# PrioCHECK® Brucella Ab

## Introduction

Brucellosis occurs worldwide and affects both humans and animals. The disease is caused by bacteria of the genus *Brucella* (Gram-negative coccobacillus). The genus comprises 6 species: *B. abortus* (7 biovars), *B. melitensis* (3 biovars), *B. suis* (5 biovars), *B. ovis*, *B. canis*, *B. neotomae* and *B. maris*. The main reservoir for *B. abortus* is cattle, the main reservoir for *B. melitensis* is sheep and goats. Many serological tests have been developed for the routine diagnosis of brucellosis. Whatever test is used, one should be aware that cross-reactions with other Gram-negative bacteria such as *Salmonella*, *Pasteurella*, *Yersinia enterocolitica*, *Escherichia coli*, *Francisella tularensis*, *Camplylobacter*, *Salmonella*, *Pasturella*, *Yersinia* enterocolitica 0:9, *Escherichia coli* O:117 and O:156 may occur. After infection with *B. abortus* or *B. melitensis*, antibodies of the IgM class appear and are followed by antibodies in the IgG classes (IgG1 and IgG2). The PrioCHECK® Brucella Ab detects antibodies of the IgG1 class in serum and milk and is a modification of the test described by Bercovich et al. (1990). The test meets the requirements of the EU directive 64/432. National guidelines for working with animal samples and laboratory animals are in veterinary diagnostic use only.

## Test Principle

A microtiterplate is coated with sonicated and inactivated *Brucella abortus* antigen. Serum samples or milk samples are dispensed in the coated wells of a microtiterplate. Antibodies, directed against *Brucella abortus* and *Brucella melitensis*, that are present in the test sample, bind to the antigen during incubation. The bound antibodies are detected using an anti-IgG monoclonal antibody, conjugated to the enzyme horseradish-peroxidase. Subsequently, the bound conjugate is visualized by adding Chromogen (TMB) Substrate. Color development measured optically at a wavelength of 450 nm shows the presence of antibodies directed against brucellosis.

## Kit Components

### Test Plate

Five Test Plates.

### Conjugate (30x) (30x concentrated, dilute before use)

One vial contains 2.5 ml Conjugate. Diluted conjugate is not stable, prepare just before use.

### Dilution Buffer (5x) (5x concentrated, dilute before use)

One vial contains 60 ml Dilution Buffer. Shelf life of dilution buffer working solution: 5 hours at 22±3°C.

## Test Procedure

### Precautions

National guidelines for working with animal samples must be strictly followed. The PrioCHECK® Brucella Ab must be performed in laboratories suited for this purpose. Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

### Dilution Buffer Working Solution

Stability of dilution buffer working solution: 5 hours at 22±3°C. Diluted conjugate is not stable, prepare just before use.

### Additional Material Required

**Component 1**

- Washing Fluid (200x)
  - (200x concentrated, dilute before use)
  - One vial contains 60 ml Washing Fluid.
  - Shelf life of washing solution: 1 week at 22±3°C.

**Component 5**

- Reference Serum 1 (Ready-to-use)
  - One vial contains 0.5 ml Reference Serum 1 (positive control).
- Reference Serum 2 (Ready-to-use)
  - One vial contains 0.5 ml Reference Serum 2 (negative control).

**Component 3**

- Stop Solution (Ready-to-use)
  - One vial contains 60 ml Stop Solution.

**Component 4**

- Additional Kit Contents:
  - 10 plate sealers
  - Package Insert
  - Certificate of analysis

**Component 2**

- Component 1
- Component 2
- Component 3
- Dilution Buffer (5x)
- Reference Serum 1 (Ready-to-use)
- Reference Serum 2 (Ready-to-use)
- Stop Solution (Ready-to-use)

## Analysis of Results:

Multiscan EX or equivalent.

**Notes**

To achieve optimal results with the PrioCHECK® Brucella Ab, the following aspects must be considered:

- **Test Procedure** protocol must be strictly followed.
- All reagents of the kit must be equilibrated to room temperature (22±3°C) before use.
- Pipette tips have to be changed for every pipetting step.
- Separate solution reservoirs must be used for each reagent.
- Kit components must not be used after their expiry date or if changes in their appearance are observed.
- Kit components of different lot numbers must not be used together.
- Demineralized or water of equal quality must be used for the test.

**SOLUTIONS TO BE MADE IN ADVANCE**

### Dilution Buffer Working Solution

The concentrated Dilution Buffer (Component 3) must be diluted 5 times (1 part Dilution Buffer + 4 parts demineralized water). The total volume of dilution buffer working solution that can be prepared is 300 ml. Stability of dilution buffer working solution: 5 hours at 22±3°C.

### Conjugate Dilution

Prepare dilution of the Conjugate (30x) (Component 2) in dilution buffer working solution. For one plate prepare 12 ml. Add 0.4 ml Conjugate (30x) to 11.6 ml of dilution buffer working solution. The diluted conjugate must be prepared just before use.

### Washing Solution

The Washing Fluid (200x) (Component 4) must be diluted 1/200 in demineralized water and is sufficient for a final volume of 12 liters of washing solution. Stability of washing solution: 1 week at 22±3°C.

## Additional Material Required

**Additional Material Required**

### Component 1

- Component 1
- Component 2
- Component 3
- Dilution Buffer (5x)
- Reference Serum 1 (Ready-to-use)
- Reference Serum 2 (Ready-to-use)
- Stop Solution (Ready-to-use)

**Notes:** See Appendix IV for sample preparation procedure and storage.

### PRE-DILUTION OF REFERENCE SAMPLES

1.1 Prepare a 1:10 dilution of Reference Sera 1, 2 and 3 (Component 5, 6 and 7) in a dummy plate by dispensing 90 µl of dilution buffer working solution to the wells C1 up to H1. Add 10 µl of Reference Serum 1 to wells C1 and D1, 10 µl of Reference Serum 2 to wells E1 and F1 and 10 µl of Reference Serum 3 to wells G1 and H1.

### PRE-DILUTION AND INCUBATION OF INDIVIDUAL SAMPLES

2.1 Bovine samples 1:10 pre-diluted

Dispense 90 µl of dilution buffer working solution to the wells and add 10 µl of bovine serum.

Sheep samples 1:5 pre-diluted

Dispense 80 µl of dilution buffer working solution to the wells and add 20 µl of sheep serum.

Goat samples 1:2.5 pre-diluted

Dispense 60 µl of dilution buffer working solution to the wells and add 40 µl of goat serum.
Brucella Ab

Dispense 80 µl of dilution buffer working solution to wells A1 and B1 of the Test Plate (Component 1).

Dispense 90 µl of dilution buffer working solution to wells C1 to H1 of the Test Plate.

Dispense 10 µl pre-diluted reference sera 1, 2 and 3 to the wells C1 till H1 of the Test Plate.

Serum sample

Dispense 10 µl undiluted sample sera (individual or bulk) to the wells of the Test Plate.

Seal and shake the Test Plate gently.

Incubate the Test Plate 60±5 minutes at 37±1°C.

Pooled sheep serum samples

Dispense 90 µl of dilution buffer working solution to the remaining wells and add 10 µl of the pre-diluted sera to each of the corresponding wells of the Test Plate.

Milk sample

Dispense 100 µl undiluted milk samples (individual or bulk) to the wells of the Test Plate.

Seal and shake the Test Plate gently.

Incubate the Test Plate 60±5 minutes at 37±1°C.

PRE-DILUTION AND INCUBATION OF POOLED TEST SAMPLES

Pre-dilution in a dummy plate

Prepare a pool of 10 serum samples (bovine, sheep or goat) by mixing 10 µl of each individual serum in the dummy plate. Do not pool samples of different species!

Shake the dummy plate gently.

Dispense 100 µl of dilution buffer working solution to wells A1 and B1 of the Test Plate (Component 1).

Dispense 90 µl of dilution buffer working solution to wells C1 to H1 of the Test Plate.

Dispense 10 µl pre-diluted reference sera 1, 2 and 3 to the wells C1 till H1 of the Test Plate.

Seal and shake the Test Plate gently.

Incubate the Test Plate 60±5 minutes at 37±1°C.

CONTINUE WITH THE FOLLOWING PROCEDURE FOR BOTH INDIVIDUAL AND POOLED SAMPLES.

INCUBATION WITH CONJUGATE

Empty the Test Plate and wash the plate 6 times with 200-300 µl washing solution. Tap the plate firmly after the last wash cycle.

Dispense 100 µl of the diluted conjugate to all wells.

Seal and shake the Test Plate 60±5 minutes at 37±1°C.

INCUBATION WITH CHROMOGEN (TMB) SUBSTRATE

Empty the Test Plate and wash the plate 6 times with 200-300 µl washing solution. Tap the plate firmly after the last wash cycle.

Dispense 100 µl of the Chromogen (TMB) Substrate (Component 8) to all wells.

Incubate the Test Plate for 15 minutes at 22±3°C.

Add 100 µl of the Stop Solution (Component 9) to all wells.

Mix the content of the wells of the Test Plate.

Note: Start the addition of Stop Solution 15 minutes after the first well was filled with Chromogen (TMB) Substrate. Add the Stop Solution in the same order and the same pace as the Chromogen (TMB) Substrate solution was dispensed.

READING OF THE TEST AND CALCULATING THE RESULTS

6.1 Measure the optical density (OD) of the wells at 450nm within 15 minutes after color development has been stopped.

6.2 Calculate the mean OD<sub>450</sub> blank (wells A1 and B1).

6.3 Calculate the corrected OD<sub>450</sub> of the Reference Sera and test samples by subtracting the mean OD<sub>450</sub> blank.

6.4 Calculate the percent positivity (PP) of Reference Sera 1, 2, 3 and of the test samples according to the formula below.

The corrected OD<sub>450</sub> of all samples are expressed as percent positivity (PP) relative to the corrected mean OD<sub>450</sub> of Reference Serum 1 (wells C1 and D1).

RESULT INTERPRETATION

Validation criteria

7.1 The mean OD<sub>450</sub> blank must be <0.150.

7.2 The corrected mean OD<sub>450</sub> of Reference Serum 1 must be ≥1.000.

7.3 The percent positivity (PP) of Reference Serum 2 must be <45.

7.4 The percent positivity (PP) of Reference Serum 3 must be >45.

Note: Not meeting any of these criteria is reason to discard the results of that specific test run.

Note: If the corrected mean OD<sub>450</sub> of Reference Serum 1 is below 1.000 possibly the Chromogen (TMB) Substrate is too cold. In that case warm the solution to 22±3°C or incubate up to 30 minutes. If the corrected mean OD<sub>450</sub> of Reference Serum 1 is above 2.000 a shorter incubation period with the Chromogen (TMB) Substrate is recommended.

Interpretation of the percent positivity

Bovine serum (individual or pooled) samples

PP <45% (negative) Brucella antibodies are absent in the test sample.

PP >45% (positive) Brucella antibodies are present in the test sample.

Bovine milk (individual or pooled) samples

PP <20% (negative) Brucella antibodies are absent in the test sample.

PP >20% (positive) Brucella antibodies are present in the test sample.

Sheep serum (individual or pooled) samples

PP <30% (negative) Brucella antibodies are absent in the test sample.

PP >30% (positive) Brucella antibodies are present in the test sample.

Goat serum (individual or pooled) samples

PP <40% (negative) Brucella antibodies are absent in the test sample.

PP >40% (positive) Brucella antibodies are present in the test sample.

Serum samples in a positive pool need to be retested individually.

REFERENCE SERUM 1 (Ready-to-use)

Hazard Code: This product is not classified according to EU regulations.

REFERENCE SERUM 2 (Ready-to-use)

Hazard Code: This product is not classified according to EU regulations.

REFERENCE SERUM 3 (Ready-to-use)

Hazard Code: This product is not classified according to EU regulations.
Component 8
Chromogen (TMB) Substrate (Ready-to-use)
Hazard Code: This product is not classified according to EU regulations.

Component 9
Stop Solution
Hazard Code: R35: Causes severe burns.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45: In case of accident or if feel unwell, seek medical advice immediately (show the label on vial).

Appendix III

References

Appendix IV

Sample preparation procedure and storage

Serum
- Samples can be stored at -20°C before testing.
- Samples can be tested individually or in a pool of 10 samples.
- Serum samples of bovine, sheep and goat are tested in different final concentrations in the ELISA test plate. The final dilution for bovine sera is 1:100, for sheep 1:50 and for goat 1:25.
- Do not pool samples of different species!
- Serum samples have to be pre-diluted in a dummy plate first.
- When titrating serum samples, two-fold serial dilutions should be prepared in dilution buffer working solution.

Milk
- Milk samples can be stored at 5±3°C before testing.
- If milk samples are not tested within 3 days after collection it is recommended to add sodium azide (0.02%) as a preservative.
- The fluid of the milk sample to be tested should be collected from underneath the creamy layer.
- Individual and bulk milk samples are tested undiluted.

Table 1: Sample dilution

<table>
<thead>
<tr>
<th>Animal Type of sample</th>
<th>Predilution in dummy plate*</th>
<th>In test plate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Individual</td>
<td>10 µl serum + 90 µl DB (1:10)</td>
<td>10 µl serum + 90 µl DB (1:100)</td>
</tr>
<tr>
<td>Sheep Individual</td>
<td>20 µl serum + 80 µl DB (1:5 )</td>
<td>10 µl serum + 90 µl DB (1:50)</td>
</tr>
<tr>
<td>Goat Individual</td>
<td>40 µl serum + 60 µl DB (1:2,5)</td>
<td>10 µl serum + 90 µl DB (1:25)</td>
</tr>
<tr>
<td>Bovine Pool of 10 sera</td>
<td>Mix 10 µl of each sera (1:10)</td>
<td>10 µl serum + 90 µl DB (1:100)</td>
</tr>
<tr>
<td>Sheep Pool of 10 sera</td>
<td>Mix 10 µl of each sera (1:10)</td>
<td>20 µl serum + 80 µl DB (1:50)</td>
</tr>
<tr>
<td>Goat Pool of 10 sera</td>
<td>Mix 10 µl of each sera (1:10)</td>
<td>40 µl serum + 60 µl DB (1:25)</td>
</tr>
<tr>
<td>Bovine Milk (individual)</td>
<td>-</td>
<td>100 µl milk sample</td>
</tr>
<tr>
<td>Bovine Milk (bulk)</td>
<td>-</td>
<td>100 µl milk sample</td>
</tr>
<tr>
<td>Reference Sera</td>
<td>10 µl serum + 90 µl DB (1:10)</td>
<td>10 µl serum + 90 µl DB (1:100)</td>
</tr>
</tbody>
</table>

*Use same dilution buffer working solution.

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