

Department:	Laboratory and Blood Bank (Serology)		
Document:	Internal Policy and Procedure		
Title:	Brucella Antibody Test		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

- 1.1 The purpose of this document is to outline the standard operating procedure (SOP) for properly processing, examining, and reporting results for Brucella Antibody Test (BAT) test.

2. DEFINITONS:

- 2.1 Brucellosis is an infectious disease caused by the bacteria of the genus Brucella. These bacteria are primarily passed among animals.
- 2.2 BAT: Brucella Antibody Test.

3. POLICY:

- 3.1 Brucellosis is an infectious disease caused by the bacteria of the genus Brucella.
- 3.2 Humans become infected by coming in contact with animals or animal products that are contaminated with these bacteria.
- 3.3 Diagnosed by in- vitro detection and quantitative estimation of specific antibodies to Brucella present in serum. BAT is used to detect, identify and quantitate specific antibodies in serum samples from patients suffering from flu- like symptoms, recurrent fever, joint pain, and fatigue.

4. PROCEDURE:

4.1 Principle of BAT test:

- 4.1.1 The BAT homologous antigens detect agglutinins which are formed in human infection to microbiological agents. If a serum sample contains this agglutinin, a visible agglutination occurs.

4.2 Specimen Requirements:

- 4.2.1 Fresh serum samples should be collected in the manner routinely used for any clinical laboratory test.
- 4.2.2 Sample collected in plain tube (Yellow/ Red) only – 3 ml.
- 4.2.3 Sample must be at room temperature upon performing the test.
- 4.2.4 Sample is stable for no longer than 72 hours after collection if stored at 2- 8oC and for longer period if frozen.

4.3 Procedure:

4.3.1 Slid agglutination method (Qualitative):

- 4.3.1.1 Bring reagents and specimens to room temperature. The sensitivity of the test may be reduced at low temp.
- 4.3.1.2 Place 50 uL of undiluted serum into clean transparent slide test.
- 4.3.1.3 Swirl the antigen vial gently before using. Add 1 drop of antigen to each circle next to the sample to be tested.
- 4.3.1.4 Mix well using applicator sticks and spread over the entire area enclosed by the circle.
- 4.3.1.5 Place the slid on the mechanical rotator at 80-100 r.p.m. and read within 1 min.

4.3.2 Slid agglutination method (titration):

- 4.3.2.1 Bring reagents and specimens to room temperature. The sensitivity of the test may be reduced at low temp.

- 4.3.2.2 Place 20 uL, 10 uL, 5 uL of undiluted serum into clean transparent slide test.
- 4.3.2.3 Shake the antigen well & add 1 drop (50 ul) to each circle next to the sample to be tested. Mix well using applicator sticks starting with the highest dilution up to the lowest. Place the slide on the mechanical rotator at 80-100 r.p.m. within 1 min. Read results immediately.

4.4 **Reading:**

- 4.4.1 Examine macroscopically the presence or absence of clumps within 1 min.
- 4.4.2 Negative: No agglutination.
POSITIVE= degree of agglutination: 1/80, 1/160, and 1/320.

4.5 **Interpretation:**

- 4.5.1 A normal result shows no antibodies to Brucella. However, during the first few days to weeks of exposure to an antigen, there may be very little antibody production. As brucellosis progresses more antibodies are produced. If antibodies are detected, there has likely been exposure to the Brucella bacteria but this does not necessarily indicate current infection. Increasing antibody levels are more likely to indicate a current infection.
- 4.5.2 Calculation:
4.5.2.1 Titers \geq 1/80 indicate infection

4.6 **Results reporting:**

- 4.6.1 Qualitative Test:
 - 4.6.1.1 Negative results are released as negative
 - 4.6.1.2 Positive results are indicated by observable agglutination in the reaction mixture
- 4.6.2 Semi- Quantitative Test:
4.6.2.1 Record the last dilution of serum with visible agglutination:

Dilution	1/80	1/160	1/320
Serum	20ul	10ul	5ul
Reagent	50ul	50ul	50ul

4.7 **Quality Control:**

- 4.7.1 The positive control should show agglutination within 2 min.
- 4.7.2 The negative control should show NO agglutination within 2 min.
- 4.7.3 Otherwise repeat the test.

5. MATERIAL AND EQUIPMENT:

- 5.1 Bacterial antigens
- 5.2 Positive control
- 5.3 Negative control
- 5.4 Mechanical rotator adjustable to 80-100 r.p.m.
- 5.5 Heater at 37°C

6. RESPONSIBILITIES:

- 6.1 All technician assigned in Serology lab.
- 6.2 The final report must be signed by section supervisor and approved by lab pathologist.

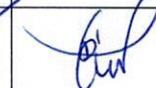
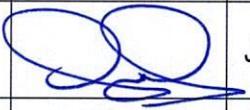
7. APPENDICES:

- 7.1 Kit insert

8. REFERENCES:

- 8.1 CRESCENT Febrile Antigens kit Pamphlet
- 8.2 Medical Laboratory Technology Methods & Interpretations 4th ed by Ramnik Saad

9. APPROVALS:

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