

Comparison of rapid commercial tests with standard tube widal test for the diagnosis of enteric fever

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Abstract

Background: Typhoid fever, caused by *Salmonella enterica* serovar Typhi, remains a major health concern in developing countries due to poor sanitation and the emergence of multidrug-resistant strains. Traditional diagnostic methods such as blood culture and Widal test have limitations in early detection and reliability.

Objective: To evaluate the diagnostic performance of three rapid serodiagnostic kits—Enterocheck IgM, SD Bioline *Salmonella* Typhi, and CTK—in comparison to the Widal tube test, focusing on sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Methods: A cross-sectional study was conducted from May to August 2013 in the Department of Microbiology & Immunology at Shri Guru Ram Rai Institute of Medical & Health Sciences and Shri Mahant Indires Hospital, Dehradun. Twenty-two serum samples from febrile patients were tested using the Widal tube test and three rapid diagnostic kits.

Results: Enterocheck IgM demonstrated the highest sensitivity (85%) and PPV (94%), while SD Bioline and CTK showed perfect specificity (100%) and PPV (100%). However, all kits had low NPV values: Enterocheck IgM (25%), SD Bioline (20%), and CTK (18%).

Conclusion: Enterochek IgM emerged as the most sensitive rapid diagnostic kit, making it a valuable tool for early detection of typhoid fever. Despite its lower specificity, its high PPV supports its use in clinical settings. Larger-scale studies incorporating blood culture validation are recommended to confirm these findings.

Keywords: Bioline, Diagnostic Kit, PPV, NPV

Introduction

Typhoid fever is a systemic infection caused by *Salmonella enterica* serovar Typhi, a human-restricted pathogen that primarily affects populations in developing countries. While *S. typhi* and *S. paratyphi* A infect only humans, other *Salmonella* serotypes are pathogenic to animals such as poultry, pigs, and cattle¹. The disease is exacerbated by poor sanitation, overcrowding, and indiscriminate antibiotic use, contributing to the emergence of multidrug-resistant strains and complicating treatment options².

Diagnosis of typhoid fever traditionally relies on blood culture and the Widal test. Blood culture, though considered the gold standard, has limited utility in early stages due to low bacterial load and infrastructure constraints³. The Widal test, despite its widespread use, suffers from moderate sensitivity and specificity, and its reliability varies across geographic regions due to antigenic cross-reactivity with other *Salmonella* serotypes and members of Enterobacteriaceae^{4,5}.

To overcome these limitations, rapid serodiagnostic tests have been developed to detect specific IgM antibodies against *S. typhi*, enabling earlier and more accurate diagnosis⁵.

Given the need for fast, reliable, and accessible diagnostic tools in resource-limited settings, this study was conducted to evaluate the sensitivity, specificity, positive predictive value (PPV), and negative predictive

value (NPV) of Enterochek WB, SD Bioline, and CTK rapid diagnostic kits, and to compare their performance with the Widal test in a hospital-based setting.

Materials and Methods

The present study was conducted in Department of Microbiology & Immunology, at Shri Guru Ram Rai Institute of Medical & Health Sciences, & at Shri Mahant Indiresch Hospital, Dehradun. All serum samples of patient suspected of enteric fever where tested for antibodies against *Salmonella* by various methods.

Sample Collection

- Before collection of the blood the area of vein puncture site is to be cleaned with the disinfectant i.e 70% alcohol.
- Then with the help of disposable syringe the blood is drawn and collected in a sterile plain vial (without anticoagulant) and allowed to clot.
- Centrifuge the blood for serum separation at 3000rpm for 3-5 min at room temperature.

Tests for Detection of Salmonella Antibodies

1. Widal Test (Span Dignostics Ltd.)
2. SD (Standard Diagnostics, Inc)⁵.
3. CTK (CTK Biotech, Inc.)⁶
4. Enterochek WB (Zephyr Biomedical)⁷

Widal Test (Slide and Tube Method)

Principle

It is an agglutination test for detection of agglutinins (H and O) in patients with enteric fever. Agglutinins against “O” (somatic) and “H” (flagellar) antigen of *Salmonella* groups are detected quantitatively employing killed suspension of appropriate organism. *Salmonella* antibodies start appearing in the serum at the end of the first week and rise sharply during the third week of enteric fever.

Rapid Slide (Screening) Test

1. Clean the glass-slide supplied in the kit properly and wipe it free of moisture.
2. Place one drop of undiluted test serum in each of the 1st four circles (1 to 4) and one drop of positive control serum in each of the last two circles (5 & 6).
3. Place one drop of antigens O, H A (H) and B (H) in circles 1,2,3 and 4 respectively and O antigen in circle 5 and any one of the H antigens (H, AH or BH) in circle 6.
4. Mix the contents with separate stick and spread to whole circle.
5. Rock the slide for one minute and observe for agglutination if agglutination is visible within one minute the test is positive, proceed for quantitative

slide test or tube test for quantitative estimation of the titre of the appropriate antibodies.

6. If no agglutination is observed the test is negative.

Quantitative Tube (Widal) Test

1. Take a set of 8 clean, dry, 10x 75mm test tube for each serum under test.
2. Dilute each serum sample and set up the test as follows.
3. Mix well. Incubate all tubes at 37° C for 16 to 20 hrs. And examine for agglutination
4. Alternatively, incubate O antigen at 50° C for 4 hrs and all H antigens for 2 hrs and take the reading
5. The antibody titre is the highest dilution of serum showing distinct agglutination

Tube Dilution chart

	1	2	3	4	5	6	7	8
Dilution	1:20	1:40	1:80	1:60	1:320	1:640	1:280	Saline Control
Normal Saline, in ml	1.9	1	1	1	1	1	1	1
Patient serum Undiluted	0.1 mix	- mix	- mix	- mix	- mix	- mix	-	-
Transfer diluted serum (1ml)	-	1	1	1	1	1	1	-
Appropriate Antigen Drops	1	1	1	1	1	1	1	1

Interpretation of results

Reading should be taken at least 30 min -1 hrs after removing the assay tubes from the incubator," O" antigen shows dice like pattern where as "H" antigen shows the characteristic floccular appearance. In negative reaction the appearance of suspension remains unchanged with a minute button of deposit at the bottom of the test tube shows a typical swirl when the tube is flicked.

In addition, the pattern of sedimented organism, reduction in opacity of supernatant as compared to saline control tube must be observed.

Agglutination titre greater than >1:80 is considered significant and suggests infection, whereas low titer are found in normal individual.

There should be a fourfold rise in titre between two serum samples collected at the acute phase and the convalescent phase.

Salmonella typhi IgG/ IgM rapid Test

Procedure of the test

1. Allow all kit components and specimen to room temperature prior to testing.
2. Add 4 drops (about 120ml) of assay buffer to each disposable test tube.

3. Using the loops provided, add 1 ml of specimen to test tube containing assay buffer.
4. Gently stir the assay buffer with the loop to ensure adequate mixing of the specimen in the assay buffer and remove the loop.
5. Remove the test strip from the foil pouch prior to use.
6. Holding the strip vertically, invert the test strip into the tube containing diluted specimen.
7. Interpret test result in 15-30 min. If the test band is very faint at 15 min, please read the result again at 30 min.

Interpretation of The Test

Negative

The control line is only visible on the test strip. No IgG and IgM antibodies were detected. Probably not typhoid.

IgG Positive

The control line and IgG line (G) are visible on the test strip. This is positive for IgG antibodies. This is indicative of previous salmonella typhi infection (in which case fever may not be due to typhoid) or relapse or reinfection.

IgM Positive

The control line (c) and IgM line (m) are visible on the test strip. This is positive for IgM antibodies to salmonella typhi. This is indicative of acute typhoid fever.

IgG and IgM Positive

The control line, IgM line (M) and IgG line (G) are visible on the test strip. This is positive for both IgM and IgG antibodies. This is indicative of acute typhoid fever (in the middle stage of salmonella typhi infection).

Invalid

The control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most

likely reasons for control line failure. Repeat the test using a new test device.

CTK Biotech

Principle

The onsite Typhoid IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of:

1. A burgundy coloured conjugate pad containing recombinant H antigen and O Antigen conjugated with colloidal gold (HO conjugates) and rabbit IgG-gold conjugate.

2. A nitrocellulose membrane strip containing two test bands (G and M band) and a control band (C band).

The M band is precoated with monoclonal anti-human IgM for the detection of IgM anti-S.typhi and paratyphi, and the C band is pre-coated with goat anti-rabbit IgG.

When an adequate volume of test specimen is dispensed into the sample well of the cassette, the test specimen migrates by capillary action across the test sample well of the cassette. IgM antibodies if present in the patient specimen will bind to the HO conjugates. The immunocomplex is then captured on the membrane of the pre-coated anti-human IgM antibody, forming a burgundy coloured M band, indicating a S.typhi or paratyphi IgM positive test result.

IgG antibodies if present in the patient specimen will bind to the HO conjugates. The immunocomplex is then captured by the pre-coated reagents on the membrane, forming a burgundy coloured G band, indicating a S.typhi or paratyphi IgG positive test result.

Absence of any test bands (M and G) suggests a negative result. The test contains an internal control (L band) which should exhibit a burgundy coloured band of the immunocomplex goat anti-rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of

the test bands otherwise, the test result is invalid and the specimen must be retested with another device.

ASSAY Procedure

Step1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 1 drop (about 30-45 ML) of specimen into the sample well making sure that there are no air. Then add 1 drop (about 35-50 ML) of sample Dilute immediately.

Step 5: Set up timer.

Step 6: Result can be read in 15 minutes.

Interpretation of Assay Result

1. **Negative OR Non- Reactive Result:** If only the C band is present, the absence of any burgundy color in the both test bands (M and G) indicated that no anti S typhi or paratyphi antibody is detected in the specimen. The result is negative or non-reactive.

2. Positive OR Reactive Result

A. In addition to the presence of C band, if only M band is developed, the test indicates for the presence of anti S. typhi or paratyphi IgM in the specimen. The result is IgM positive or reactive.

B. In addition to the presence of C band, if only G band is developed, the test indicates for the presence of anti S. typhi or paratyphi IgG in the specimen. The result is IgG positive or reactive.

C. In addition to the presence of C band, both M and G bands are developed, the test indicated for the

presence of anti S. typhi or paratyphi IgG and IgM in the specimen. The result is both IgG and IgM positive or reactive.

3. **Invalid:** if no C band is developed, the assay is invalid regardless of any burgundy color in the test band as indicated below. Repeat the assay with a new device.

Enterocheck -WB

Principle: The principle of immunochromatography is a unique two sites immunoassay on a nitrocellulose membrane. The conjugate pad contains two components- Anti Human IgM antibody conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test specimens flow through the membrane test assembly, the anti- human IgM antibody-colloidal gold conjugate complexes with the S. typhi specific IgM antibodies in the specimen and travels on the membrane due to capillary action. This complex moves further on the membrane to the test region (T) where it is immobilized by the S. typhi specific LPS Ag coated on the membrane leading to formation of a pink to pink purple colored band. The absence of this colored band in the test region indicated a negative test result.

The unreacted conjugate and unbound complex, if any along with the rabbit globulin colloidal gold conjugate move further on the membrane and are subsequently immobilized by the anti-rabbit antiserum coated on the membrane at the control region (c), forming a pink to pink purple colored band. This control band acts as a procedural control and serves to validate the result.

Test Procedure

1. Bring the Kit component of ENTEROCHECK – WB device to room temperature before testing.

2. Open a foil pouch by tearing along the “notch”.

3. Remove the testing device and the sample loop. Once opened, the device must be used immediately.

4. Label the device with specimen identity.
5. Place the testing device on a flat horizontal surface.
6. Carefully dispense 5 ml of serum into the specimen part 'A' using a micropipette. Or using the 5ml sample loop provided, dip the sample container and blot the sample in the specimen part 'A'.
7. Add five drops of sample running buffer into the buffer part 'B'.
8. At the end of 15 min read result as follow.

Result Interpretation

Negative Result: If IgM antibodies to S.typhi are not present, only one colored bond appears in the control window (c).

Positive Result: If IgM antibodies to S. typhi are present two colored bands appear in the test (T) and control (c). The intensity of the test band may be more or less then the control band, depending upon the concentration of IgM Ab in specimen.

Invalid Result: The test is invalid if no band is visible at fifteen min. The test should also be considered invalid if only test band appears and no control band appears. Verify the test procedure and repeat the test with a new device.

Results

The present study was conducted in the Department of Microbiology & Immunology, at Shri Guru Ram Rai Institute of Medical & Health Sciences, & at Shri Mahant IndiresH Hospital, Dehradun from May 2013 to August 2013. Overall 22 serum samples from patients with fever were included in study.

Table 1: Comparison of CTK with Widal tube test

Tests	CTK Positive	CTK Negative	Total
Widal Positive	11	9	20
Widal	0	2	2

Negative			
Total	11	11	22

Table 2: Comparison of SD with Widal tube test

Tests	SD Positive	SD Negative	Total
Widal Positive	12	8	20
Widal Negative	0	2	2
Total	12	10	22

Table 3: Comparison of Enterochek IgM with Widal tube test

Tests	Enterochek IgM Positive	Enterochek IgM Negative	Total
Widal Positive	17	3	20
Widal Negative	1	1	2
Total	18	4	22

Table 4: Comparative Evaluation of the Diagnostic Test for Typhoid fever

Tests	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
CTK	55%	100%	100%	18%
SD	60%	100%	100%	20%
Enterochek IgM	85%	50%	94%	25%

Discussion

This study evaluated the diagnostic performance of three commercially available rapid diagnostic kits—CTK, SD Bioline, and Enterochek IgM—against the Widal tube test using 22 serum samples from febrile patients at Shri Guru Ram Rai Institute of Medical & Health Sciences and Shri Mahant IndiresH Hospital, Dehradun.

- **CTK** showed a sensitivity of 55% and specificity of 100%, with a PPV of 100% and NPV of 18%.
- **SD Bioline** demonstrated slightly higher sensitivity at 60%, with identical specificity (100%), PPV (100%), and NPV (20%).
- **Enterochek IgM** outperformed both in sensitivity (85%) and PPV (94%), though its specificity was lower at 50%, and NPV was 25%.

These findings suggest that while CTK and SD Bioline are highly specific and reliable in confirming typhoid cases, they may miss a significant number of true cases due to lower sensitivity. In contrast, Enterochek IgM is more effective in identifying true positives, making it a better screening tool, especially in early-stage illness.

The high PPV across all kits indicates strong diagnostic confidence when the test result is positive. However, the low NPV values highlight the risk of false negatives, underscoring the importance of clinical correlation and consideration of epidemiological context. Enterochek IgM's higher sensitivity makes it particularly valuable in early diagnosis, which is crucial for timely treatment and reducing complications.

These results align with previous studies. Kinikar et al. reported Enterochek WB sensitivity of 89.47% and specificity of 96.87%, supporting its utility in early detection⁸. Manufacturer data from Tulip Diagnostics also emphasize its role in identifying IgM antibodies specific to *S. typhi* lipopolysaccharide, a marker of recent infection⁷.

The study was limited by its small sample size and the absence of blood culture, the gold standard for typhoid diagnosis. While the Widal test served as a comparative baseline, its known limitations must be considered when interpreting results⁴.

Among the rapid diagnostic kits evaluated, Enterochek IgM demonstrated superior sensitivity and PPV, making it a more effective tool for early diagnosis of typhoid fever. However, its lower specificity suggests it should be used in conjunction with clinical assessment and, where possible, confirmatory testing. Larger-scale studies incorporating blood culture validation are recommended to further substantiate these findings and guide diagnostic protocols in endemic regions.

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