Determination of antibodies associated to Brucella Melitensis by means of coloured bacterial suspension on slide

TEST SUMMARY
Slide and tube agglutination test for the qualitative and semi-quantitative detection of antibodies associated to Brucella Melitensis infections.

SAMPLES
Fresh clear serum. Stability 7 days at 2-8°C or 3 months at –20°C.

Do not freeze repeatedly.
The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolized or lipemic samples.

BRUCELLA MELITENSIS SLIDE
To titre

Titre

Sample
NaCl 9 g/L

1. Prepare a row of tube test for each sample as follows:

2. Prepare 1 tube for Positive Control and 1 tube for Negative Control with 0.1 ml of control + 0.9 ml of NaCl 9 g/L each.

3. Add 50 µl (1 gtt) of suspension to each tube.

4. Mix thoroughly and incubate tube test at 37°C for 24 h.

RESULTS INTERPRETATION
SLIDE AGGLUTINATION
Examine macroscopically the absence or presence of agglutination after 1 minute by comparing the results with the Positive and Negative control.

Agglutination into time established means positivity.

Homogeneous suspension with no visible agglutination means negativity.

For each positive result it is advisable to confirm the titre with the test-tube titration.

The results obtained with slide titration method are roughly equivalent to those which would occur in tube test with serum dilutions. Respectively: 1/20 – 1/40 – 1/160 – 1/320 – 1/640.

TUBE AGGLUTINATION
Examine macroscopically the absence or presence of agglutination by comparing the results with the tubes of Positive and Negative control.

Partial agglutination is a sign of positive reaction.

The level of the serum examined is due to the most higher dilution in which is showed a feeble positivity.

REFERENCE VALUES
Titre ≥ 1/80 indicate a recent infection.

In case of a positive result with a low titre, it is significant for the diagnosis verify the increase of titre between samples taken at a distance of days.

If the titre remains unchanged it may be a previous contact or previous vaccination.

A single positive result has less significance than the demonstration of a rising or falling antibodies titre as evidence of infection.

The level of “normal” agglutinins to these organisms varies in different countries and different communities. It is recommended that each laboratory establish its own reference range.

NOTE
In some geographical areas with a high prevalence of febrile antibodies, it is recommended to dilute the sample 1/4 with NaCl 9 g/L before to perform the assay.

As with any diagnostic procedure, if the results are incompatible with the clinical presentation, the physician should evaluate the data obtained using this test by comparing them with other clinical information.

In vitro diagnostic use only.

CALIBRATION/QUALITY CONTROL
There is not any International Reference for the sensitivity standardization of these reagents. For this reason, LTA uses an internal control that contains animal serum with antibodies against Brucella, and titred with commercial reagents of certified quality.

Use of control sera is recommended as reference; the positive control ought to show a partial or complete agglutination, instead the negative control ought to show no agglutination.

Controls should be ever used to distinguish an eventual agglutination of the bottom of reagent.

Controls should be used as described in procedures or even to be treated as samples (dilution, etc.).