Introduction

*Brucella* is a genus of fastidious, gram-negative, nonmotile, small coccobacilli (rods) resemble Haemophilus in appearance, with complex growth requirements. Growth is often promoted by elevated carbon dioxide concentration. The several species, obtained from different animal reservoirs, have only minor cultural and antigenic differences. The M antigen is more prominent in *Brucella melitensis* and the A antigen in *Brucella abortus*. All species are obligatory mammalian pathogens, able to grow in phagocytes and causing chronic infection. *Brucella abortus* is a brucella that causes abortion in cattle. It may be shed in milk by apparently healthy animals and is thereby transmitted to man, in whom it causes an infection that may be acute, relapsing, chronic, or subclinical. *Brucella melitensis* is a brucella obtained from the milk and urine of apparently healthy goats. It causes Malta fever. *Brucella neotomae* is obtained from the desert wood rat. *Brucella ovis* is obtained from sheep. *Brucella suis* is obtained from pigs. It occasionally infects humans.

*Brucella* causes brucellosis, or undulant fever, it is an infection that causes fever. *Brucella* is transmitted via animals (zoonosis), specially cattle, and grows well in phagocytes. Animal tissue or unpasteurized milk is the usual means by which humans are infected. Cases are rare, often go undetected, and develop into chronic and recurrent episodes of the systemic disease. Culture isolation is difficult so that diagnosis is usually based upon laboratory serology. But antibody does not confer immunity. Immunity to brucella is conferred by activated macrophages and lymphokines derived from them. *Brucella antibodies cross-react with Vibrio cholerae and Francisella tularensis*. Direct agglutination assay is a commonly used serologic test for *Brucella* infection.

Stained brucella suspensions are used to detect, identify and quantitate specific antibodies in sera. They are standardized, smooth suspension of killed bacteria which have been stained to facilitate reading of agglutination tests. Stained brucella suspensions are used primarily in the investigation of pyrexia, and are suitable for used in rapid slide tests, standard Widal tests anti-human globulin (Coombs's test) and complement fixation test. Serological tests are based on the fact that antibodies in serum, produced in response to exposure to bacterial antigens, will react with bacterial suspensions which carry homologous antigens. In the diagnosis of febrile disease, such tests should be used in parallel with appropriate cultural techniques for the isolation and identification of the causative organism.
PRINCIPLE

Stained, standardized, smooth suspensions of killed bacteria (brucella) are agglutinated when mixed with samples containing specific antibodies to brucella.

REAGENTS

1. Stained Brucella suspensions, contains approximately $10^{10}$ bacteria/ml and contain 0.25% formalin and 0.01% thiomersal as preservative.
2. Positive serum “control”, Which is known to have antibodies against brucella
3. Negative serum “control”, Which is known to have NO antibodies to brucella.

SPECIMENS

Use serum (clotted blood) or plasma which should be fresh (stored for max 8 days at +2 to +8 C) or stored in the deep freeze. Deep frozen samples (at −25 C or below) must have been frozen within 24 hours after collection and must be used within 3 months; repeated thawing and freezing is to be avoided. Serum samples should be completely coagulated and should contain no particles or traces of fibrin after centrifugation. Highly lipaemic samples or frozen smaples which become turbid after thawing should be clarified by centrifugation (10 min, at approx. 15,000 x g) before use in the test.

METHODS

A. Rapid Screening Test

1. Bring the patient specimens are reagents to room temperature
2. Place 0.02 ml of undiluted serum in a 3 cm diameter circle on a white tile.
3. Add one drop of the appropriate well-shaken suspension using the dropper provided
4. Mix by stirring for a few seconds and spread to fill the whole area of the circle on the tile
5. Slowly rotate for one minute and observe for agglutination.

*If agglutination is visible within one minute, a significant titer should be obtained in a confirmatory tube test. The reaction is roughly equivalent to that obtained in a tube agglutination test with a serum dilution of 1 in 80. However, care should be taken in case a prozone (antibody excess) phenomenon is encountered (when a negative reaction will be obtained with a serum which has a high antibody titer). From time to time it is advisable to test the suspension in a complete slide titration or tube agglutination test with a known positive serum, for example Brucella abortus or Brucella melitensis antiserum, and a negative control serum. If a suspension agglutinate with a known negative serum, or fails to react with a known positive serum it should be discarded. A prozone phenomenon may be encountered.
B. Rapid Slide Titration

1. Using a 0.2 ml pipette, deliver 0.08, 0.04, 0.02, 0.01 and 0.005 ml of undiluted serum into a row of 3 cm diameter circles on a white tile.

2. Using the dropper provided, add one drop of the appropriate well-shaken suspension to each serum aliquot.

3. Mix by stirring for a few seconds with a wooden applicator stick, proceeding from the mixture containing 0.005 ml serum to that containing 0.08 ml serum, spreading the contents to fill the circles.

4. Rotate the tile slowly and read agglutination at one minute.

• The reactions obtained are roughly equivalent to those which would occur in a tube agglutination test with serum dilutions of 1:20, 1:40, 1:80, 1:160, 1:320 respectively, although significant discrepancies are sometimes observed. It is possible for a serum which is reactive in a tube agglutination fail to react on a slide. It is strongly recommended that a tube test should be performed with a sample if results are obtained which do not conform to clinical indications. False results may be obtained if the reagents are not allowed to reach room temperature before use. Also, false positive reactions are likely if the test is read more than one minute after mixing.

C. Tube Agglutination Test

1. Make one row of serum dilutions for each antigen to be tested as shown in the following “Table”, using saline or 0.25 % phenol saline as diluent. Mix the contents of tube 1 and transfer 1 ml to tube 2. Repeat for each tube, up to but not including tube 8, finally discarding 1 ml from tube 7.

2. Add one drop of the appropriate suspension to each tube of a given row using the dropper provided. Do not dilute the suspension before use.

3. Mix and incubate at 37 C for 24 hour.

4. Examine for agglutination.

• In a positive reaction there is obvious granular agglutination. In a negative reaction and in the saline control the appearance of the suspension should be unchanged, and show a typical swirl when the tube is flicked. The titer is the dilution of serum in the last tube showing agglutination. As a positive control, a dilution series of Brucella abortus or Brucella melitensis antiserum may be included. These are not, strictly speaking, standard sera and although titers should approach those given on the bottle labels, the exact titers may not always be obtained. A prozone phenomenon may sometimes be encountered.

<table>
<thead>
<tr>
<th>Tube number</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>Diluent ml</td>
<td>1.9</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Patient serum</td>
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<td>1 ml serial dilutions</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></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>
INTERPRETATION

Agglutinations are found in a high proportion of normal individuals and titers of less than 1 in 80 are of doubtful significance.

A rising or falling titer is more significant than a single high titer. It is difficult to differentiate between a Br. Abortus and a Br. Melitensis infection by serological tests, but from the point of view of treatment, this distinction is not necessary.

False positive reactions may occur with sera from patients infected with Pasteurella tularensis or vaccinated with Vibrio cholerae.

References

1. MUREX Diagnostic Limited
2. J. H. L. PLAYFAIR “Immunology at a Glance”.
3. AUSTYN & WOOD “Principle of Cellular and Molecular Immunology”.
4. D. P. STITES etc. “Basic & Clinical Immunology”.

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