**PRINCIPLE**

The Rose Bengal Test or buffered *Brucella* antigen test (BBA) is a rapid slide agglutination procedure developed for the direct detection of *Brucella* antibodies in human and animal sera 1-3. The bacterial suspension is reactive with both immunoglobulin G and immunoglobulin M antibodies being the former detected earlier (sub-clinical infections) and over a large period during the disease (chronic stage) than the conventional tube agglutination test. The assay is performed by testing the buffered suspension (pH 3.6) of *B. abortus* strain coloured with Rose Bengal against unknown sera. The presence or absence of a visible agglutination, indicates the presence or absence of antibodies in the samples tested.

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>R</th>
<th>Rose Bengal Antigen. Bacterial suspension of <em>B. abortus</em> stained with Rose Bengal and buffered at pH 3.6. Contains 0.95 g/L of sodium azide.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL+</td>
<td><em>Brucella</em>. Anti-Brucella animal serum with an agglutinating activity of appr. 100 IU/mL. Contains 0.95 g/L of sodium azide</td>
</tr>
<tr>
<td>CONTROL-</td>
<td>Animal serum with an agglutinating activity &lt;10 IU/mL. Contains 0.95 g/L of sodium azide.</td>
</tr>
</tbody>
</table>

*Warning:* The reagents in this kit contain sodium azide. Do not allow to contact with skin or mucous membranes.

**PACKAGING CONTENTS**

<table>
<thead>
<tr>
<th>REF</th>
<th>kit 100 tests.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2210005</td>
<td>1 vial Rose Bengal Antigen, 1x1 mL Brucella Positive control, 1x1 mL Negative control, 3 Test cards and 2x50 disposable stirrers.</td>
</tr>
<tr>
<td>2210010</td>
<td>1 vial Rose Bengal Antigen</td>
</tr>
</tbody>
</table>

**STORAGE AND STABILITY**

Store at 2-8°C. Do not freeze. Frozen reagents could change the functionality of the test. Antigen and Controls are stable until the expiry date stated on the label.

**REAGENT PREPARATION**

Antigen and Controls are ready to use.

**SAMPLES**

Fresh, clear serum. After the clear serum has been separated it may be stored at 2-8°C up to one week or for longer periods at −20°C.

**QUALITY CONTROL**

Positive and negative controls should be run daily following the steps outlined in the procedure, in order to check the optimal reactivity of the antigen.

The positive control should produce clear agglutination. If the expected result is not obtained, do not use the kit.

**EXPECTED VALUES**

As an orientation point, 25 IU/mL is accepted internationally as the upper limit of the normal range. As reference ranges are subject to many influencing variables which may differ for each population investigated, each laboratory should establish its own reference range.
CLINICAL SIGNIFICANCE

In the serological diagnosis of brucellosis in humans, the Rose Bengal Test appears to have its main value in epidemiological surveys to delineate potential risk of infection in various population groups. In the veterinary field, however, it is used as a diagnostic test in cattle and swine populations where incidence of brucellosis is relatively high and, as a screen procedure in low incidence areas. Anti-Brucella antibodies testing has a high diagnostic value on a tentative diagnosis made on the basis of case history and clinical findings.

ANALYTICAL PERFORMANCE

- The minimum detectable unit (analytical sensitivity) is of approximately 25 IU/mL, tested against a 2º International Preparation of anti-Brucella abortus serum (NIBS/WHO).
- Diagnostic specificity: 100%.
- Prozone effect: No prozone effect was detected up to 1000 IU/mL.
- Results obtained with this reagent did not show significant differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Hemoglobin (<10 g/L), lipemia (<10 g/L) and rheumatoid factors (<300 IU/mL) do not interfere. Bilirubin (>2.5 mg/dL) interferes. Other substances may interfere.

LIMITATIONS OF PROCEDURE

- In areas where there has been much strain 19 vaccination, the Rose Bengal Test yields a high proportion of false positive reactors, positive sera being subjected to confirmatory tests.
- Biological false negative reactions can occur in early primary infections and in late stages of the disease. In cattle during the incubation period, the results of serological tests may well be negative although such animals may abort afterwards.

NOTES

1. The sensitivity of the test may be reduced at low temperatures. The best results are achieved at 15-25ºC.
2. Delays in reading the results may result in over-estimation of the antibody present.
3. Tube agglutination is still the method of reference for serological diagnosis of human bovine brucellosis, even in animals vaccinated with strain 19 vaccine, provided they are vaccinated as calves. Except for swine brucellosis, in which Rose Bengal Test has proven the most satisfactory diagnostic procedure, the complement fixation test is considered the most specific serological method for the diagnosis of brucellosis in cattle, sheep and goats, being used as a supplemental test on samples that give a suspicious titre on agglutination (1:20≤30 IU/mL), or as a confirmatory test on sera that are positive to the Rose Bengal Test.

SOURCES OF ERROR

- Bacterial contamination of controls and specimens as well as freezing and thawing of the antigen may lead to false positive results.
- Traces of detergent in the test cards may give false positive results. Wash used cards first under tap water until all reactants are removed and then with distilled water. Allow to dry in air, avoiding the use of organic solvents as they may impair the special finish on the slide.
- The Rose Bengal Antigen must not be used beyond its expiry date because a prolonged storage can affect the sensitivity of the suspension.

REFERENCES


QUALITY SYSTEM CERTIFIED

ISO 9001  ISO 13485

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