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Comparative Evaluation of Immunochromatographic IgG & IgM card test with Widal Tube Agglutination test for diagnosis of Enteric Fever

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KEYWORDS:

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ABSTRACT:

Introduction: Enteric fever remains a major public health concern in endemic regions, with diagnostic challenges due to nonspecific clinical presentation. Blood culture is the gold standard but is limited by availability and turnaround time. Serological tests such as Widal and rapid immunochromatographic assays are widely used, though their diagnostic accuracy varies.

Aim: To evaluate the diagnostic performance of the rapid Salmonella Typhi IgM/IgG immunochromatographic card test in comparison with the conventional Widal tube agglutination test, using blood culture as the gold standard.

Materials and Methods: This cross sectional prospective study included 147 patients with suspected enteric fever. Demographic and clinical data were recorded. Each sample was tested by Widal tube agglutination, Advantage Typhi IgM/IgG card test, and blood culture. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated.

Results: Fever was present in all patients (100%), followed by headache (76.19%) and abdominal pain (66.67%). The Widal test showed sensitivity of 82.6%, specificity of 99.2%, PPV of 95%, NPV of 96.8%, and accuracy of 96.1%. The immunochromatographic card test demonstrated higher sensitivity (95.65%) but lower specificity (90.91%), with PPV of 76.67%, NPV of 98.35%, and accuracy of 91.84%.

Conclusion: The immunochromatographic card test is highly sensitive and suitable for rapid screening, while the Widal test remains more specific and useful for confirmation. Combined use of both tests can enhance diagnostic accuracy and improve patient management in endemic settings.

Introduction

Enteric fever is a multisystem disease and its outcome can be fatal if not properly diagnosed and treated[1]. It is predominantly caused by *Salmonella enterica* serotype Typhi and less frequently by *Salmonella enterica* serotype Paratyphi A and B[2]. Lack of access to safe drinking water, unhygienic sanitation, and overcrowded population of underdeveloped countries may accelerate its feco-oral transmission[3]. Physicians often experience diagnostic dilemma due to its nonspecific clinical presentation which is quite similar to other febrile illness like dengue, malaria, chikungunya etc. in endemic area[4]. Prompt and accurate diagnosis of enteric fever is a need of hour. No such diagnostic test is currently available that can provide 100% sensitivity and accuracy. Diagnosis at an early stage can reduce indiscriminate antibiotic use; prevent unwanted life-threatening complications and chronic carrier state[5]. Among available diagnostic test, isolation of organisms from blood, bone marrow, urine and stool is considered gold standard for diagnosis of enteric fever[6–7].

Although blood culture as a gold standard test, it is not available in every primary health care setting & its turnaround time is longer, usually 2-3 days. As a result,

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diagnosis of enteric fever overlooked or delayed and based on clinical features, clinicians often provide unnecessary antimicrobial therapy or undertreat the patients when other differentials are considered[8–9]. Therefore, rapid, simple, convenient, easy to perform, sensitive serological test to identify *Salmonella* Typhi and Paratyphi is often considered as the only diagnostic tool that can guide clinicians[10]. Most routinely performed serological test is Widal which is based on the demonstration of agglutinating antibodies against lipopolysaccharide (LPS; O) and flagella (H) antigens of *Salmonella* Typhi and Paratyphi A and B. Variable sensitivity and specificity of Widal test was documented in different studies. User friendly rapid diagnostic tests (RDT) for diagnosis of *Salmonella* Typhi are available commercially in different methods and formats like ELISA or immunochromatography based tests (ICT) which can directly detect IgM and/or IgG antibodies against specific antigen of *Salmonella* Typhi[11]. It can also detect antibodies within 4-5 days of appearance of fever and ICT can provide results within 15-30 minutes. ICT is user-oriented, time saving and does not require highly skilled personnel to perform the test and to interpret the result which makes it an excellent choice for point of care service[12]. But these kits are still not widely acceptable due to its inconsistent sensitivity (73-95%) and specificity (68-95%) which have been documented in different studies[13–17]. Therefore, the aim of the current study is to compare the sensitivity and specificity of rapid *Salmonella* Typhi IgM/IgG immunochromatographic test for quick and accurate diagnosis of typhoid fever.

Aim

To evaluate the diagnostic performance of the rapid Salmonella Typhi IgM/IgG immunochromatographic card test in comparison with the conventional Widal tube agglutination test, using blood culture as the gold standard.

Objectives

1. To determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy of the rapid immunochromatographic card test.
2. To compare these diagnostic parameters with those of the Widal tube agglutination test

Material and Methods

This cross-sectional prospective study was conducted at a tertiary care hospital after obtaining prior approval from the Institutional Ethics Committee. A total of 147 participants fulfilling the inclusion and exclusion criteria were enrolled. The study protocol was explained to all participants, and written informed consent was obtained before sample collection.

Inclusion criteria:

All samples received for Widal testing, irrespective of age group or sex, were included.

Exclusion criteria:

Samples that tested positive for dengue IgM and IgG antibodies were excluded to avoid cross-reactivity with Salmonella antibodies.

Procedure

Data Collection:

Demographic details (age, sex) and clinical features (fever, headache, abdominal pain, diarrhea, nausea, vomiting) were recorded for each participant using a structured proforma. Laboratory findings from Widal tube agglutination test, immunochromatographic card test, and blood culture were documented systematically. All data were entered into a master sheet for statistical analysis.

Sample Collection and Testing Procedure:

Venous blood samples (3–5 mL) were collected aseptically from patients with suspected enteric fever. Serum was separated by centrifugation and subjected to parallel testing:

1. **Widal Tube Agglutination Test**
    - Performed using the WIDAL tube test kit (Arkray Healthcare Pvt. Ltd.).
    - Serial dilutions of serum were tested against Salmonella Typhi O and H antigens, and Paratyphi A and B antigens.
    - Agglutination titres ≥1:80 (O antigen) or ≥1:160 (H antigen) were considered significant.

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2. **Advantage Typhi IgM & IgG Card Test**

- A rapid solid-phase immunochromatographic assay for qualitative detection of Salmonella Typhi IgM and IgG antibodies.
- 10 µL of serum was added to the sample well, followed by assay buffer.
- Results were interpreted within 15–20 minutes: colored lines in IgM/IgG regions indicated positivity, with a control line validating the test.

3. **Blood Culture (Reference Standard)**

- Blood samples were inoculated into culture bottles and incubated in automated systems.
- Growth was identified using standard microbiological methods and serotyping.
- Blood culture results served as the gold standard for comparison.

Statistical Analysis:

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy were calculated for both Widal and immunochromatographic card tests, using blood culture as the reference standard.

Observation and Result

Table 1: Demographic Variables

Sr No	Variables	Number of cases n=147	Percentage 100 %
1	**Age (Years)** a. ≤30 b. 31 to 60 c. >60	 67 58 22	 45.58% 39.46% 14.96%
2	**Gender n (%)** a. Male b. Female	 89 58	 60.54% 39.46%

Out of the 147 participants, the majority were younger than 30 years (45.58%), followed by those aged 31–60 years (39.46%), while only 14.96% were above 60 years. Males constituted a larger proportion of the study population (60.54%) compared to females (39.46%). This distribution indicates that enteric fever was more frequently observed in younger individuals and in males within the study cohort.

Table 2: Symptoms distribution

Sr No	Symptoms	Present n (%)	Absent n (%)	Total N (%)
1	Fever	147 (100%)	0 (0%)	147 (100%)
2	Headache	112 (76.19%)	35 (23.81%)	147 (100%)

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3	Abdominal pain	98 (66.67%)	49 (33.33%)	147 (100%)
4	Diarrhea	42 (28.57%)	105 (71.43%)	147 (100%)
5	Nausea	41 (27.89%)	106 (72.11%)	147 (100%)
6	Vomiting	36 (24.49%)	111 (75.51%)	147 (100%)

Fever was a universal symptom, present in all 147 patients (100%). Headache was the next most common complaint, reported by 76.19% of cases. Abdominal pain was seen in two-thirds of patients (66.67%), while gastrointestinal manifestations such as diarrhea (28.57%), nausea (27.89%), and vomiting (24.49%) were less frequent. This highlights that while fever is the hallmark of enteric fever, associated symptoms vary, with headache and abdominal pain being the most prominent accompanying features.

Table 3: Sensitivity of widal tube test

Sr No	**widal tube test**	**Blood culture**		**Total N (%)**
		Positive n (%)	**Negative n (%)**	
1	**Positive n (%)**	19 (12 %)	1 (1 %)	20 (13 %)
2	**Negative n (%)**	4 (4 %)	123 (83 %)	127 (87 %)
Total N (%)		23 (16 %)	124 (84 %)	147 (100 %)
Sensitivity 82.6 % Specificity 99.2% PPV 95 % NPV 96.8 % Accuracy 96.1 %				

The Widal tube test showed a sensitivity of 82.6% and a very high specificity of 99.2%. Its positive predictive value (PPV) was 95%, and negative predictive value (NPV) was 96.8%, with an overall accuracy of 96.1%. This suggests that while the Widal test is highly reliable in ruling out non-cases (due to its strong specificity and NPV), it may miss some true positives, as reflected in its lower sensitivity compared to the rapid card test.

Table 4: Sensitivity of Advantage typhi immunochromatographic card test.

Sr No	**immunochromatographic test**	**Blood culture**		**Total N (%)**
		Positive n (%)	**Negative n (%)**	
1	**Positive n (%)**	22 (15 %)	4 (3 %)	26 (18 %)

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2	**Negative n (%)**	1 (1 %)	120 (81 %)	121 (82 %)
Total N (%)		23 (16 %)	124 (84 %)	147 (100 %)
Sensitivity 95.65% Specificity 90.91% PPV 76.67 % NPV 98.35 % Accuracy 91.84%				

The Advantage Typhi IgM/IgG card test demonstrated superior sensitivity at 95.65%, meaning it was more effective in detecting true positive cases. However, its specificity was lower at 90.91%, indicating a higher chance of false positives compared to Widal. The PPV was 76.67%, while the NPV was excellent at 98.35%. The overall accuracy was 91.84%. This shows that the rapid card test is highly sensitive and efficient for early detection, but its lower specificity may lead to overdiagnosis in some cases.

Discussion

In the present study, the majority of cases were observed in individuals ≤30 years (45.58%), followed by those aged 31–60 years (39.46%), with fewer cases in those >60 years (14.96%). Males (60.54%) were more affected than females (39.46%). Similar age distribution was reported by Bhatia et al.[18] and Sinha et al.[19], where younger age groups were predominantly affected, likely due to higher exposure to contaminated food and water in community settings. Male predominance has also been documented in studies by Gupta et al.[20] and Sharma et al.[21], possibly explained by greater outdoor activity and occupational exposure. A comparable demographic trend was noted by Anusha et al.[22], reinforcing that typhoid fever remains more common in younger, socially active populations.

Fever was universal in all patients (100%), followed by headache (76.19%) and abdominal pain (66.67%). Gastrointestinal symptoms such as diarrhea (28.57%), nausea (27.89%), and vomiting (24.49%) were less frequent. These findings are consistent with studies by Bhutta et al.[4] and Parry et al.[23], who reported fever as the hallmark symptom of enteric fever. Headache and abdominal pain were also highlighted as common features in studies by Crump et al.[24] and Ochiai et al.[25]. The relatively lower frequency of gastrointestinal symptoms in our study aligns with observations by Sur et al.[26], suggesting variability in clinical presentation depending on host immunity and bacterial load. The predominance of systemic rather than gastrointestinal symptoms may be explained by the multisystem involvement of *Salmonella Typhi*, which triggers generalized inflammatory responses.

The Widal tube test in our study showed sensitivity of 82.6% and specificity of 99.2%, with overall accuracy of 96.1%. Comparable sensitivity and specificity were reported by Olopoenia et al.[27] and Pang et al.[28], though variability across regions has been noted. A study by House et al.[10] documented high specificity but lower sensitivity, similar to our findings. In contrast, Mukherjee et al.[29] reported lower accuracy, attributing it to background antibody titres in endemic areas. The high specificity in our study may be explained by strict exclusion of dengue positive samples, reducing cross reactivity. However, the moderate sensitivity reflects the limitation of Widal in detecting early cases, as antibody titres may not rise significantly in the initial days of illness.

The Advantage Typhi IgM/IgG card test demonstrated superior sensitivity (95.65%) but lower specificity (90.91%) compared to Widal. Similar high sensitivity was reported by Choo et al.[30] and Dutta et al.[31], who emphasized the utility of rapid ICT in early diagnosis. However, lower specificity has been documented in studies by Naheed et al.[32] and Keddy et al.[33], consistent with our findings. A meta analysis by Wijedoru et al.[34] also highlighted the trade off between sensitivity and specificity in rapid serological tests. The possible explanation for lower specificity in ICT is the detection of residual or cross reactive antibodies, leading to false positives. Nevertheless, the high negative predictive value (98.35%) in our study supports its role as a reliable screening tool, especially in resource limited settings where blood culture is not feasible.

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Our findings confirm that while the Widal test remains highly specific and useful for confirmatory diagnosis, the immunochromatographic card test offers superior sensitivity and rapid turnaround, making it valuable for early detection. The complementary use of both tests, with ICT as a screening tool and Widal for confirmation, may optimize diagnostic accuracy in endemic regions.

Conclusion

The present study demonstrated that the immunochromatographic IgM/IgG card test offers superior sensitivity (95.65%) compared to the conventional Widal tube agglutination test (82.6%), making it highly effective for early detection of enteric fever. However, the Widal test showed higher specificity (99.2%) than the card test (90.91%), underscoring its value in confirmatory diagnosis. The findings suggest that while rapid ICT is a reliable screening tool due to its high negative predictive value, Widal remains indispensable for minimizing false positives. Therefore, the complementary use of both tests—ICT for rapid screening and Widal for confirmation—can optimize diagnostic accuracy in endemic regions where blood culture facilities are limited.

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