Cromatest

LINEAR

Rose Bengal CE

CONTENTS			
REF	2210005	Rose Bengal	100 Tests
	2210010	Rose Bengal	100 Tests
For *in vitro* diagnostic use only			

Rose Bengal

Determination of anti-Brucella antibodies

SLIDE TEST

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

R — **Rose Bengal Antigen**. Bacterial suspension of *B. abortus* stained with Rose Bengal and buffered at pH 3.6. Contains 0.95 g/L of sodium azide.
T+, Xi, C R: 24/25-34-35

CONTROL + — **Brucella.** Anti-*Brucella* animal serum with an agglutinating activity of appr. 100 IU/mL. Contains 0.95 g/L of sodium azide.

CONTROL - — Animal serum with an agglutinating activity <10 IU/mL. Contains 0.95 g/L of sodium azide.

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

PACKAGING CONTENTS

REF 2210005, kit 100 tests.
1 vial Rose Bengal Antigen, 1x1 mL Brucella Positive control, 1x1 mL Negative control, 3 Test cards and 2x50 disposable stirrers.

REF 2210010, kit 100 tests.
1 vial Rose Bengal Antigen.

STORAGE AND STABILITY

Store at 2-8ºC. 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 2-8ºC up to one week or for longer periods at –20ºC.

MATERIAL REQUIRED

- Automatic pipettes.
- Mechanical rotator, adjustable at 100 r.p.m.
- Laboratory alarm clock.

PROCEDURE

1. Bring the test reagents and samples to room temperature (Note 1).
2. Resuspend the antigen vial gently. Aspirate dropper several times to obtain a thorough mixing.
3. Place 1 drop (50 µL) of the serum under test into one of the circles on the card. Dispense 1 drop of positive control serum and 1 drop of negative control serum into two additional circles.
4. Add 1 drop of Rose Bengal Antigen to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide slowly either by hand or by means of a mechanical rotator (100 r.p.m.) for a period of **4 minutes** (Note 2).
7. Observe immediately under a suitable light source for any degree of agglutination.

Reading

Nonreactive: Smooth suspension with no visible agglutination, as shown by negative control (Note 3).

Reactive: Any degree of agglutination visible macroscopically. All reactive specimens should be further tested to clarify the situation (Note 3).

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.

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CLINICAL SIGNIFICANCE[4,5]

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)[6].
- Diagnostic specificity: 100%.
- Prozone effect: No prozone effect was detected up to 1000 IU/mL.
- Results obtained with this reagent did not show significative 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[7].

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

1. Rose, J.E. and Roepke, M.H. Am. J. Vet. res. 18: 550 (1957).
2. Nicoletti, P. J. Am. Vet. Med. Assoc. 151: 1778 (1967).
3. Caces, E., De Lauture, H., Vol, S., Tichel, J. and Boulard, P. Comp. Immun. Microbiol. Infect. Dis. 1: 107 (1978).
4. Rusell, A.O., Patton, C.M. and Kaufmann, A.F. J. Clin. Micr. 7: 454 (1978).
5. Morgan, W.J.B., Mackinnon, D., Lawson, G.R. and Cullen, G.A. Vet. Rec. 85: 636 (1969).
6. Bull. Wld. Hlth. Org. 361: 20 (1967).
7. Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4th Edition. AACC Press (1995).

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