SVANOVIR® *Brucella*-Ab C-ELISA

Brucella **C-ELISA**
Antibody Test

Contents	Art. No. SV-104893	Art. No. SV-104894
Microtitre plate Microtitre plates (96 wells) coated with non-infectious S-LPS *Brucella abortus* antigen (sealed and stored dry)	2 (Strips) 12 x 8	10 (Plates)
Conjugate Ready-to-use (horseradish peroxidase conjugated goat anti-mouse IgG antibodies)	1 x 25 mL	1 x 100 mL
Monoclonal Antibodies (mAb) Lyophilised	2 (Bottles)	10 (Bottles)
PBS-Tween Solution 20 x concentrate	1 x 125 mL	3 x 125 mL
Sample Dilution Buffer Ready-to-use	2 x 25 mL	2 x 100 mL
Substrate Solution (Tetramethylbenzidine in substrate buffer containing H_2O_2) - STORE IN THE DARK!	1 x 20 mL	1 x 100 mL
Stop Solution Contains sulphuric acid (2M) - DANGER! 	1 x 10 mL	2 x 25 mL
A. Positive Control Serum - Contains preservatives	1 x 0.1 mL	1 x 0.3 mL
B. Negative Control Serum - Contains preservatives	1 x 0.1 mL	1 x 0.3 mL
C. Weak Positive Control Serum - Contains preservatives	1 x 0.1 mL	1 x 0.3 mL

This manual covers the following SVANOVIR® *Brucella*-Ab C-ELISA kits: Article number SV-104893 and SV-104894

Brucella **C-ELISA**
Antibody Test

Name and Application
SVANOVIR® *Brucella*-Ab C-ELISA is an Enzyme Linked Immunosorbent Assay (ELISA) for the detection of specific antibodies to *Brucella abortus*, *Brucella melitensis* and *Brucella suis* in bovine, ovine, caprine and porcine serum, respectively. In addition it minimizes the cross reaction with other gram negative bacteria. In cattle this assay is capable of discriminating between *Brucella* infected animals and animals vaccinated with *Brucella* strain 19.

General information
Brucella is the causative agent of brucellosis, a zoonosis of world-wide importance, that is also called Malta fever, Mediterranean fever (*B. melitensis*) or Bang's disease (*B. abortus*) which affects both humans and animals. The main domestic animals that are affected are cattle, sheep, goats and pigs. A number of wildlife species, such as bison, elk, reindeer and caribou, can also be infected.
Ovine and caprine brucellosis is primarily caused by *B. melitensis*. Patologically and epidemiologically *B. melitensis* infection in sheep and goats is very similar to *B. abortus* infection in cattle.
Persistent lifelong infections are common and are spread through shedding in the reproductive and mammary secretions. Because of it's economic impact on animal health and the risk to the human population most countries have a Brucellosis Control Program. This program usually involves vaccination of young or mature animals with strain 19 and/or a slaughter program of infected/exposed animals based on a reaction to conventional serological assays. None of these assays are capable of distinguishing animals that are vaccinated from those that are infected resulting in the slaughter of some vaccinated animals. The only definitive diagnosis is bacterial culture of the causative organism. However, this is a time consuming and expensive procedure.

Principle
The kit procedure is based on a solid phase competitive ELISA. In this procedure, the samples together with a mouse monoclonal antibody (mAb) specific for an epitope on the o-polysaccharideportion of the S-LPS antigen, are exposed to *Brucella abortus* smooth lipopolysaccaride (S-LPS) coated wells on microtiter plates. If *Brucella* antibodies are present in the test sample they will bind to the antigens in the well and block these antigenic sites. If *Brucella* antibodies are absent in the sample, these sites will remain free and the mAb which was added together with the sample will bind to these free antigenic sites. After an incubation period the unbound materials are removed by rinsing and a goat anti- mouse horseradish peroxidase (HRP) conjugated IgG is added to the plate. The HRP conjugate will bind to the specific mAb in absence of *Brucella* antibodies in the sample. Unbound materials are removed by rinsing prior to the additon of the substrate. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate. A negative result is indicated by the development of a blue colour. The reaction is stopped by addition of stop solution; the colour changes to yellow. The result can be read by a microplate photometer, where the optical density (OD) is measured at 450 nm.
Sera from strain 19 vaccinated cattle do not compete with the mAb because of their specificity and lower affinity, leading to negative reaction. However, in some cases samples taken before 6 months post vaccination may react positively.

Materials needed but not provided

1. Precision pipettes
2. Disposable pipette tips
3. Distilled, deionised or any similar high quality water
4. Wash bottle, multichannel pipettor or plate washer
5. Container: 1 to 2 litres for PBS-Tween
6. Microplate photometer, 450 nm filter

Specimen information

Serum:

5 µL of blood serum or plasma is needed for each sample well. Fresh, refrigerated, or previously frozen serum or plasma may be tested.

Preparation of reagents

PBS-Tween Buffer:

Dilute the PBS-Tween Solution 20 x concentrate 1/20 in distilled water. Prepare 500 mL per plate by adding 25 mL PBS-Tween solution to 475 mL distilled water and mix thoroughly.

N.B. Please check that there is no crystal precipitation in the bottle. If crystals are seen, please warm and shake well.

mAb Solution:

Reconstitute freeze dried mAb with 6 mL Sample Dilution Buffer. Add the buffer carefully into the bottle. Prepare immediately before use. Mix gently – do not use vortex mixer!
Reconstituted mAb may be frozen and stored at -20ºC for up to 4 weeks. The reconstituted mAb may not be frozen and thawed more than twice.

Precautions

1. Carefully read and follow all instructions.
2. Store the kit and all reagents at 2-8°C (36-46°F).
3. All reagents should equilibrate to room temperature 18-25°C (64-77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction manuals from different test kit batches.
6. Care should be taken to prevent contamination of kit components.
7. Do not use test kit beyond date of expiry.
8. Do not eat, drink, or smoke where specimens or kit reagents are handled.
9. Use a separate pipette tip for each sample.
10. Do not pipette by mouth.
11. Include positive, negative and weak positive serum controls on each plate or test strip series.
12. Use only distilled, deionised or any similar high quality water for preparation of reagents.
13. When preparing the buffers, etc., measure the required volume.
14. The Stop Solution contains sulphuric acid, which is corrosive.*
15. All unused biological materials should be disposed according to the local, regional and national regulations.

Recommendations!

There is always a surplus volume of liquid reagent. The volume mentioned on the label is the minimum obtainable.
Strips with broken seal can be resealed together with the desiccant and stored at 2-8°C (36-46°F) for up to 4 weeks.

Procedure

1. All reagents should equilibrate to room temperature 18-25˚C (64-77˚F) before use.
2. Add Samples

A. Add 45 µL of Sample Dilution Buffer into each well that will be used for serum samples, serum controls and conjugate controls.

B. Add 5 µL of positive, weak positive and negative serum controls, into each of the appropriate wells, respectively. For confirmation purposes it is recommended to run the control sera in duplicates.

C. Add 5 µL of Sample Dilution Buffer into two appropriate wells (designated as Conjugate Control, Cc).

D. Add 5 µL of test sample to each of the appropriate wells. The samples can be tested in singlicates or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

3. Add 50 µL of mAb-Solution into all wells used for controls and samples.

   Note! The time difference between control/sample and mAb-Solution addition must not exceed 10 minutes.

4. Seal the plate and mix the reagents thoroughly for 5 minutes, either by using a plate shaker or by tapping the sides of the plate.
5. Incubate the plate at room temperature 18-25˚C (64-77˚F) for 30 minutes.
6. Rinse the plates/strips 4 times with PBS-Tween Buffer: fill up the wells at each rinse, empty the plate and tap hard to remove all remains of fluid.
7. Add 100 µL of Conjugate Solution into each well. Seal the plate and incubate at room temperature 18-25˚C (64-77˚F) for 30 minutes.
8. Repeat step #6.
9. Add 100 µL Substrate Solution to each well and incubate for 10 minutes at room temperature 18-25˚C (64-77˚F). Begin timing after the first well is filled.
10. Stop the reaction by adding 50 µL of Stop Solution to each well and mix thoroughly. Add the Stop Solution in the same order as the Substrate Solution was added in step #9.
11. Measure the optical density (OD) of the controls and samples at 450 nm in a microplate photometer (use air as a blank). Measure the OD within 15 minutes after the addition of Stop Solution to prevent fluctuation in OD values.

Calculations

1. Calculate the mean OD values for each of the controls and samples.
2. Calculate the percent inhibition (**PI**) values for controls as well as samples, using the following formula:

$$PI = 100 - \frac{(OD_{\text{Samples or Control}} \times 100)}{OD_{\text{Conjugate Control Cc}}}$$

Interpretation of the results

Criteria for test validity

To ensure validity, the values of the controls must fall within the following limits:

OD	**Cc**	**0.75 - 2.0**
PI	**Positive control**	**80 - 100**
PI	**Weak Positive control**	**30 - 70**
PI	**Negative control**	**<30**

For invalid test, technique may be suspect and the assay should be repeated.

Interpretation

The status of a test sample is determined as follows:

PI	**Status**
< 30 %	**Negative**
≥ 30 %	**Positive**

References

1. OIE Manual of standards for Diagnostics Tests and Vaccines, 4th Edition 2000.
2. Nielsen, K. *et al* (1992). Enzyme Immunoassay: Application to diagnosis of bovine brucellosis. Monograph, Agriculture Canada, ISBN 0-662-19838-7.
3. Nielsen, K., Duncan, J.R., (1990). Animal Brucellosis. CRC Press, ISBN 0-8493-5878-7.
4. Nielsen, K. and Gall, D. (1994). Advances in the diagnosis of bovine brucellosis: Use of Enzyme Immunoassays. The Genetic Engineer and Biotechnologist, 14 (1) 25-39.
5. Nielsen, K. *et al* (1994). Comparison of Enzyme Immunoassays for the Diagnosis of Bovine Brucellosis. Vet Preventive Med.
6. A Competetive Enzyme Immunoassay for the Detection of Serum Antibody to *Brucella abortus*. APHD Standard Protocol, Version 2.0, 20-Sept-94.

***DANGER: Stop solution (sulphuric acid)**
May be corrosive to metals. Causes skin irritation. Causes serious eye irritation.

Keep only in original container. Wear eye protection/ face protection. Wear protective gloves.
IN CASE OF CONTACT WITH EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician. If eye irritation persists: Get medical advice/ attention.
IN CASE OF CONTACT WITH SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse. If skin irritation occurs: Get medical advice/attention. Absorb spillage to prevent material damage.

Symbols

REF	Article No.
LOT	Serial (batch) No.
	Temperature limit
	Expiry date
	Number of tests
	See manual
	Manufacturer

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