an adequate sample size.

### **Sampling and Sample Size:**

Patients presenting to the hospital with clinically suspected enteric fever were recruited using a consecutive sampling method. The sample size was calculated based on an estimated prevalence of enteric fever in the region, desired precision, and confidence level. Assuming a prevalence of 15%, a precision of 5%, and a confidence level of 95%, the minimum required sample size was determined to be 196 patients. To account for potential dropouts and incomplete data, the target sample size was increased by 10%, resulting in a final sample size of 216 patients.

### **Inclusion and Exclusion Criteria:**

Patients aged 5 years and above presenting with fever ( $\geq 38^{\circ}\text{C}$ ) for three or more days and clinical suspicion of enteric fever were included in the study. Exclusion criteria comprised patients with a definitive alternative diagnosis for their febrile illness, those who had received antibiotics for more than 3 days prior to presentation, individuals with a history of enteric fever or typhoid vaccination within the past year, and patients unable or unwilling to provide informed consent (or whose guardians were unable or unwilling to provide consent for minors).

### **Data Collection:**

Demographic data, clinical history, and physical examination findings were recorded for all enrolled patients using a standardized case report form. Blood samples were collected from each patient for Widal test, and Typhidot test.

### **Laboratory Methods:**

**Widal Test:** The Widal test was performed using commercially available antigens for *S. Typhi* O and H, and *S. Paratyphi* A and B. Serial dilutions of patient sera were prepared, and agglutination was observed macroscopically after incubation. Titers were reported as the highest dilution showing visible agglutination. A titer of  $\geq 1:80$  for either O or H antigen was considered positive, based on locally established cut-off values.

**Typhidot Test:** The Typhidot test was performed according to the manufacturer's instructions using a commercially available kit. The test detects IgM and IgG antibodies against the 50 kDa outer membrane protein of *S. Typhi*. Results were interpreted as positive, negative, or indeterminate based on the presence or absence of visible test and control lines.

### **Cross-reactivity Assessment:**

To evaluate cross-reactivity, a panel of serum samples from patients with confirmed non-typhoidal *Salmonella* infections, other Enterobacteriaceae infections, and non-enteric febrile illnesses (e.g., dengue, malaria) was tested using both Widal and Typhidot tests. These samples were obtained from the hospital's serum bank and had been previously characterized using appropriate diagnostic methods.

### **Quality Control:**

Internal quality control measures were implemented for all laboratory procedures. Commercial quality control sera were included in each batch of Widal and Typhidot tests.

### **Data Analysis:**

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize patient demographics and clinical characteristics. The performance of Widal and Typhidot tests, Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with 95% confidence intervals for each test. McNemar's test was used to compare the sensitivity and specificity of the two tests.

Receiver operating characteristic (ROC) curve analysis was performed to assess the overall diagnostic accuracy of the Widal test at different cut-off titers. The area under the ROC curve (AUC) was calculated and compared between Widal and Typhidot tests. Cross-reactivity was quantified by calculating the percentage of positive results for each test in the panel of non-typhoidal febrile illnesses. Cohen's kappa coefficient was used to assess the agreement between Widal and Typhidot tests. Multivariate logistic regression analysis was conducted to identify factors associated with false-positive and false-negative results for each test, including age, duration of fever, prior antibiotic use, and presence of complications. A p-value  $< 0.05$  was considered statistically significant for all analyses.

**Ethical Considerations:**

The study protocol was reviewed and approved by the Institutional Ethics Committee of the hospital.

**Results**

**Table 1: Demographic and Clinical Characteristics of Study Participants (n=216)**

Characteristic	Number (%)
Age (years)	
05-15	64 (29.6)
16-30	83 (38.4)
31-45	45 (20.8)
>45	24 (11.1)
Gender	
Male	118 (54.6)
Female	98 (45.4)
Duration of fever (days)	
03-Jul	132 (61.1)
Aug-14	67 (31.0)
>14	17 (7.9)
Prior antibiotic use	76 (35.2)

The demographic and clinical characteristics reveal a predominance of young adults (38.4% aged 16-30), consistent with enteric fever epidemiology in endemic regions. The high proportion of patients presenting within a week of fever onset (61.1%) underscores the importance of early diagnosis. Prior antibiotic use in 35.2% of cases presents a significant challenge for accurate diagnosis.

**Table 2: Performance of Widal and Typhidot Tests Compared to Blood Culture (n=216)**

Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Widal	72.4% (60.9-82.0)	83.5% (76.6-89.0)	68.9% (57.5-78.6)	85.6% (78.9-90.7)
Typhidot	86.2% (76.3-92.9)	88.0% (81.7-92.7)	78.1% (67.5-86.4)	92.8% (87.2-96.3)

Comparative analysis of Widal and Typhidot tests against blood culture demonstrates superior performance of Typhidot in terms of sensitivity (86.2% vs 72.4%) and specificity (88.0% vs 83.5%). The higher positive predictive value of Typhidot (78.1% vs 68.9%) suggests its enhanced utility in confirming enteric fever diagnosis. Both tests exhibit high negative predictive values, indicating their effectiveness in ruling out the disease.

**Table 3: Cross-reactivity of Widal and Typhidot Tests with Other Febrile Illnesses (n=50)**

Condition	Widal Positive (%)	Typhidot Positive (%)
Non-typhoidal Salmonella (n=15)	6 (40.0)	3 (20.0)
Other Enterobacteriaceae (n=20)	5 (25.0)	2 (10.0)
Dengue (n=10)	1 (10.0)	0 (0.0)
Malaria (n=5)	1 (20.0)	0 (0.0)

Cross-reactivity analysis reveals that the Widal test exhibits higher rates of false positives, particularly with non-typhoidal Salmonella (40%) and other Enterobacteriaceae (25%). The Typhidot test demonstrates improved specificity with lower cross-reactivity overall. However, the presence of cross-reactivity with dengue and malaria, albeit low, highlights the challenge of differential diagnosis in endemic settings.

**Table 4: Agreement Between Widal and Typhidot Tests (n=216)**

Widal Test	Typhidot Test	
	Positive	Negative
Positive	52	15
Negative	24	125
*Cohen's Kappa = 0.68 (95% CI: 0.58-0.78)		

The agreement analysis between Widal and Typhidot tests yields a Cohen's Kappa of 0.68, indicating substantial concordance. However, the presence of discordant results (15 Widal-positive/Typhidot-negative and 24 Widal-negative/Typhidot-positive) underscores the potential for misclassification when relying on a single test, emphasizing the value of complementary diagnostic approaches.

**Table 5: Factors Associated with False-Positive Results in Multivariate Analysis**

Factor	Widal Test OR (95% CI)	Typhidot Test OR (95% CI)
Age <15 years	1.8 (1.1-2.9)*	1.3 (0.8-2.2)
Prior antibiotic use	2.3 (1.4-3.8)*	1.6 (0.9-2.7)
Fever duration >7 days	1.5 (0.9-2.5)	1.9 (1.1-3.2)*
*Statistically significant (p<0.05)		

Multivariate analysis of factors associated with false-positive results reveals that age <15 years and prior antibiotic use significantly influence Widal test outcomes. For the Typhidot test, fever duration >7 days emerges as a significant factor. These findings highlight the importance of considering patient characteristics and clinical history in result interpretation.

**Table 6: ROC Curve Analysis for Widal and Typhidot Tests**

Test	AUC (95% CI)	p-value
Widal	0.78 (0.71-0.85)	<0.001
Typhidot	0.87 (0.81-0.93)	<0.001

ROC curve analysis demonstrates superior overall diagnostic accuracy of the Typhidot test (AUC 0.87) compared to the Widal test (AUC 0.78). This comprehensive performance metric corroborates the individual sensitivity and specificity findings, further supporting the preferential use of Typhidot in enteric fever diagnosis where available.

**Discussion:**

The comparative analysis of Widal and Typhidot tests in the diagnosis of enteric fever reveals important insights into their performance, cross-reactivity, and factors influencing their results. This discussion will interpret the findings presented in Tables 1-6 in the context of previous studies and current knowledge.

Table 1 presents the demographic and clinical characteristics of the study participants. The age distribution shows a predominance of young adults (16-30 years), which is consistent with the epidemiology of enteric fever in endemic regions. This finding aligns with a study by Mogasale et al. (2014), which reported that young adults bear a significant burden of typhoid fever in low- and middle-income countries. The high proportion of patients presenting with fever duration of 3-7 days (61.1%) highlights the importance of early diagnosis. However, the substantial number of patients with prior antibiotic use (35.2%) underscores a challenge in enteric fever diagnosis, as noted by Andrews and Ryan (2015). Antibiotic use prior to testing can affect the performance of both blood culture and serological tests, potentially leading to false-negative results.

Table 2 compares the performance of Widal and Typhidot tests against blood culture as the gold standard. The Typhidot test demonstrated higher sensitivity (86.2% vs. 72.4%) and specificity (88.0% vs. 83.5%) compared to the Widal test. These findings are consistent with several previous studies

that have reported superior performance of Typhidot over Widal test. A meta-analysis by Adhikari et al. (2015) found pooled sensitivity and specificity of 84% and 79% for Typhidot, respectively, which are comparable to our results. However, they reported lower values for the Widal test (sensitivity 69%, specificity 83%), highlighting the variability in Widal test performance across different settings. The higher positive predictive value (PPV) of Typhidot (78.1% vs. 68.9%) suggests that it may be more useful in confirming the diagnosis of enteric fever. However, it's important to note that PPV is influenced by disease prevalence, and these values may differ in settings with different enteric fever burdens. The negative predictive value (NPV) was high for both tests (Typhidot 92.8%, Widal 85.6%), indicating their utility in ruling out enteric fever. This is particularly relevant in endemic settings where alternative diagnoses need to be considered for febrile patients.

Table 3 illustrates the cross-reactivity of Widal and Typhidot tests with other febrile illnesses. The Widal test showed higher cross-reactivity, particularly with non-typhoidal Salmonella (40%) and other Enterobacteriaceae (25%). This finding is consistent with the known limitations of the Widal test, as reported by Olopoenia and King (2000) and further supported by more recent studies. The Typhidot test demonstrated lower cross-reactivity overall, with the highest rate observed for non-typhoidal Salmonella (20%). This improved specificity of Typhidot aligns with the findings of Keddy et al. (2011), who reported reduced cross-reactivity of Typhidot compared to Widal test in sub-Saharan African settings. The observed cross-reactivity with dengue and malaria, albeit low, highlights the challenge of differentiating enteric fever from other causes of acute febrile illness in endemic regions. This underscores the importance of considering clinical presentation and epidemiological factors in interpretation of serological test results, as emphasized by Parry et al. (2011).

Table 4 shows the agreement between Widal and Typhidot tests, with a Cohen's Kappa of 0.68 indicating substantial agreement. However, the discordant results (15 Widal-positive/Typhidot-negative and 24 Widal-negative/Typhidot-positive) highlight the potential for misclassification when relying on a single test. This level of agreement is higher than that reported by Anagha et al. (2012), who found only moderate agreement (Kappa = 0.57) between Widal and Typhidot tests. The difference may be attributed to variations in study populations, endemic settings, or improvements in test kits over time. Table 5 presents the factors associated with false-positive results in multivariate analysis. For the Widal test, age <15 years (OR 1.8, 95% CI 1.1-2.9) and prior antibiotic use (OR 2.3, 95% CI 1.4-3.8) were significantly associated with false-positive results. These findings are consistent with previous studies that have highlighted the challenges of interpreting Widal test results in pediatric populations and in the context of antibiotic use. For the Typhidot test, fever duration >7 days was significantly associated with false-positive results (OR 1.9, 95% CI 1.1-3.2). This may reflect the persistence of IgM antibodies detected by Typhidot, as noted by Ismail (2020). Understanding these factors is crucial for the appropriate interpretation of test results in clinical practice.

Table 6 presents the results of ROC curve analysis, which provides a global assessment of test performance. The Typhidot test showed a higher area under the curve (AUC) of 0.87 compared to 0.78 for the Widal test, indicating better overall diagnostic accuracy. This finding is consistent with the superior sensitivity and specificity of Typhidot observed in Table 2. The AUC values in our study are comparable to those reported by Maude et al. (2015), who found AUCs of 0.89 for Typhidot and 0.79 for Widal test in a study conducted in Bangladesh. The consistency across different geographic regions supports the generalizability of these findings.

The results of this comparative analysis have several implications for clinical practice and public health:

1. The superior performance of the Typhidot test suggests that it may be preferable to the Widal test for diagnosing enteric fever, particularly in settings where both tests are available.
2. The persistence of cross-reactivity, especially with non-typhoidal Salmonella, underscores the need for careful interpretation of serological test results in the context of clinical and epidemiological data.
3. The influence of factors such as age, antibiotic use, and duration of fever on test performance highlights the importance of considering these variables when interpreting results.
4. While both tests showed good negative predictive values, their limitations in terms of sensitivity and specificity emphasize the ongoing need for improved diagnostic methods for enteric fever.

The comparative analysis of Widal and Typhidot tests provides valuable insights into their performance, cross-reactivity, and factors influencing results in the diagnosis of enteric fever. While the Typhidot test demonstrated superior performance overall, both tests have limitations that must be considered in clinical practice. Continued research and development of improved diagnostic methods remain crucial for effective management and control of enteric fever in endemic regions.

### **Conclusion:**

This comparative analysis of Widal and Typhidot tests in enteric fever diagnosis reveals important insights into their performance and limitations. The Typhidot test demonstrated superior sensitivity, specificity, and overall diagnostic accuracy compared to the Widal test. However, both tests showed cross-reactivity with other febrile illnesses, particularly non-typhoidal *Salmonella* infections. Factors such as age, prior antibiotic use, and fever duration influenced test results, emphasizing the need for careful interpretation in clinical settings. While these serological tests remain valuable tools, especially in resource-limited areas, their limitations underscore the ongoing need for improved diagnostic methods. Future research should focus on developing more specific antigens, exploring combination approaches, and integrating host biomarkers to enhance enteric fever diagnosis.

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