TYPHIDOT
(3 HOURS)

Dot EIA test for specific detection of IgG & IgM to *Salmonella typhi*

**INTENDED USE**
*TYPHIDOT* is a Dot EIA assay designed for the qualitative detection of specific IgM and IgG antibodies against a specific outer membrane antigen of *Salmonella typhi* in human serum. It is intended to be used as *in vitro* diagnostic of typhoid fever. The results obtained should not be the sole determinant for clinical decision.

**SUMMARY AND EXPLANATION OF THE TEST**
Typhoid fever is an infectious disease caused by a bacterium, *Salmonella typhi*. It continues to be a major health problem especially in the Asia Pacific region, the Indian subcontinent, Central Asia, Africa and South America. Definitive clinical diagnosis of typhoid is unreliable because typhoid fever symptoms mimic other diseases with fever that are common in this part of the world. Clinical presentations vary tremendously among patients and cover a wide spectrum, hence the need for a good laboratory test. In addition, an accurate diagnosis of typhoid at an early stage is important not only for an aetiological diagnosis for the patient but also to identify individuals that might serve as a source of infection. Thus all cases of fever should be tested for typhoid and a rapid laboratory tests will be required.

Current methods to diagnose typhoid are by isolation of *S. typhi* from body fluids and by detection of antibody using the Widal test. Both methods are time-consuming with variable sensitivity. The culture method has a sensitivity of 48-78% but among the paediatric age group the sensitivity could be lower. Although the Widal test is widely used, its many limitations lead to difficulties in its interpretation especially in areas of high endemicity.

*TYPHIDOT* is a revolutionary method of diagnosis. Invented in Malaysia, it is the first known qualitative antibody detection test design for the rapid diagnosis of typhoid fever. Using the Dot EIA technique, presence of IgM and IgG antibodies against a specific antigen on the outer membrane of *S. typhi* are detected. A control clinical field trial among febrile children admitted to a hospital in an endemic area showed that *TYPHIDOT* when compared to culture is sensitive (95%) and did not show any cross-reactions. Despite being used in a study population with a high prior probability to typhoid, *TYPHIDOT* showed a high negative predictive value of 96.1% and offers the advantages of speed (3 hour), simplicity and early diagnosis.

**PRINCIPLE OF THE TEST**
*TYPHIDOT* is a Dot EIA assay. The presence of IgM and IgG antibodies made against a specific antigen on the outer membrane of *Salmonella typhi* are detected by incubating nitrocellulose strips dotted with the specific antigen protein with the patient sera and control sera (normal sera and culture positive typhoid sera). To visualise the antigen-antibody complex, the strips are simultaneously incubated with peroxidase-conjugated anti-human IgM and IgG. Upon addition of the chromogenic substrate, the results can be read visually. Positive reading is indicated by the BLUE colour as intense or more intense than that of the positive control. Total assay time is 3 hour.

**MANUFACTURER**
Revongen Corporation Center, No.12A, Jalan TP5, Taman Perindustrian UEP, 47600 Subang Jaya, Selangor, Malaysia.
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Fax : 603-8025 1637/1354
Email : info@reszonics.com
Website : www.reszonics.com

**ORDER INFORMATION**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
<th>Packing Size</th>
</tr>
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<tbody>
<tr>
<td>TF-ED0102</td>
<td>TYPHIDOT (3 HOURS)</td>
<td>28 tests / kit</td>
</tr>
</tbody>
</table>
REAGENTS AND MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Reagents and Materials</th>
<th>28 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predotted antigen strips</td>
<td>56 strips</td>
</tr>
<tr>
<td>Sample diluent (A1)</td>
<td>30 ml</td>
</tr>
<tr>
<td>Washing buffer (10X) (A2)</td>
<td>20 ml</td>
</tr>
<tr>
<td>Buffer for Anti-Human IgM*HRP (B1)</td>
<td>20 ml</td>
</tr>
<tr>
<td>Buffer for Anti-Human IgG*HRP (B2)</td>
<td>20 ml</td>
</tr>
<tr>
<td>Colour reagent Substrate A (C1)</td>
<td>10 ml</td>
</tr>
<tr>
<td>Colour reagent Substrate B (C2)</td>
<td>30 ml</td>
</tr>
<tr>
<td>Anti-Human IgM*HRP (IgM)</td>
<td>100 µl</td>
</tr>
<tr>
<td>Anti-Human IgG*HRP (IgG)</td>
<td>55 µl</td>
</tr>
<tr>
<td>Positive control</td>
<td>110 µl</td>
</tr>
<tr>
<td>Negative control</td>
<td>110 µl</td>
</tr>
<tr>
<td>Reaction tray</td>
<td>1</td>
</tr>
<tr>
<td>Worksheet</td>
<td>2</td>
</tr>
<tr>
<td>Instruction manual (product insert)</td>
<td>1</td>
</tr>
</tbody>
</table>

MATERIALS REQUIRED BUT NOT SUPPLIED
- Sample collection and preparation device and equipment
- Measuring cylinder (100 ml)
- Micropipettes (2-200 µl and 1000 µl) and sterile micropipette tips
- Small conical flasks (100 ml) or equivalent
- Forceps
- Wash bottles
- Filter paper
- Distilled water
- Rocker platform (optional)
- Discard jar containing bleach or equivalent disinfectant
- Aspirator
- Aluminium foil
- 1 sterile tube or bottle
- 1 conical flask covered with aluminium foil or a dark reagent bottle
- Clock or timer

WARNINGS AND PRECAUTIONS
1. For *in vitro* diagnostic use only.
2. Do not do kit beyond the expiration date.
3. Do not mix reagent from different Batch numbers.
4. Avoid microbial contamination of reagents when removing aliquots from the reagent vials. Sterile disposable pipette tip is recommended.
5. Handle all specimens as being potentially infectious. Dispose all materials that come in contact with the specimen as infectious waste.
6. The controls have been tested free of HIV and viral hepatitis. It is however recommended that the reagents and human sera be handled using established good laboratory working practices.

STORAGE AND STABILITY
Store at 2-8°C. Keep away from direct sunlight, strong light, moisture and heat.

SAMPLE COLLECTION AND PREPARATION
Serum and plasma are separated from whole blood according to standard procedure.

ASSAY PROCEDURE
1. Allow kit to warm to room temperature (minimum 23°C) before doing the test.
   
   Important Notes: before opening the vial for reagent at small volume, it is recommended to gently tap the vial on a hard surface or briefly centrifuge the vial to dislodge any liquid in the cap.

2. Preparation of reagents
   a. Washing buffer
      Dilute 20 ml of washing buffer (10X) (A2) into 180 ml of distilled water to produce a final concentration of 1X. The diluted washing buffer (1X) is sufficient for 28 tests. Store it separately at 2-8°C and use when necessary.
   b. Prediluted conjugates
      Prepare fresh before use. Based on the number of tests (including controls), add the recommended volume of buffer for anti-human IgM*HRP (B1) into a sterile tube/bottle. Then add the recommended volume of anti-human IgM*HRP and mix well (refer to table 1 below).
      Prepare the prediluted anti-human IgG*HRP using the same procedure as prediluted anti-human IgM*HRP. Use buffer for anti-human IgG*HRP (B2) and anti-human IgG*HRP instead (refer to table 2 below).

3. Procedure for diagnostics test
   a. Divide the reaction tray into two (2) columns. Mark one column as M and one column as G. Should be brought to room temperature before mixing. Avoid exposing these reagents to strong light during incubation or storage. Based on the number of tests (including controls), add the recommended volume of substrate B into a reagent bottle or a flask covered with aluminium foil. Then add the recommended volume of substrate A and mix well (refer to table below).
   
   Table 1 – preparation of prediluted anti-human IgM*HRP

<table>
<thead>
<tr>
<th>No. of test</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1 (ml)</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>11</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>IgM (µl)</td>
<td>10</td>
<td>15</td>
<td>25</td>
<td>30</td>
<td>40</td>
<td>45</td>
<td>55</td>
<td>60</td>
<td>70</td>
</tr>
</tbody>
</table>

   Table 2 – preparation of prediluted anti-human IgG*HRP

<table>
<thead>
<tr>
<th>No. of test</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2 (ml)</td>
<td>2</td>
<td>3.2</td>
<td>5.2</td>
<td>6</td>
<td>8</td>
<td>9.2</td>
<td>11.2</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>IgG (µl)</td>
<td>5</td>
<td>8</td>
<td>13</td>
<td>15</td>
<td>20</td>
<td>23</td>
<td>28</td>
<td>30</td>
<td>35</td>
</tr>
</tbody>
</table>

   c. Colour development reagent
      Prepare fresh before use. Substrate A (C1) and substrate B (C2) should be brought to room temperature before mixing. Avoid exposing these reagents to strong light during incubation or storage. Based on the number of tests (including controls), add the recommended volume of substrate B into a reagent bottle or a flask covered with aluminium foil. Then add the recommended volume of substrate A and mix well (refer to table below).

   Table 3 – Preparation of colour development reagent

<table>
<thead>
<tr>
<th>No. of test</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 (ml)</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
<td>3.5</td>
<td>4.0</td>
<td>5.6</td>
</tr>
<tr>
<td>C2 (ml)</td>
<td>2.5</td>
<td>5.0</td>
<td>7.5</td>
<td>10.0</td>
<td>12.5</td>
<td>15.0</td>
<td>17.5</td>
<td>20.0</td>
<td>22.4</td>
</tr>
</tbody>
</table>

   Note: The M and G strips are for testing for the presence of IgM and IgG respectively. Make sure that there is an M and a G strip (2 strips) for each patient or control serum.
c. Place both the IgM and IgG strips for each patient into 1 reaction well so that the strips are exposed to the same sera. Perform similarly for the positive and negative control sera.

d. Add 990 µl of sample diluent (A1) into the reaction wells with strips.

e. To the appropriate reaction well, add 10 µl of either positive control, negative control or test sera to achieve a final serum dilution of 1:100.

f. Gently shake tray to mix. Make sure all strips are with the marked side up, fully immersed in the solutions and not in contact with each other.

g. Incubate on a rocker platform (optional) for 1 hour. If a rocker platform is not available, shake the tray gently every 5-10 minutes.

h. Aspirate the solution in the reaction well into a discard jar containing disinfectant. Add 1 ml of diluted washing buffer (1X) into each well and shake for 5 minutes. Repeat this step for 3X.

i. Place the strips labeled M into the wells (2 strips/ well) in column M in the reaction tray and place the strips labeled G into the wells (2 strips/ well) in column G in the reaction tray. **Do not allow the strips to dry.**

j. Using sterile micropipettes tip, add 1 ml of the diluted Anti-Human IgM into the wells in column M and 1 ml of the diluted Anti-Human IgG into the wells in column G.

k. Cover the tray with aluminium foil and incubate for 1 hour at room temperature on a rocker platform. **Note: 30 minutes before the end of incubation, prepare colour development reagents.**

l. Add 1 ml of the prepared colour development reagent into each well. Cover the tray and incubate on the rocker platform.

m. Allow 15 minutes for colour development. If no colour is observed, allow development for a maximum of 30 minutes.

n. Stop the reaction by aspirating the solution in the reaction well into the discard jar and briefly rinse the strips in distilled water (3X).

o. Place the M and G strips for test serum and controls onto filter paper to blot off excess water. Read the results. If results reading cannot be done immediate, store the strips submerged in distilled water for up to 1 day.

p. Rinse the tray thoroughly with distilled water. Store dry for reuse.

q. For a permanent record, paste the dried strips in the relevant positions on the worksheet provided.

**QUALITY CONTROL**

1. Positive and negative controls are included and must be included in each batch of test run.

2. The strips should have a white background with only the 2 dots appearing where applicable.

3. The test should be repeated under the following conditions:
   a. If the strips show a blue background making interpretation difficult.
   b. If the colour intensity of the negative control is similar to that of the positive control.
   c. If the colour intensity of the test serum is high but not equal to the positive control. Request for a second serum specimen at least 2 days later so that a higher serum titer would be available for detection.

4. For borderline cases where the colour intensity of the dots produced by the test sera is in between lighter than or equivalent to those of the positive control, repeat test with serum specimen collected one or two days later.

5. For borderline cases where the colour intensity of the dots produced by the test sera is in between lighter than or equivalent to those of the positive control, repeat test with serum specimen collected one or two days later.

**INTERPRETATION OF RESULTS**

To interpret the results of TYPHIDOT for typhoid, the colour intensity of the dots produced by the test sera must be equivalent to or greater than those of the positive control. Please follow all guides below:

1. Always compare each M strip of the test sera with the M strip of positive control. Similarly, compare the G strip of the test sera with the G strip of positive control.

2. When comparing colour intensity, compare the dot on the left of the test strip with the dot on the left of the positive control strip. Similarly, compare the colour intensity of dot on the right of the test strip (next to the line marked on the strip) with the colour intensity of the dot on the right of the positive control strip. Only when both dots on the test strip are as dark as or are darker than their corresponding dots on the positive control strips can the result be reported as positive.

3. If one of the dots on the test strip is lighter compared to the corresponding dot on the positive control strip, the result should be reported as negative.

4. For borderline cases where the colour intensity of the dots produced by the test sera is in between lighter than or equivalent to those of the positive control, repeat test with serum specimen collected one or two days later.

5. For borderline cases where the colour intensity of the dots produced by the test sera is in between lighter than or equivalent to those of the positive control, repeat test with serum specimen collected one or two days later.

<table>
<thead>
<tr>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific antigen Control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
</tr>
<tr>
<td></td>
<td>Negative control</td>
</tr>
<tr>
<td>Test result: Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Test result: Negative</td>
</tr>
</tbody>
</table>

5. Read both M and G strips for each test sera. Positive result on M and G strips indicate presence of IgM and IgG antibodies against *S. typhi* respectively, and vice versa for negative results. Clinical interpretation is as following:

<table>
<thead>
<tr>
<th>Results</th>
<th>Clinical interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM positive; IgG negative</td>
<td>Acute typhoid fever</td>
</tr>
<tr>
<td>IgM and IgG positive</td>
<td>Acute typhoid fever (in the middle stage of infection)</td>
</tr>
<tr>
<td>IgM negative; IgG positive</td>
<td>Implications for the presence of IgG antibodies, may be due to:</td>
</tr>
<tr>
<td></td>
<td>1. current infection</td>
</tr>
<tr>
<td></td>
<td>2. previous infection</td>
</tr>
<tr>
<td></td>
<td>3. relapse or re-infection</td>
</tr>
<tr>
<td></td>
<td>(refer to limitations of the test)</td>
</tr>
<tr>
<td>IgM and IgG negative</td>
<td>Probably not typhoid</td>
</tr>
</tbody>
</table>


LIMITATIONS OF THE TEST

1. This product is designed for use with human serum only.
2. The test is a qualitative assay and is not for quantitative determination of antibodies concentration levels. The intensity of the dot does not have linear correlation with the antibody titer of the specimen.
3. The results obtained should only be interpreted in conjunction with other diagnostic results and clinical information.
4. High IgG concentration may give false negative for IgM because specific IgG will drastically reduce binding of specific IgM to the antigen.
5. In case of only IgG antibodies is presence, it is important that clinical interpretation be made together with the clinical symptoms in order to diagnose if the patient is currently having typhoid. Based on the clinical presentations, a clinical decision has to be made by the attending doctor. Blood culture results, if positive, a few days later will prove whether the clinical decision is right or not.

PERFORMANCE CHARACTERISTICS

TYPHIDOT has been evaluated in many endemic area including Malaysia, India, Indonesia, Pakistan and Philippines. Most of the study reported sensitivity and specificity of more than 90%.

REFERENCES