INTENDED USE
Immunospec FEBRILE ANTIGENS (bacterial agglutination antigens) are bacterial suspensions for use in either slide or tube agglutination tests to detect the presence of bacterial agglutinins associated with bacterial infection or previous exposure to a related organism. Two test procedures are recommended, the rapid slide agglutination test and the tube agglutination test. The rapid slide test is recommended as a screening procedure and should be used to establish the presence or absence of homologous antibody. If antibody is present in the serum specimen, then the tube procedure should be used to establish antibody titer.

METHOD A: RAPID SLIDE TEST

1. Obtain a clear transparent glass slide and divide it into 1/3 inch squares with a Wax pencil or a diamond tipped pencil. A small window pane can be used for this purpose. The use of ring slides is also recommended.
2. Using a suitable pipette, add the following amounts of serum to be tested from Left to right to consecutive squares or rings: .08m1; .04m1; .02m1; .01m1; .005m1.
3. Serum should be clear and unheated. Repeat this procedure with positive and negative control sera.
4. Shake the antigen gently to insure a uniform suspension.
5. Add one drop of antigen suspension just below each quantity of serum.
6. Mix the serum and antigen well using a piece of applicator stick. Use separate applicator sticks for each serum quantity or use the same stick and proceed from right to left. Each mixture should form an area approximately 1/2 inch by 1 inch.
7. Rotate slide by hand or on a mechanical shaker at 150 RPM for 2-3 minutes.
8. Observe for agglutination using any good indirect light against a dark background.
9. A positive serum of known titer and a negative serum should be included as controls.

RESULTS
The degree of agglutination is recorded as follows:

4+ -100% of the organisms are agglutinated
3+ -75% of the organisms are agglutinated
2+ -50% of the organisms are agglutinated
1+ -25%/ of the organisms are agglutinated
± -Less than 25% of the organisms are agglutinated
Negative -No agglutination is observed

Although the slide test is not recommended to establish titer, the quantity of serum giving 50% agglutination can be used to establish the approximate equivalent to the tube test dilutions shown below:

<table>
<thead>
<tr>
<th>Serum Volume</th>
<th>Approximate Tube Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>.08m1</td>
<td>1:20</td>
</tr>
<tr>
<td>.04m1</td>
<td>1:40</td>
</tr>
<tr>
<td>.02m1</td>
<td>1:80</td>
</tr>
<tr>
<td>.01m1</td>
<td>1:160</td>
</tr>
<tr>
<td>.005m1</td>
<td>1:320</td>
</tr>
</tbody>
</table>

METHOD B: TEST TUBE TITRATION TEST

1. Place ten 12x75mm test tubes in a suitable rack.
2. Add 1.9ml of 0.9% sodium chloride solution to the first tube.
3. Add 1.0ml of 0.9% sodium chloride solution to the remaining tubes.
4. Add 0.1ml of the serum to be tested to the first tube. Mix well and transfer 1.0 ml of the diluted serum from the first tube to the second tube. Repeat this procedure until all ten tubes contain serial two-fold serum dilutions of 1:20 through 1:10, 240. Remove 1.0m1 of the diluted serum from tube10 and discard. Tube No. 1 is considered a 1:20 dilution. Repeat this procedure with positive and negative control sera.
5. Place one tube at the end of the series of dilution tubes and add 1.0 ml of the 0.9% sodium chloride solution used to dilute the serum. Label this tube "saline control".
6. Mix the antigen suspension well by gently shaking the bottle. Add one drop of antigen to each tube.
7. Shake the rack well to mix the antigen and serum and place in a water bath. The recommended time and temperature of incubation is as follows:

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Temperature (°C)</th>
<th>Time of Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella &quot;O&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>45° 50ºC</td>
<td>18 hours</td>
</tr>
<tr>
<td>Group B</td>
<td>45° 50ºC</td>
<td>18 hours</td>
</tr>
<tr>
<td>Group C</td>
<td>45° 50ºC</td>
<td>18 hours</td>
</tr>
<tr>
<td>Group D (Typhoid O)</td>
<td>45° 50ºC</td>
<td>18 hours</td>
</tr>
<tr>
<td>Salmonella &quot;H&quot; a</td>
<td>45° 50ºC</td>
<td>2 hours</td>
</tr>
<tr>
<td>Salmonella &quot;H&quot; b</td>
<td>45° 50ºC</td>
<td>2 hours</td>
</tr>
<tr>
<td>Salmonella &quot;H&quot; c</td>
<td>45° 50ºC</td>
<td>2 hours</td>
</tr>
<tr>
<td>Salmonella &quot;H&quot; d (Typhoid H)</td>
<td>45° 50ºC</td>
<td>2 hours</td>
</tr>
<tr>
<td>Brucella abortus and Brucella melitensis</td>
<td>37° C</td>
<td>48 hours</td>
</tr>
<tr>
<td>Proteus OX2, OX19, &amp; OXK</td>
<td>45° 50ºC</td>
<td>18 hours</td>
</tr>
</tbody>
</table>

STABILITY
Three years at refrigerated temperature.

SPECIMENS
Sera should be clear and should not be heated.

PROCEDURE
Materials provided: FEBRILE ANTIGENS of choice.

Materials required but not provided:

- Transparent plain glass ring slide
- Serum pipettes
- Applicator sticks
- Test Tubes
- NaCl solution, 0.9%
- Mechanical rotator (optional)
NOTE: Typhoid H and other Salmonella flagellar antigens should be incubated for 2 hours at 45°- 50ºC followed by 18 hours at 2°-8°C before final reading.

8. After incubation, carefully remove the rack containing the test tubes and observe for agglutination. The use of an indirect light source against a black background will give optimal conditions for reading tube test.

9. Record the test results as follows:
   - 4+ - All the organisms appear clumped on the bottom of the tube and the supernatant fluid is clear.
   - 3+ - Approximately 75% of the organisms are clumped and the supernatant is slightly cloudy.
   - 2+ - Approximately 50% of the organisms are clumped and the supernatant is moderately cloudy.
   - 1+ - Approximately 25% of the organisms are clumped and the supernatant is cloudy.
   - Negative - No agglutination is observed and suspension appears cloudy.

10. Record titer of reactive serum as the last dilution which gives a 2+ reaction.

**PRECAUTIONS**

1. For greater proficiency in test interpretation, always include positive and negative serum controls as well as a saline control in each test protocol.
2. All sera to be tested should be clear and free from bacterial contamination.
3. Do not heat sera prior to testing.
4. Shake antigen vial well before use to insure a smooth, uniform suspension.
5. Antigen should be stored in refrigerator at 2°-8ºC when not in use.
6. Antigen should not be frozen.

**QUALITY CONTROL PROCEDURE**

The use of positive control sera tested in parallel with unknown test serum specimens is recommended to assure the laboratory worker that the bacterial antigen in use is capable of reacting with its homologous antibody. Immunospec’ positive control sera have a titer of 1:80 or more with homologous antigens.

**LIMITATIONS OF THE PROCEDURE**

1. Agglutinins are not always produced in bacterial infections.
2. Cross reaction may occur in certain pathologies. For instance, Tularemia infections may produce agglutinins to Brucella antigens.
3. Vaccinations for several diseases may produce cross reacting agglutinins. Typhus vaccination may produce antibodies to Proteus antigens.

**NOTE**

Accurate diagnosis of disease depends on a close working relationship between the clinical laboratory and the physician. A rise in antibody titer between acute phase serum specimens and convalescent phase serum specimens accompanied by the usual signs and symptoms of a given disease in a patient is the best basis for accurate diagnosis.

**EXPECTED VALUES**

When viable bacteria are introduced into a susceptible host, an immune response generally occurs which is capable of producing antibodies called agglutinins. These agglutinins are capable of reacting specifically with suspensions of Salmonella species responsible for the infection, causing them to agglutinate. Agglutinins are produced slowly during the acute phase of infection and continue to form during the convalescent phase of infection. The titer of the concentration of antibody rises considerably between acute infection and convalescence. Therefore, a rise in titer between serum collected during the acute or febrile stage of infection and serum collected during the convalescent stage can be of diagnostic significance. The following table indicates the agglutinin titers normally expected during the course of infection with several bacterial species.