



IDvet Rose Bengal Antigen

Technical and validation data

Introduction

Brucellosis is a disease caused by *Brucella*, a genus of Gram-negative bacteria which are small non-motile, non-encapsulated coccobacilli functioning as facultative intracellular parasites. There are several different species of *Brucella*, each with slightly different host specificity, including *B. melitensis* (goats and sheep), *B. abortus* (cattle and bison), *B. suis* (pigs), and *B. ovis* (sheep).

Brucellosis infection of cattle causes abortion or premature calving of recently infected animals between the fifth and eighth month of pregnancy. Infected cows frequently suffer from retained afterbirth and are difficult to get rebred and sometimes become sterile. Brucellosis is spread from the vaginal discharge of an infected cow or from an aborted fetus. Abortions, retained placenta, weak calves and infertility frequently occur. Milk produced from an infected cow may also harbour the organism. The infected milk creates a public health hazard, as this is the organism that causes undulant fever in humans.

Serological testing is used as part of disease eradication programs. The Rose Bengal rapid slide agglutination method is a rapid, cost-effective technique for the detection of anti-*Brucella abortus*, *melitensis*, or *suis* antibodies in bovine, ovine, caprine, and porcine serum.

It is an easy-to-implement method capable of screening large numbers of sera.

Test procedure

- ♦ Mix 20 to 30 µl of Rose Bengal antigen and 30 µl of serum on a slide.
- ♦ After 4 minutes under slight agitation, the presence of specific antibodies is demonstrated by the formation of agglutinates that are visible to the naked eye. In the absence of specific antibodies, the mixture remains homogeneous.

Test specifications

| | |
|-------------------------------|---|
| Product code: | RSA-RB |
| Description: | <p>Suspension of <i>Brucella abortus</i> biovar 1 Weybridge strain No 99, inactivated by heat and phenol and coloured with Rose Bengal stain in an acidified buffer.</p> <p>The test makes use of the principle of slide agglutination in a buffered acidified medium (pH 3.6) which reduces the appearance of non-specific agglutination. The Rose Bengal stain facilitates the reading of agglutinates.</p> <p>Calibrated on the OIEISS International <i>Brucella</i> standard serum and the national secondary serum to be positive against a 1:45 dilution and negative against a 1/55 dilution, as per the European directive CEE 64/432 and OIE requirements.</p> |
| Species / sample type: | Detects anti- <i>Brucella abortus</i> , <i>melitensis</i> , or <i>suis</i> antibodies in bovine, ovine, caprine, and porcine serum. |
| Kit format: | 10 ml, allowing for the analysis of 330 sera (30 µl per sample). |

Analytical sensitivity

The IDvet RB antigen 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, as per the European directive CEE 64/432 and OIE requirements.

Specificity

Given the existence of cross-reactions between *Yersinia enterocolitica* and *Brucella abortus*, positive serological reactions may be observed with the Rose Bengal antigen, even in disease-free herds.

1200 sera from disease-free herds from the Brittany, Aveyron and Hérault regions in France were tested by the IDvet antigen, and another commercial antigen.

Results:

- ♦ Out of the 1200 sera tested, only 10 sera gave non-specific agglutinations with the IDvet antigen.
- ♦ The same 1200 sera tested with the competitor's antigen resulted in 12 non-specific agglutinations.
- ♦ 8 sera gave non-specific reactions with both antigens.
- ♦ The specificity of the IDvet Rose Bengal can be considered equivalent to that of the other commercial antigen tested in this study.

Sensitivity

Analysis of 20 sera from infected animals, including:

- 8 sera from the CERTIBIO serum panel from the French reference laboratory ANSES (samples SR3, and SR6 – 12)
- 12 sera from animals from infected regions in Southern Italy. These sera gave positive results with other commercial Rose Bengal antigens, and *Brucella abortus* was isolated from the animals.

Results:

- ♦ All 20 sera tested gave positive results with the IDvet RB antigen.

Repeatability and Reproducibility

Repeatability was evaluated through the analysis of a weak positive serum tested several times by different operators, during different test runs, and on different days.

Results:

- ♦ The serum was found positive each time it was tested.

Robustness

The antigen was tested by different laboratories and operators, and results obtained were comparable to those obtained by another commercial antigen.

Conclusion

The IDvet Rose Bengal rapid slide agglutination antigen is a rapid and reliable method for the detection of anti-*Brucella abortus*, *melitensis*, or *suis* antibodies in bovine, ovine, caprine, and porcine serum.

References

- ♦ Alton G., Jones L., Angus R., Verger J. TECHNIQUES FOR THE BRUCELLOSIS LABORATORY. INRA. Paris 1988.
- ♦ Règlement (CE) n°535/2002 of the commission of March 21st, 2002, modifying Annex C of the Directive 64/432/CEE and decision 2000/330/CE.
- ♦ OIE Terrestrial Manual 2009; 2.4.3; Bovine brucellosis.