Introduction
In many developing countries (like middle-east Asian countries and Bangladesh) and India, enteric fever is an endemic disease.[1‑3] *Salmonella* Paratyphi A, B and C, and *Salmonella* Typhi cause enteric fever. Out of these, the latter is more frequent and severe. It has an incubation time of seven days to 21 days. The signs and symptoms include headache, fever, constipation and vague pain in the abdomen. Sometimes skin rashes may also occur.[1‑3]

In the usual way, the diagnosis is clinical but clashes with various differentials like dengue, malaria, infectious mononucleosis, chikungunya, leptospirosis, acute gastroenteritis, rickettsial diseases, etc.[4‑6] This may be problematic at the level of primary care physicians while dealing with patients of fever. Gold standard diagnostic culture is time-consuming with a fainter sensitivity of 60–80% because of less volume of bacteria in the blood and the start of possible antimicrobial therapy. Moreover, it decreases more in the infection’s second week by 10–20%.[7‑9] Therefore, there is a requirement for a quicker serological diagnosis, which may make the core of diagnosis practically.

Various antibody and antigen-based tests are included in the serological testing. The most commonly used test is the Widal
test, but it cannot detect antibodies in the infection’s first week.\cite{10,12} Rapid diagnostic kits are used now with the new developments in the medical field. Examples of these medical kits include TyphiDot (IgM and IgG), Enteroscreen, Typhipoint, IgM-dipstick, and Tubex. These kits detect IgM/IgG antibodies indirectly against the Salmonella species antigens; however, their diagnostic accuracy also remains varied [sensitivity 73–95% and specificity 68–95%].\cite{4,7,8}

In this study, we evaluated the role of the latest commercially available serodiagnostic tests (immunochromatographic assay manufactured by Labcare diagnostic Pvt. Ltd. and lateral flow immunoassay manufactured by Medsource ozone biomedicals Pvt. Ltd.) in enteric fever. The study was done with an objective to assess and compare the diagnostic accuracy of rapid tests (antigen vs. antibody) in the early detection (less than five days of fever) and late detection (more than five days of fever) of S. Typhi and S. Paratyphi against gold standard blood culture. The study results shall guide the primary care physician about the relevant choice of the diagnostic test to be applied for the correct diagnosis of enteric fever (depending upon the time of fever when the patient presents to the hospital) – which shall allow for prompt and appropriate management.

**Methods**

A comparative study was done in the Department of Microbiology of a tertiary care hospital, New Delhi, from 1/11/2016 to 31/03/2018, where all cases of documented fever (three or more days) were consecutively selected and were screened for enteric fever. Patients with prescription and intake of any antibiotics in the conceding seven days were excluded. Institutional Ethical approval was taken while laying down the protocol before beginning the study.

For sample size calculation, the Maude et al.\cite{10} study was taken as reference, which observed a sensitivity and specificity of 59% and 61%, respectively, for LifeAssay RDT. A final sample size of 180 patients was taken to maintain 90% power of the study, keeping 5% alpha error and 20% margin of error. Duly informed written consent was taken from the patients to enroll them in the study.

The demographic characteristics (age, gender) and clinical symptoms (fever and duration) of the study patients were noted. Blood samples of the study group were collected after obtaining written informed consent. Two samples were collected; the first sample was taken within five days of fever and the second sample, after five days of fever, which was processed in the Microbiology Department for 1) WIDAL TEST (tube agglutination method using colored TYDAL reagent, Tulip diagnostic Pvt. Ltd.) 2) Antibody testing (LATERAL FLOW IMMUNOASSAY) 3) Blood culture (BacT/Alert system) and 4) Antigen testing (IMMUNOCHROMATOGRAPHIC ASSAY)

For blood culture, around 10–20 ml of blood of the patient (for adult) and 1–5 ml (for child) was collected in BacT/Alert bottle under strict aseptic precautions. For serological testing, around 3–5 ml of blood was collected aseptically, which was processed by centrifugation, and the serum was used to test it for the Widal test, lateral flow immunoassay and immunochromatographic assay. If any delay was suspected, the serum was stored at 2–8°C for up to three days or frozen at –20°C for a longer period.

Taking blood culture as the gold standard investigation, the “specificity (Sp), sensitivity (Sn), negative predictive values (NPV), positive predictive value (PPV) and diagnostic accuracy” of the serological tests were calculated. Based on blood culture, antibiotic sensitivity was also recorded.

The outcome measures were the better serological tests in the first five days of fever and the latter five days of fever.

**Statistical analysis**

The data analysis was done using the specialized software named “Statistical Package for Social Sciences (SPSS) software”, IBM manufacturer, Chicago, USA, version 21.0. For this, the data was first entered into a spreadsheet of the Microsoft EXCEL program. The data presentation for the study was done as mean with standard deviation (SD) and number (%).

Comparison of Rapid Ab, Rapid Ag, Widal test and Blood culture in fever less than five days and in fever more than five days was performed using the Chi-square test. Sn, Sp, PPV, NPV and diagnostic accuracy of rapid Ag test, rapid Ab test, and Widal test was calculated and compared using the Chi-square test. Inter-rater kappa agreement was used to find out the strength of association between Rapid Ab, Rapid Ag, Widal tests. For statistical significance, a p value less than 0.05 was considered significant.

**Results**

The mean (SD) age of the study patients was 16.42 (12.53) years, with 113 (62.78%) patients recruited from pediatric OPD (0–18 yr) and 67 (37.22%) patients from medicine OPD (more than 18 years).

The gender distribution showed a slight male preponderance (58.33% males vs. 41.67% females). Fever was the commonest universal complaint, with other complaints being loose motions (2.78%), vomiting (2.22%), pain abdomen (1.11%) and headache, body ache, coughing, poor appetite, pain in both legs present in a single case each (0.56%). [Table 1]

Positive blood culture was reported in 58 (32.22%) cases, among which 49 cases had S. Typhi, five cases had S. paratyphi, and four cases had mixed infection.

In 58 confirmed cases of enteric fever with blood culture (gold standard), at fever less than five days duration, rapid Ag test
was positive in 68.97% cases, rapid Ab test was positive in 48.28% cases, and Widal test was positive in 46.55% cases \( (P = 0.026) \). This trend in positivity was reversed in cases with fever more than five days history as Rapid Ag test was positive in only 12.07% cases, rapid Ab test was positive in 86.21% cases and Widal test was positive in 79.31% cases \( (P\)-value less than 0.0001) as shown in Table 2.

Among the suspected cases \((n = 122)\) of enteric fever (who had sterile blood cultures), there was no significant difference in the positivity of various tests (rapid antigen test, rapid antibody test, Widal test) in fever less than and more than five days of duration \([42 (34.43\%) \text{ vs. } 48 (39.34\%) \text{ vs. } 47 (38.52\%), \ P = 0.696]\).

For the rapid antigen test, rapid antibody test, and Widal test, the diagnostic accuracy was 45.56%, 42.22% and 41.11%, respectively, which was comparable for diagnosing enteric fever in cases with less than five days of fever \( (P = 0.675) \), as shown in Table 3. For fever more than five days, the rapid antigen test showed a significantly lower diagnostic accuracy (15%) as compared to the rapid antibody test (61.11%) and the Widal test (66.11%) \( (P < 0.0001) \).

There was a mild negative kappa agreement between rapid antigen test/rapid antibody test \( (K = -0.329\) and rapid antigen test/Widal test \( (-0.287)\).

All isolates of S. Typhi were sensitive to Ce (cefotaxime), Ca (ceftazidime), Nt (netilmicin), Ao (aztreonam) and PT (piperacillin + tazobactam) and 100% resistant to Na (nalidixic acid). Also S. Typhi was 95% sensitive to C (chloramphenicol), 80% sensitive to Ak (amikacin), 80% sensitive to G (gentamicin), 40% sensitive to E (erythromycin), 88% sensitive to T (tetracycline), 97.15% sensitive to Co (cotrimoxazole), 72.41% sensitive to Of (ofloxacin), 92.31% sensitive to A (ampicillin), 56.52% sensitive to Cf (ciprofloxacin) and 97.50% sensitive to Ci (ceftaxalone).

All isolates of S. Paratyphi were sensitive to C (chloramphenicol), PT (piperacillin + tazobactam), T (tetracycline), Co (ciprofloxacin), and 50% isolates showed sensitivity to Ak (amikacin) and G (gentamicin). 83.33% of S. Paratyphi were sensitive to A (ampicillin) and 85.71% sensitive to Cf (ciprofloxacin).

### Discussion

A slightly high but comparable diagnostic accuracy was seen in the index study of the rapid antigen test when compared with the Widal test and rapid antibody test among culture-positive patients during the first week of enteric fever. The rapid antigen test had a diagnostic accuracy of 41.11%, which is in line with the Maude et al[29] study (three RDT were assessed with accuracy varying from 24–59% of all).

### Table 1: Distribution of Socio-demographic characteristics of study subjects

| Socio-demographic characteristics | Frequency | Percentage |
|----------------------------------|-----------|------------|
| Age in years | 16.42±12.53 |
| Gender | | |
| Female | 75 | 41.67% |
| Male | 105 | 58.33% |
| Presenting Complaints | | |
| Fever (102-104°F) | 180 | 100.00% |
| Headache | 1 | 0.56% |
| Body ache | 1 | 0.56% |
| Loose motion | 5 | 2.78% |
| Cough | 1 | 0.56% |
| Cold | 0 | 0.00% |
| Pain abdomen | 2 | 1.11% |
| Vomiting | 4 | 2.22% |
| Poor appetite | 1 | 0.56% |
| Constipation | 0 | 0.00% |
| Pain B/L legs | 1 | 0.56% |

### Table 2: Comparison of Rapid Ab, Rapid Ag and Widal test in confirmed cases (58 cases) of enteric fever

| Test | Positive | Negative | \( P \) |
|------|----------|----------|---------|
| Fever <5 days | | | |
| Rapid Ag test | 40 (68.97%) | 18 (31.03%) | 0.026 |
| Rapid Ab test | 28 (48.28%) | 30 (51.72%) | |
| Widal test | 27 (46.55%) | 31 (53.45%) | |
| Fever >5 days | | | |
| Rapid Ag test | 7 (12.07%) | 51 (87.93%) | <0.0001 |
| Rapid Ab test | 50 (86.21%) | 8 (13.79%) | |
| Widal test | 46 (79.31%) | 12 (20.69%) | |

### Table 3: Comparison of diagnostic accuracy of rapid Ag test, rapid Ab test and Widal test

| Variables | Rapid Ag test | Rapid Ab test | Widal test |
|-----------|---------------|---------------|------------|
| Fever <5 days | | | |
| Sensitivity | 68.97% | 48.28% | 46.55% |
| Specificity | 34.43% | 39.34% | 38.52% |
| Positive predicted value | 48.78% | 36.84% | 36.49% |
| Negative predicted value | 81.63% | 71.15% | 70.75% |
| Diagnostic accuracy | 45.56% | 42.22% | 41.11% |
| \( P \) | 0.675 | | |
| Fever >5 days | | | |
| Sensitivity | 12.07% | 86.21% | 79.31% |
| Specificity | 16.39% | 40.18% | 59.84% |
| Positive predicted value | 45.45% | 38.66% | |
| Negative predicted value | 66.67% | 88.57% | 80.33% |
| Diagnostic accuracy | 15% | 61.11% | 66.11% |
| \( P \) | <0.0001 | | |

Overall, the antigen kit is better to be used in the initial days with a high sensitivity of 68.97% compared to 12.07% in the latter days of fever. Also, the rapid antibody test and Widal test are not much of practical use in the first five days of fever as they have
low sensitivities of 48.28% and 46.55%, respectively. This goes against the common norm of application of the Widal test for enteric fever diagnosis by Family care physicians irrespective of the duration of fever.[10]

In Indonesia, LifeAssay RDT was assessed, which had a sensitivity of 62.1% and specificity of 97.8%.[13] In Cambodia, it was seen in another study that LifeAssay RDT had a sensitivity and specificity of 55% and 98%, respectively.[14] It was seen in the present study that higher specificity is against previous studies. These studies further stress the practical use of rapid serological tests.

The interpretation of the results must be done with the consideration that blood culture sensitivity was low as positive blood culture for S. typhi/paratyphi was shown only in 32.22% cases (all the cases were taken as confirmed cases of enteric fever). Negative culture results were obtained for the remaining patients (these cases were considered as suspected cases of enteric fever). Marginally higher culture positivity (56%) was reported by Maude et al.,[15] but it was still thought to be on the lower side. Enteric fever cases have low sensitive blood cultures as antibodies are used irrationally and in large quantities, and also, it is tough to take huge quantities of blood for cultures from kids.[16] Therefore, in these cases, PCR evaluation is done initially, as it is difficult to do culture isolation in the early phase; however, more research is needed for this claim.[10]

Since blood culture positivity is low and it takes three to five days for the final report, rapid antigen tests or RDTs can be considered since they have good sensitivity for diagnosis.

Through this study, the Widal test’s practicality is being addressed as it is the most frequent and inexpensive test in use. This test is the most relevant as well it is based on the antibody detection principle, which forms 5–7 days post fever. Surprisingly, it was found to be positive in 46.55% of the cases (≤ 5 days of fever), having an initial TO and TH titer of more than 1:160. Immunologically sensitized or hyperimmune population is thought to be responsible for this finding as they are exposed to S. Typhi/Paratyphi and other Salmonellae continuously.[17] As in most of the cases, the second sample in the latter part of the fever is not sent to the lab; therefore, this observation holds practical importance. Moreover, we observed that the sensitivity of the Widal test shifted to 79.31% after five days of fever which was consistent with the antibody principle, and the values were in comparability to Rapid antibody test findings, as both these tests are based on antibody detection. This was in line with the findings of a recent study published in 2021,[19] where the sensitivity of the Widal test was 90% and of the rapid antibody test was 72.73%. Even in another latest study by Ousene K et al. (2021),[18] the sensitivity of the Widal test was 94.44%, which was higher than the rapid antibody test (80.56%), but the overall specificity of the Widal test was significantly lower than rapid antibody testing (48.35% vs. 94.03%). However, the differentiation on the basis of the duration of fever was not assessed in any of those studies, as done in the present study. Overall, findings indicate that the Widal test carries a lower diagnostic power for enteric fever in comparison to rapid antibody testing. Moreover, the Widal test can be superseded by rapid antibody testing for confirmation of suspicious clinical cases of enteric fever in the latter part of the fever (≥ 5 days).

Typhoid being a common disease, with its differentials with other fevers, becomes a common concern in the practice of a primary care physician. The use of rapid diagnostic tests (of varied kits) is rampant—because of the different prices—such that the diagnostic accuracy and validity if left far behind. Moreover, the use of these kits (antigen and antibody) remains unrestricted to the time periods of onset of fever, thereby leading to inaccurate results. This study stresses the specific use of rapid antigen tests and rapid antibody tests in the initial and latter five days of typhoid fever, respectively, by the primary care physician, as it provides faster results in adjunct to the gold standard blood culture. To date, no specific study has focused on the comparative analysis of rapid diagnostic tests for enteric fever based on the time periods of onset of fever, thereby allowing for misdiagnosis in routine medical practice. The present study holds novelty in this regard as it witnesses a different sensitivity of both tests during different time periods of enteric fever.

Strength and limitations of the study

Good sample size was taken in this study, and it was tried to include all the patients who came to the hospital with fever. However, the size of the sample remained small because of the low positivity rate of the culture. The diagnostic tests were done in duos during and later five days of fever, which made it possible for us to know a preferable test during different phases of fever. A mild negative kappa agreement among rapid antigen test/Widal test (−0.287) and rapid antibody test/rapid antigen test (K = −0.329) was seen in both phases of the fever, which shows that in the latter week of the fever, antibody detection is viable; whereas, in the first week of the fever, antigen detection is viable. The diagnostic impasse continues as both tests are joined since there is mild positivity of antibody tests in the first five days.

Conclusion

It is safe to conclude that in the initial five days of fever, the rapid antigen test has a higher sensitivity of 68.97%; whereas, in cases with more than five days of fever, the Widal test has a higher sensitivity of 79.31% and the rapid antibody test has a sensitivity of 86.21%.

The gold standard for enteric fever diagnosis is blood culture, but the Widal test is instrumental in diagnosis. Rapid antigen and antibody tests showed promising results in early diagnosis of enteric fever, even in cases where blood culture is negative. However, more research is required to further back our findings and to make better guidelines for the diagnosis of enteric fever.
Key points

• Rapid antigen tests mark a high sensitivity of accurate diagnosis in the initial five days of typhoid fever.
• Rapid antibody tests (with or without Widal tests) hold a superior sensitivity for diagnosis after five days of onset of enteric fever.
• Blood culture, though it may be the gold standard, sensitivity falls short of being a screening test. Thus it requires the application of adjunctive rapid diagnostic tests in the practice of a primary care physician for correct and early diagnosis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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