

SOP EXAMPLE

Canine Brucellosis Antibody Test Kit - D-TEC® CB

PURPOSE: The rapid slide agglutination test (RSAT) for the diagnosis of *Brucella canis*

REQUIRED SUPPLIES / EQUIPMENT:

Reagents A - Positive Control

Reagent B - Antigen

Reagent C - 2-Mercaptoethanol, (0.2M solution)

Reaction cards

Pipettes

Stir Sticks

PROCEDURE:

Procedure for Rapid Slide Agglutination Test (RSAT)

1. ENSURE REAGENTS AND SAMPLE ARE AT ROOM TEMPERATURE (70-78°F; 21-25°C) prior to testing.
2. Cut card on dotted line. Two circles are used to perform each test.
3. Place one drop of Reagent A, Positive Control (Red Cap), in one circle of the supplied card.
4. Use a disposable plastic pipette to place one drop of test serum in another circle. Each test kit contains disposable pipettes to prevent serum cross contamination. Do not dispose of pipette until the 2ME-RSAT is completed.
5. Thoroughly mix Reagent B, Antigen (White Cap), by vigorously shaking the vial. Add one drop of the Reagent B, Antigen, close to each serum drop, being careful not to touch the serum with the dropper tip.
6. Mix each antigen-serum combination with a separate end of a stir stick, spreading to fill the circular area. Do not allow the positive control test to touch the unknown serum sample.
7. Gently rock the card for 10-15 seconds. Place on a flat surface and observe for agglutination for no longer than two minutes. If the serum is negative (absence of agglutination), the animal is considered not to be infected with *B. canis*. If the card tests positive, perform the 2ME-RSAT.

Procedure For 2-Mercaptoethanol-Rapid Slide Agglutination Test (2ME-RSAT)

1. Add 2 drops of Reagent C, 2-Mercaptoethanol (Yellow Cap) to a tube containing 2 drops of the serum to be tested, and mix well.

2. Place 1 drops of this mixture on a new reaction circle.
3. Add 1 drop of Reagent B, Antigen as above to the serum solution and mix with stir stick as described above.
4. Gently rock the card for 10-15 seconds. Place on a flat surface and observe for agglutination for no longer than 2 minutes. When the RSAT-positive sample also tests positive by 2ME-RSAT, the animal is presumptively diagnosed as being infected with *B. canis*. Blood should be subjected to cultural examination for *B. canis*. When the RSAT-positive samples tests negative by 2ME-RSAT, the animal may be in the early stage of *B. canis* infection, or alternatively, its serum may contain non-specific agglutinins to *B. canis*. To distinguish between these two conditions, a second serum sample should be collected in approximately thirty days and retested by the 2ME-RSAT procedure. Only if this sample tests positive should the animal be presumptively diagnosed as having *B. canis* infection. Blood should then be subjected to cultural examination for *B. canis*.

Definitive diagnosis of canine brucellosis is based upon isolation of *B. canis* from the animal.

NEGATIVE TEST

No agglutination within two minutes

POSITIVE TEST

Agglutination within two minutes

REFERENCE(S):

Canine Brucellosis Antibody (package insert), Test Kit, D-TEC® CB, Synbiotics Corporation, pp 1-2

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