Brucellosis
Rose Bengal Test

Standard Operating Procedure

SAFETY PRECAUTIONS

The laboratory shall take all precautions in order to guarantee the necessary safety, for both the operator and the environment, against the biological and chemical hazards due to the activities conducted according to this document.

1 Scope

The present document describes a technique aiming at detecting antibodies specific of smooth *Brucella* species (especially *B. abortus, B. melitensis* and *B. suis*) by the Rose Bengal test in animal sera (ruminants, equidae, suidae, camelidae and carnivores, both wild and domestic, in particular).

2 Normative references

a) Bovine brucellosis, *In*: The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees), 6th Edition 2008, Chapter 2.4.3, OIE, Paris, 624-659. http://www.oie.int/eng/normes/mmanual/2008/pdf/2.04.03_BOVINE_BRUCELL.pdf

b) Commission Decision of 10 December 2008 (2008/984/EC) amending Annex C to Council Directive 64/432/EEC and Decision 2004/226/EC as regards diagnostic tests for bovine brucellosis *(notified under document number C(2008) 7642)*. Official Journal of the European Union, L 352/38-L 352/45.

c) ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories.

d) *OIE Quality Standard and Guidelines for Veterinary Laboratories, 2nd Edition, 2008,* OIE, Paris.

e) Norme Française (French Standard) NF U 47-003 - Méthodes d'analyse en santé animale, Recherche d'anticorps contre la brucellose par la technique de l'épreuve à l'antigène tamponné, avril 2009, AFNOR, France.

3 Definitions

- **Series of tests**

Implementation of all of the analytical phases of a technique carried out continuously or intermittently, separated by short interruptions, by the same operator(s), in the same location, with the same equipment and the same reagents.

4 Principle and reaction

4.1 Principle

The Rose Bengal Test is one of the buffered *Brucella* antigen tests. It is a rapid agglutination test.
The reaction mixture consists of 50 % serum and 50 % antigen (0.5 % phenol-saline suspension of *Brucella abortus* biovar 1, strain 99, inactivated, stained with Rose Bengal and buffered to pH = 3.65 ± 0.05).

4.2 Reaction

4.2.1 Method

The method used is the rapid plate agglutination test.

4.2.2 Antigen (Ag)

The supplier must certify that the activity of the antigenic preparation has been standardised against the International primary (OIEISS) or National secondary standard, with the technique established in this document and according to OIE requirements.

Antigen is used at a volume of 25-30 µl.

4.2.3 Tested sera

Sera are tested neat, at a volume of 25-30 µl.

4.2.4 Control sera
Negative and positive control sera are to be used neat at a volume of 25-30 µl.

4.3 Controls
4.3.1 Control Sera
Positive and negative control sera are both included in each series of tests.

5 Diluents, culture media, reagents and other products

5.1 Diluents
Not applicable.

5.2 Culture media
Not applicable.

5.3 Reagents
5.3.1 Antigen
The antigen is available commercially, for veterinary use, to be stored according to the supplier's instructions. This antigen should never be stored at a temperature ≤ 1°C.

5.3.2 Control sera
5.3.2.1 Positive control sera (commercial or lab. prepared).
It is advisable to use a weak positive serum as control. If this is not easily available, available strong positive serum should be diluted in negative serum in order to obtain a weak reaction.
5.3.2.2 Negative control sera (**commercial or lab. prepared**).

5.4 Other products
5.4.1 Water
Not applicable.

6 Equipment and plastic/glass ware
Conventional serology laboratory equipment and in particular:

6.1 Temperature controlled refrigerator at 5°C ± 3°C.

6.2 Distribution and dilution device with a suitable volume range and accuracy.

6.3 White tile, enamel or plastic plate, or in a WHO haemagglutination plate.

6.4 Appropriate plate shaker, if possible with a 3-D or rocking movement.

6.5 Mixing device (plastic or glass rod or combs).

6.6 Timer or chronometer.

7 Sampling
Not applicable.

8 Preparation of the sample for analysis
The preparation of the serum sample shall comply with the requirements of the OIE Manual.

9 Operating procedure

9.1 Test
9.1.1 Preparation of the antigen
- Bring the antigen (5.3.1) to room temperature before use (*ca*. 30 minutes);
- Shake the antigen bottle well, but gently in order to obtain a homogeneous suspension.

It is advisable to re-heat only enough antigen for the tests that are to be performed and to maintain the rest in the standard storage conditions, since the antigen should not be exposed for long periods of time to room temperature and is sensitive to repetitive temperature changes.

9.1.2 Test and control sera

- If necessary, thaw test and control sera (5.3.2)
- Bring them to room temperature before use (*ca*. 30 minutes).

NOTE Test sera must be satisfactory in quality. Haemolysed or unclear sera might produce results that are difficult to interpret.

9.1.3

- Place the same volume (25-30 µl) of neat serum (if possible non-inactivated) and of antigen side by side on a plate;
- Mix thoroughly and rapidly the serum and the antigen;
- Shake lightly the plate for 4 minutes.
    - If a rocking shaker is used, the reaction mixtures are made following an oval shape which axes are tilted according to the plate axes.
    - If a 3-D shaker is used, the reaction mixtures are made following a round shape.

NOTE Orbital shakers must not be used (otherwise the antigen is centrifuged to edges of the reaction mixture).

The size of the reaction mixture should be such that its thickness be thin enough to enable an easy reading of the reaction and wide enough to limit evaporation during the reaction time. It is advisable that the size of the reaction mixture fit in a 15-20 mm-side square (see Figure 1).

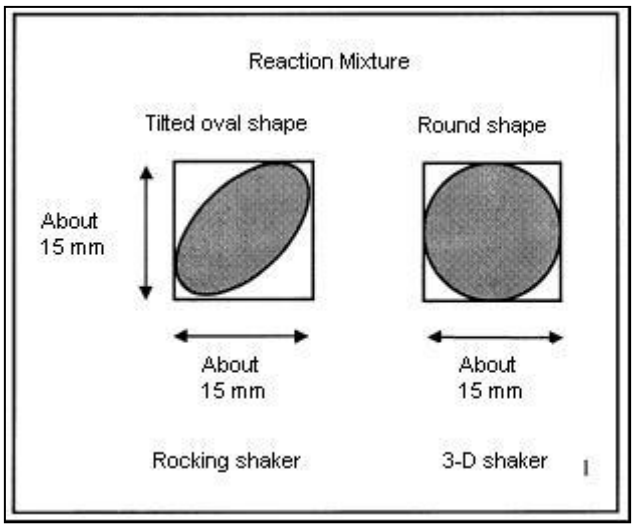

Figure 1

9.2 Reading

Reading is made immediately, with good lightning and with the naked eye.
Agglutinates revealed after 4 minutes ± 10 % should not be taken into consideration.

9.3 Interpretation

9.3.1 Expression of results

The results are expressed as follows:

- No agglutination: negative
- Any visible agglutination: positive
- Flocculates (false agglutination): un-interpretable or unreadable

NOTE: Flocculates (easily distinguished from true agglutinates by their aspect) are not rare when testing dog, pig or wild animal sera.

9.3.2 Interpretation

Not applicable

10 Storage and disposal of samples

Each laboratory should set up the provisions for the correct storage of samples until their disposal.

Sera prepared from the whole blood samples received at the lab. must be stored at 5°C ± 3°C. It is advisable for all blood samples to be centrifuged and stored whenever possible without the clot. For long-lasting storage, it is advisable to freeze sera without clot at ≤ -16°C.

Decontamination and disposal of samples must be performed in accordance to in-force regulations.

11 Restitution of results

For the laboratory's clients, the restitution of the results is made qualitatively: negative, positive or un-interpretable (or unreadable).

12 Precision

The use of a positive reference material (secondary or working standard) that gives a weak positive reaction during each series of tests enables to check the sensitivity and reproducibility of tests conditions. The expected reaction of this reference test material must be effectively obtained.

13 Analysis report

The analysis report must comply with the requirements of ISO/IEC 17025.