5. The membrane is laminated with an adhesive tape to prevent surface evaporation. Air pockets or patches may appear, which do not interfere with the test results. Presence of a band at the test region even if low in intensity or formation is a positive result.

6. The deliberate slow reaction kinetics of CORE S. TYPHI IGM is designed to maximize and enhance reaction time between sample capture and tracer elements to improve test sensitivity.

7. Most positive results develop within 15 minutes. However, certain sera sample may take a longer time to flow. Therefore, negatives should be confirmed only at 30 minutes. Do not read results after 30 minutes.

8. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

9. CORE S. TYPHI IGM should be used as a screening test in clinically suspected cases only, and its results should be confirmed by other supplemental method before taking clinical decisions.

**BIBLIOGRAPHY**


SPECIMEN COLLECTION AND PREPARATION
1. CORE S. TYPHI IGM uses human serum / plasma / whole blood as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. For whole blood, collect blood with a suitable anticoagulant such as EDTA or Heparin or Oxalate and use the freshly collected blood.
4. Whole blood should be used immediately and should not be frozen.
5. Though fresh specimen is preferable, in case of delay in testing, it may be stored at 2-8 °C for maximum up to 24 hrs.
6. If serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum.
7. Repeated freezing and thawing of the specimen should be avoided.
8. Do not use turbid, lipaemic and hemolysed serum/plasma.
9. Do not use hemolysed, clotted, contaminated, viscous/turbid specimens.
10. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
11. Refrigerated specimens must be brought to room temperature prior to testing.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS
1. Bring the kit components of CORE S. TYPHI IGM 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 µl of whole blood / serum / plasma into the specimen port “A” using a micropipette or the sample loop provided. Dip the sample loop in the sample container and blot the sample in the specimen port “A”.
7. Add five drops of sample running buffer into the reagent port “B”.
8. At the end of 15 minutes, read results as follows:

Negative Result
If IgM antibodies to S.typhi are not present, only one coloured band appears in the Control Window (C).

Positive Result
If IgM antibodies to S.typhi are present, two coloured bands appear in the Test (T) and Control Windows (C). The intensity of the test band may be more or less than the Control band, depending upon the concentration of IgM antibodies in specimen.

Invalid Result
The test is invalid if the Control band is not visible at fifteen minutes. Verify the test procedure and repeat the test with a new device.

TEST PERFORMANCE
Internal Evaluation
In an in-house study, the performance of CORE S. TYPHI IGM was evaluated using a panel of fifty specimens of WIDAL-positive (of varying reactivity) and WIDAL-negative sera in comparison with a commercially available DOT ELISA test kit. The results of the evaluation are as follows:

<table>
<thead>
<tr>
<th>Specimen Data</th>
<th>Total</th>
<th>CORE S. TYPHI IGM</th>
<th>Commercially available Dot ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of specimen tested</td>
<td>50</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>Number of Positive tested</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Number of Negative tested</td>
<td>44</td>
<td>43</td>
<td>44</td>
</tr>
</tbody>
</table>

Based on this evaluation:
Sensitivity of CORE S. TYPHI IGM : 100%
Specificity of CORE S. TYPHI IGM : 97.7%

External Evaluation-I
Seventy samples that were blood-culture positive, blood-culture negative sera and potentially cross-reacting sera were evaluated with CORE S. TYPHI IGM. The results of the evaluation are as follows:

<table>
<thead>
<tr>
<th>Specimen Data</th>
<th>Total</th>
<th>No. of Positives</th>
<th>No. of Negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood-culture positive sera</td>
<td>29</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Blood-culture negative sera</td>
<td>10</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Potentially cross-reacting negative sera</td>
<td>31</td>
<td>3</td>
<td>28</td>
</tr>
</tbody>
</table>

Based on this evaluation:
Sensitivity of CORE S. TYPHI IGM : 79.3%
Specificity of CORE S. TYPHI IGM : 90.2%

External Evaluation-II (Specificity & Precision study)
Thirty blood-culture negative sera were tested with CORE S. TYPHI IGM. The following are the results:

<table>
<thead>
<tr>
<th>Specimen Data</th>
<th>Total</th>
<th>No. of Positives</th>
<th>No. of Negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood-culture negative sera</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

Based on this evaluation:
Specificity of CORE S. TYPHI IGM : 100%

Intra-assay Precision study
One blood-culture positive sample was assayed 10 times on the same day. Results: No variation in results was observed indicating 100% correlation.

Inter-assay Precision study
One blood-culture positive sample was assayed 3 times on 3 different days. Results: No variation in results was observed indicating 100% correlation.

REMARKS
1. In some studies it has been reported that IgM antibodies to S.typhi persist for about 4 months post infection. Therefore, results within four months from an endemic area should be interpreted with caution.
2. The following chart would explain the IgM seroresponse in S.typhi infected subjects after onset of fever.

<table>
<thead>
<tr>
<th>Detectable IgM Response</th>
<th>Onset of Fever</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-6 days</td>
<td>43.50%</td>
</tr>
<tr>
<td></td>
<td>6-9 days</td>
<td>92.90%</td>
</tr>
<tr>
<td></td>
<td>&gt;9 days</td>
<td>100%</td>
</tr>
</tbody>
</table>

3. A negative result, i.e., the absence of detectable IgM antibody does not rule out recent or current infection. However, if S. typhi infection is still suspected, obtain a second specimen 5-7 days later and repeat the testing.
4. Specific IgG may compete with the IgM for sites and may result in a false negative. Conversely, rheumatoid factor in the presence of specific IgG may result in a false positive reaction.