

# MegaRSAT® BRUCELLA

canis ad us. vet.

In vitro diagnosticum



Rapid Slide Agglutination Test-kit for the qualitative detection of antibodies against *Brucella canis* in serum of the dog

## INSTRUCTIONS FOR USE



Specialists in Veterinary Laboratory Supplies

Supplied by Vetlab Supplies Ltd



## 1. INFORMATION OF THE TEST-KIT

### TEST-KIT COMPONENTS

1 test-kit MegaRSAT® BRUCELLA canis contains:

- 2 glass slides with 6 antigen wells each, reusable
- 0.75 ml Positive Control (PC, cap with red dot)
- 0.75 ml Negative Control (NC, cap with green dot)
- 2.5 ml Antigen (white cap)
- 1.5 ml 2-Mercaptoethanol (cap with blue dot)
- 25 stirring rods
- 75 disposable plastic pipettes (20 µl)
- 25 reagent tubes
- 1 instructions for use

### STABILITY AND STORAGE



Store at  
2-8°C



Expiry date  
– see label

### APPLICATION



For veterinary use only



In vitro diagnosticum



Follow instructions for use precisely



Lot number



Do not use test-kit components from different kits, lot numbers or beyond stated expiry date.

### LIABILITY

The entire risk due to the performance of this product is assumed by the purchaser. The manufacturer shall not be liable for indirect, special or consequential damages of any kind resulting from the use of this product.

## 2. INTRODUCTION

*Brucella canis* is a gram-negative bacterium appearing worldwide and being a potential danger for dogs and humans (zoonosis).

Although the prevalences are very low or *Brucella canis* is partially seen rather obliterated in countries with high breeding standards, increased attention must be paid to brucellosis, especially in dog breedings. By mating with dogs from abroad (with lower breeding standards), brucellosis can be imported easily and unnoticed.

The pathogen mainly propagates via infectious abort material or vaginal fluids, by mating or vertically from bitch to puppies. Infected animals show failure in gravidity or infertility as well as atypical symptoms (e.g. uveitis). In about 75% of the cases, females abort after 45 to 55 days of gestation. Early embryonic death and absorption or abortion 10 to 20 days after mating is reported, too. These abortions may go unnoticed, and the female then is often presented with the preliminary report "failure to conceive".

In males, the main signs are epididymitis, testicular atrophy and a moist scrotal dermatitis, in addition to bad semen quantity (especially with chronic brucellosis) and quality.

Besides to missing or misunderstood symptoms, antibody levels in chronic animals can drop under the limit of detection. Hence, breeding dogs should be routinely tested for antibodies with serological methods to prevent the danger of propagation via venereal transmission.

Being fast, simple and reliable, MegaRSAT® BRUCELLA canis enables the veterinarian to have a complete on-site predication of the brucellosis status of the single animal or the complete breeding. Therapeutic and prevention measures can be applied immediately, adapted to dog and breeder needs.

## 3. INFORMATION ON THE SPECIMEN MATERIAL

20 µl (1 drop of attached plastic pipette) 15–25°C warm serum (S) are needed. Whole blood, native blood without anticoagulant and plasma cannot be used.

Homogenise the sample material well before use.

Non-cooled (15–25°C), S should be tested within 4 hours. At 2–8°C, S can be stored up to 4 days. Serum samples can be permanently stored at minimum –20°C.

Keep in mind that the sample material, as well as all used test-kit components, should have reached room temperature at the time of application. Do not leave reagents at room temperature for too long.

## 4. SPECIMEN PREPARATION

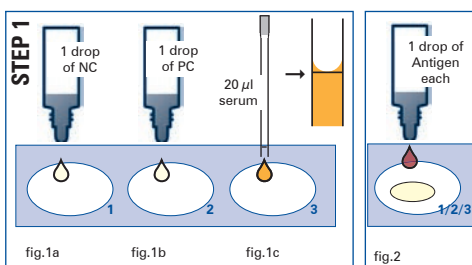
No specimen preparation necessary.

### 5a. TEST PROCEDURE STEP 1

- Remove the glass slide from the test-kit shortly before use. Place it on a flat surface.
- Put 1 drop (20 µl) of Negative Control (NC) onto the first antigen well of the glass slide (fig.1a) and 1 drop (20 µl) of Positive Control (PC) onto the second antigen well of the glass slide (fig.1b). Hold dropper bottles vertically.
- Draw sample up to the mark (≈ 20 µl sample volume) using the disposable plastic pipette. The meniscus must be above the black line (fig.1c). Place the whole sample volume (20 µl) onto the third antigen well of the glass slide (fig.1c, hold pipette vertically).
- Mix Antigen thoroughly and put 1 drop (20 µl) each onto every used antigen well of the glass slide (fig.2). Hold dropper bottle vertically. Make sure that the dropper does not come into contact with controls and sera.
- Mix the antigen-serum mixture (ASM) on each well with a stirring rod. Use the whole area of the antigen well. Wipe off the stirring rod between each ASM thoroughly (dry swab or alcohol swab). Make sure that NC, PC and serum do not come in contact with each other.
- Rotate the glass slide slightly for 10–15 seconds. Place it on a flat surface.

## 7. PRECAUTIONS FOR USERS

- The guidelines for working in medical laboratories must be observed. It is recommended to wear disposable gloves and other personal protective equipment (protective clothing, possibly a face mask). Wash and disinfect hands after completing the test.
- Label sample material and associated glass slide to ensure a precise assignment.
- Use a new pipette, a new stirring rod and a new glass slide for each sample.
- The glass slides can be disinfected and washed after use and used for the next test.
- The reagents contain low concentrations of toxic sodium azide as a preservative, therefore avoid skin/eye contact and/or ingestion.
- The sample material must be seen as potentially infectious and disposed of accordingly, together with the used test-kit components.
- Brucella canis* is infectious to humans. Therefore, extreme caution is required when examining such sera.

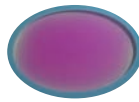


### 5b. READING OF THE TEST RESULT STEP 1

Observe the ASM for a maximum of two minutes, shaking it slightly when necessary, to see whether an agglutination becomes visible.

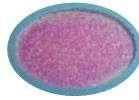
#### NEGATIVE TEST RESULT (fig.3)

No agglutination visible.



#### POSITIVE TEST RESULT (fig.4)

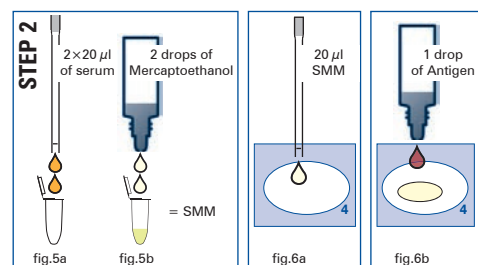
Agglutination visible. Continue with 5c.



### 5c. TEST PROCEDURE STEP 2 (Mercapto)

#### Verification of positive results from step 1

- Put 2 drops (2×20 µl) of serum with the a new pipette (also see fig.1c) into a reagent tube (fig.5a).
- Add 2 drops (2×20 µl) of 2-Mercaptoethanol to the 2 drops of serum (fig.5b). Mix well (snapping or vortexing the reagent tube).
- Put 1 drop (20 µl) of this serum-mercaptoethanol-mixture (SMM) with a new pipette (also see fig.1c) onto the fourth antigen well of the glass slide (fig.6a).



4. Mix Antigen thoroughly and put 1 drop (20 µl) onto the SMM (fig.6b). Make sure **not** to touch the SMM with the dropper.

5. Mix the antigen-SMM mixture (ASMM) on the antigen well with the again wiped stirring rod. Use the whole area of the antigen well. Make sure that the sample does not get in contact with the samples in the other antigen wells.

6. Rotate the glass slide slightly for 10–15 seconds. Place it on a flat surface.

### 5d. READING OF THE TEST RESULT STEP 2

Observe the ASMM for a maximum of two minutes, shaking it slightly when necessary, to see whether an agglutination becomes visible.

#### NEGATIVE TEST RESULT (see fig.3)

No agglutination visible.

#### POSITIVE TEST RESULT (see fig.4)

Agglutination visible.

## 9. INFORMATION FOR THE INTERPRETATION

The interpretation of the test result should always be based on anamnestic and clinical data as well as the therapy and prophylaxis possibilities.

### Negative test result

- Dog had no contact with *Brucella canis*.
- In chronic infected animals, antibody titres cannot always be detected by single testing. Therefore, breeding dogs should be serologically tested routinely (multiple testing) to minimize the danger of venereal propagation of the pathogen.

### Positive test result after 5b, but not after 5d

- Early brucellosis infection stage (< 2–4 weeks post infection!). Dog has not yet produced IgG antibodies in a detectable concentration.
- Serum contains non-specific agglutinins against *B. canis* (non-infected).

◆ To differentiate between these two possibilities, after 28 days a second serum sample should be obtained and the test with 2-mercaptoethanol (5c) should be repeated. If this test is positive, the dog is infected with *B. canis* with high probability.

### Positive test result after 5d

- Dog is infected with *Brucella canis* with high probability.
- To find out if the antibody reaction is based on an acute or chronic brucellosis, two serum samples should be taken 2–4 weeks apart for testing with IFAT (e.g. MegaFLUO® BRUCELLA canis) and/or Agar Gel Immunodiffusion (AGID) etc. A substantial titre increase in the IFAT or AGID is indicative for an ongoing Brucellosis.