PRINCIPLE

The CROMATEST bacterial suspensions are prepared specifically to detect, identify and quantitate serum agglutinins developed during some febrile infections such as brucellosis, salmonellosis and certain rickettsiosis.\(^1\)\(^-\)\(^3\)

The suspensions have been stained (somatic in blue and flagellar in red) or have colorless presentation for some specific antigens (Note 1).

The tube agglutination is carried out by preparing two-fold dilutions of serum in saline solution and then an equal volume of standardized antigenic suspension. After incubation the highest dilution of the serum that causes agglutination in the presence of the specific antigen is determined.

The tube test is considered a reference method\(^4\)\(^-\)\(^8\) and as such becomes indispensable in clarifying equivocal agglutinations that may have obtained through the rapid slide test, as well as for the accurate determination of variation in serum antibody levels that occur during the different stages of the infection.

REAGENT COMPOSITION

**Febrile Antigen.** Stabilized suspension of stained or colorless killed bacteria in a buffered solution. Contains 0.95 g/L of sodium azide.

**Agglutinin-Positive.** Non-titered polyvalent antiserum produced in rabbits. Contains 0.95 g/L of sodium azide.

**Agglutinin-Negative.** Non-reactive animal serum. Contains 0.95 g/L of sodium azide.

**Warning:** The reagents in this kit contain sodium azide. Do not allow to contact with skin or mucous membranes.

PROCEDURE

**1.** Place 6 to 8 tubes into a rack for each antigen to be tested.

**2.** Using saline solution as diluent prepare a row of two-fold dilutions of the test serum as shown in the following table:

<table>
<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>0.9</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>(µL)</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
<td>6.25</td>
<td>3.125</td>
<td>1.562</td>
<td>0.781</td>
</tr>
<tr>
<td>Bacterial Suspension (µL)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Final dilution</td>
<td>1:20</td>
<td>1:40</td>
<td>1:80</td>
<td>1:160</td>
<td>1:320</td>
<td>1:640</td>
<td>1:1280</td>
<td>Control</td>
</tr>
</tbody>
</table>

2. Add 0.5 mL of the appropriate bacterial suspension previously shaken to each tube of given row and mix (Note 3). Final serum dilutions are shown in the above table.

3. Incubate at 37ºC in the waterbath for 24 hours, or with the somatic tritrations at 50ºC for 4 hours and with the flagellar tritrations at 50ºC for 2 hours, before reading.

4. Examine macroscopically for agglutination.

**Reading**

Read the results of all control tubes first. Remove 2-3 tubes at a time from the rack, hold them in front of a suitable source of light and observe every tube for clearing of the supernatant fluid and amount and character of the sediment and agglutinate particles. After examining the sediment pattern shake the tube gently.
Negative reaction: In the antigen control and negative reaction the appearance of the suspension should be unchanged, and show a typical swirl when the tube is flicked.

Positive reaction: In a positive reaction there is an obvious agglutination and a variable degree of clearing of the supernatant fluid. Flagellar agglutinins (H) produce large floccular aggregates which are easily broken up, whereas somatic agglutinins (O) produce granular or small flaky aggregates. The end point titer of the serum is designated as the highest dilution that will cause agglutination of a known bacterial antigen. The next dilution should be negative.

QUALITY CONTROL
Positive and negative sera as well as Suspension Control tubes should be run daily to check the operativity of the system.

EXPECTED VALUES
Salmonella and Brucella: Titer levels greater than 1/80 (somatic and brucella antigens) and 1/160 (flagellar antigens) indicates recent infection. Proteus: Titer of less than 1/160 should not be considered significant. A single positive result has less clinical significance than the demonstration of a rising or decreasing titre between successive serum specimens taken days apart.

CLINICAL SIGNIFICANCE
Febrile Antigen is a term which has been accepted generally as referring to bacterial suspensions representative of a number of pathogenic microorganisms to human, involved in some bacterial infections (brucellosis, salmonellosis and certain rickettsiosis) which are accompanied by a fever in the host. The best option to establish the etiology of an infectious disease is by isolation and identification of the pathogenic microorganisms to human, involved in some bacterial infections (brucellosis, salmonellosis and certain rickettsiosis) which are accompanied by a fever in the host.

ANTALITICAL PROCEDURE
- There is not a Reference Material for the sensitivity standardization of these reagents. For this reason, Linear Chemicals adjust the sensitivity of their reagents against to specific antiseria and commercial reagents of certified quality.
- Prozone effect: False negative results may be obtained with sera containing a high titer of antibodies. A dilution of these sera will give a positive result.
- Results obtained with this reagent did not show significative differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Hemoglobin (<10 g/L), bilirubin (<20 mg/dL), lipemia (<10 g/L) and rheumatoid factors (<300 IU/mL) do not interfere.

LIMITATIONS OF PROCEDURE
- In early stages of the disease as in immunodeficient patients or under antibiotic treatment, false reactions may occur.
- Cross reactions with Brucella have been reported in cases of infection or vaccination with some strains of Campylobacter, Francisella tularensis, Yersinia enterocolitica y Salmonella. Serum from patients with chronic active liver disease and from narcotic addicts appear to contain broad non-specific activity to the Widal antigen1-2.

NOTES
1. Detailed instructions are available for the application of these reagents to microplate. Request the technical bulletin to your distributor.
2. The sera must be used in the unheated state. If inactivated by heat, some thermolabile agglutinins may be destroyed.
3. Allow all reagents and samples to reach room temperature before use. Shake thoroughly the reagents before using.

REFERENCES