Brucella Rose Bengal Test

An in vitro diagnostic test kit for detection of antibodies against Brucella abortus and Brucella melitensis in serum of cattle, sheep and goats

INTRODUCTION:

The Brucella Rose Bengal Test is a rapid slide agglutination test kit for the qualitative and semi quantitative determination of Brucellosis through detection of antibodies against all smooth Brucella in human and animal serum. The reagent is a Brucella bacterial antigen that when bound to Brucella antibodies becomes visible to the naked eye, in serum samples of cattle, sheep, goats, pigs, camels, buffalo, reindeer, yaks, elk, South American camelids and humans as well, but one must consider that the Brucella Rose Bengal Test has only been validated on cattle, sheep and goat serum samples and the test is calibrated on the OIEISS International *Brucella* standard serum and the national secondary serum to be positive against a 1/45 dilution and negative against a 1/55 dilution, according to the requirements of the EU directive 64/432/EEC. The RBT is very sensitive and recommended by the EFSA as one of the standard tests in the EU legislation on intra-Community trade for bovines, sheep and goats. However cross-reactions between Brucella antigens and other organisms may occur. These include Yersinia enterocolitica, Escherichia coli and Francisella tularensis, it could sometimes give a positive result because of previous Brucella (abortus S19 or melitensisRevl) vaccination.

TEST PRINCIPLE:

The Rose Bengal is a Brucella bacterial antigen made of a stained inactivated and buffered suspension of Brucella abortus (either 1119-3 or S-99 strains). The test depends on the ability of the Rose Bengal antigen suspension to bind indiscriminately to antibodies against all smooth Brucella abortus and Brucella melitensis strains, mainly IgM and IgG antibodies, which appear in serum predominantly in the initial and late stages of Brucella infection respectively.

Such binding results in the agglutination of the stained bacterial antigen. When this occurs, the aggregates become clearly visible to the naked eye. The presence or absence of a visible agglutination indicates the presence or absence of antibodies in the test sample.

KIT COMPONENTS:

Component 1:
Rose Bengal Antigen
One vial of 10 ml (for 10 ml kit) or 50 ml bottle (for 50 ml Kit) or 100 ml bottle (for 100 ml kit) Brucella abortus antigen stained with Rose Bengal, Lactate-Buffered (pH 3.65 ±.05) in Phenol(0.5%)
Saline (0.85%).
Component 2:
Positive control
One vial contains 1.0 ml Brucella abortus Positive Control serum. Component 3:
Negative control
One vial contains 1.0 ml Brucella abortus Negative Control serum.
Additional kit components
(10 cards -20 sticks -1 dropper)

Notes:

Store kit at 5±3°C until expiry date. See kit label for expiry date. Exposure of the test components to excessive temperatures and direct sunlight should be avoided.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

SAMPLE COLLECTION

Use fresh serum obtained by centrifugation of clotted blood. The sample may be stored at 5±3°C for 48 hours before performing the test. If longer storage is required, store at -20°C for up to 6 weeks. Thawed samples must be mixed prior to testing.

Do not use haemolysed, contaminated or lipaemic serum for testing, as this will adversely affect the assay.

REAGENT PREPARATION

All reagents should be brought to room temperature (22±3°C) and mixed prior to use. Do not induce foaming.

LIMITATIONS OF USE

The use of samples other than serum has not been validated in this test. Cross-reactions between Brucella antigens and other organisms have been reported. These include Yersinia enterocolitica 0:9, Escherichia coli, Francisella tularensis. Group N Salmonella (0:30), Escherichia coli (0:157 and 0:116), Pseudomonas maltophilia, and Vibrio cholerae.

A prozone may occasionally occur due to very high antibody titers. If this is suspected, dilute the serum 1/20 in saline and retest. Always include a known positive and negative serum in the test panel as part of the normal laboratory quality control procedure.

Both Brucella abortus and Brucella melitensis share common Brucella antigens. A sample giving a positive result with the Rose Bengal reagent should be confirmed by either complement fixation test and/or Indirect ELISA test.

TEST PROCEDURE:

Precautions

National guidelines for working with animal samples must be strictly followed. The Brucella Rose Bengal Test must be performed in laboratories suited for this purpose.

Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated. Chemical hazard data are available in section "Safety Regulations and R8iS Statements" (Appendix II).

Notes

To achieve optimal results with the Brucella Rose Bengal Test, the following aspects must be considered:

- Use a separate disposable tip for each sample to prevent cross contami- nation.
- Replace caps on all reagents immedi- ately after use. Prior to the start of the assay, bring all reagents to room temperature (22±3°C). mix all reagents by vortexing or repeated inversion or swirling.
- For use by operatives with at least a minimum of basic laboratory training.
- Do not use damaged or contaminated kit components.
- Examine the test slide macroscopically under a strong light source after 4 minutes. Kit controls or known level value samples should be tested with each test run. The kit negative control should give a negative result after
  4 minutes. The kit positive control should give a positive result at a titer of 1/4 +/- one double dilution after 4 minutes. If levels of controls or users known samples do not give expected results, test results must be considered invalid. '

A- QUALITATIVE METHOD:

1- Allow kit reagents and serum to equili- brate to room temperature (22±3°C).

2- Mix all reagents by vortexing or repeat- ed inversion or swirling.

3- Transfer one drop (30 pi) of serum to the test circle on the slide. Use a separate disposable tip for each sample to prevent cross contamination.

4- Shake the Rose Bengal antigen suspen- sion, then add one drop of suspension to the test circle, using the dropper provided. Do not allow the dropper to contact any of the tested samples.

5- To minimize any delay between adding of the Rose Bengal antigen suspension
to the first and the last serum samples, it is recommended that no more than two slides or cards should be set up by the operator at a time. Where each slide can take 6 samples, a maximum of 12 samples are tested at one time or else use a ceramic tile.

6- Immediately after the addition of the last drop of Rose Bengal antigen to the slide, mix thoroughly the drops in each circle, using the sticks provided in the kit ensuring coverage of the test circle with the mixture. Use a separate disposable stick for each sample to prevent cross contamination.

7- Gently and evenly rotate the test slide for 4 minutes by hand or on a rotator at a rate of 30 rotations per minute, whilst examining the test slide for agglutination.

8- Read immediately for any agglu- tination, when reading the slide it is recommended to tilt the slide back and forth over a fluorescent light source of even intensity

B- SEMI-QUANTITATIVE METHOD:

1- Prepare serial dilutions (1/2, 1/4, 1/8, 1/16, 1/32, 1/64 etc.) using isotonic sa- line, phenol saline or the Negative Control included in the kit.

2- Transfer one drop (30 pi) of each se- rum dilution to the test circle on the slide.

3- Shake the Rose Bengal antigen suspen- sion, then add one drop of suspension to the test circle, using the dropper provided. Do not allow the dropper to contact any of the tested samples.

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4- To minimize any delay between adding of the Rose Bengal antigen suspension to the first and the last serum samples, it is recommended that no more than two slides or cards should be set up by the operator at a time. Where each slide can take 6 samples, a maximum of 12 samples is tested at one time or else use a ceramictile.

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6- Gently and evenly rotate the test slide for 4 minutes by hand or on a rotator at a rate of 30 rotations per minute, whilst examining the test slide for agglutination.

7- Read immediately for any agglutination, when reading the slide it is recommended to tilt the slide back and forth over a fluorescent light source of even intensity.

RESULTS & INTERPRETATION

After 4 minutes the Negative Control from the kit should give a negative result and the Positive Control should give a positive result.

Any visible reaction in the test serum is considered to be positive unless there has been excessive drying around the edges. Positive and negative working standards should be included in each series of tests.

For method A:
A positive result seen as agglutination of the antigen with undiluted sample indicates the presence of antibodies at a concentration of greater than or equal to 22.2 IU/ml.

For method B:
The serum antibodies level can be approx- imately calculated by multiplying the highest dilution factor (i.e. 2, 4, 8 or 16) that tests positive by the detection limit mentioned above (22.2 IU/ml), to give a titer in the IU/ml.

e.g. if the sample remains positive up to a dilution of 1/8, the serum Burcella antibody Rose Bengal titer is 8 x 22.2 = approximately 178 IU/ml.

False negative reactions occur rarely, mostly due to prozoning (prozoning = secondary phenomenon where the con- centration of antigen or antibody, or both, are outside the zone of equivalence).

APPENDIX I

Notice
This manual is believed to be complete and accurate at the time of publication. In no event shall Arcomex be liable for incidental or consequential damage in connection with or arising from the use of this manual.

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APPENDIX II

Safety Regulations and R8iS Statements National Safety Regulations must be strictly followed.
R8iS Statements.

Component 1:
Rose Bengal Antigen
The classification is according to the lat- est editions of the EU- lists, and extended by company and literature data.

Component 2:
Positive Control
The classification is according to the lat- est editions of the EU- lists, and extended by company and literature data.

Component 3:
Negative Control
The classification is according to the lat- est editions of the EU- lists, and extended by company and literature data.

APPENDIX III

References

1, OIE Terrestrial Manual 2016, Chapter 2.1.4 Brucellosis(Brucella Abortus, B.melitensis and B.suis) Infection with B.Abortus, B. melitensis and B. suis.

2, Brucellosis in humans and animals World Health Organization in collab- oration with the Food and Agriculture Organization of the United Nations and World Organisation for Animal Health © World Health Organization 2006.

3, Scientific Opinion on "Performance of Brucellosis Diagnostic Methods for Bo- vines, Sheep and Goats The EFSA Journal (2006) 432; 1 - 44.

4, Epidemiosurveillance of brucellosis. Rev. sci. tech. Off. int. Epiz., 2013, 32 (1), 199-205.

Package insert

Kit of 10 ml Rose Bengal Antigen Kit of
50 ml of Rose Bengal Antigen Kit of 100
ml of Rose Bengal Antigen

For in vitro veterinary diagnostic laboratory use only. Store at 5±3°C

CONTACTS

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