INTERPRETATION

Calculation of the Ratio

For the wash solution (WS) and each control, calculate the mean (M) of ODs obtained. To obtain corrected OD for each sample (S) and control, subtract the M OD obtained for WS from the OD obtained for each sample or from the M OD obtained for each control. To obtain **Ratio**, divide each sample's corrected OD by the positive control's (P) corrected OD.

$$\frac{OD_S - M\ OD_{WS}}{M\ OD_P - M\ OD_{WS}} = RATIO$$

Validity Criteria

The following criteria must be met in order to validate the test:

- Negative control ratio must be less than 0.25.
- Positive control's corrected OD must be greater or equal to 0.75.

Interpretation:

- Sample ratio less than 0.45 is considered *N.caninum*-Ab negative.
- Sample ratio greater or equal to 0.60 is considered *N.caninum*-Ab positive.
- Sample ratio less than 0.60 but greater or equal to 0.45 is considered *N.caninum*-Ab suspicious. A second serum/milk sample could be collected 2 weeks later and retested.

BIBLIOGRAPHY

Dubey JP, Schares G. Diagnosis of bovine neosporosis. Vet. Parasitology. 2006, 140, 1-34.

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Neospora caninum Antibody Test Kit, ELISA
Bovichek® *Neospora serum and milk*
Insert

2015-11-05

This is an immunoenzymatic assay for the detection of antibodies against *Neospora caninum* in bovine serum and milk.

Neospora caninum (*N. caninum*) is an obligate intracellular protozoan parasite that has been confused previously with *Toxoplasma gondii*. The dog is the definitive host. Infection can be acquired by ingesting food and water contaminated with oocysts excreted in feces of dogs, by ingesting infected tissues, or transplacentally. Vertical transmission is a major route of transmission in cattle and dogs. In dairy cattle, *N. caninum* is a major cause of abortion in many countries. Calves may be aborted; stillborn; born underweight, weak, or paralyzed; or they may become paralyzed within 4 weeks of birth. Nonsuppurative encephalitis is the main lesion in aborted fetal tissues. Abortion can occur throughout gestation, and some cows may abort again. Diagnosis of *N. caninum* abortion consists in identifying the organism in tissue sections of aborted foetus by immunohistochemistry or PCR. A number of serological assays, such as ELISA, have been developed to detect *N. caninum* antibodies. They are useful to identify chronically infected cows which represent an important reservoir of the organism.

PRINCIPLE OF THE TEST

To detect the presence of antibodies against *N. caninum* in bovine serum or milk, controls and prepared serum or milk samples are incubated in wells coated with *N. caninum* antigen (Ag). The antibodies (Ab) specific to *N. caninum* present in positive serum or milk samples will bind to the Ag in the wells. After several washes to eliminate unbound substances, a conjugate (an Ab coupled to an enzyme) targeted at bovine Ab is added. After incubation, the excess of this conjugate is eliminated by a second wash and its attachment is revealed with a chromogenous substrate. The conjugate, if present, reacts with the substrate and a green color develops. The reaction is then stopped and the optical densities are read. The intensity of the color allows the determination of the status of sample tested. A negative sample will show a weak reaction (pale green) whereas a strong positive will show a strong reaction (dark green). All shades of green between dark and pale represent various degrees of positivity.

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MATERIAL

Components	Quantity
• 12 strips of 8 wells coated with *N. caninum* Ag	2
• Ready-to-use positive control	2.5 mL
• Ready-to-use negative control	2.5 mL
• Concentrated conjugate	125 µL
• Concentrated wash solution (10X)*	2 X 100 mL
• Ready-to-use substrate	30 mL
• Ready-to-use stop solution*	30 mL

* Crystals may form when stop solution and wash solution are kept at 2-7ºC. This will not affect the efficiency of the products. However, crystals have to be dissolved by bringing the solutions to room temperature and agitating.

The materials provided are sufficient for testing up to 180 samples.

Materials Required but not Provided:

- Purified water
- Adjustable single- and multi-channel pipettes
- Single-use pipette tips
- Test tubes for sample dilution
- ELISA 96-well microplate reader equipped with 405 nm filter
- Containers for preparation of solutions

PRECAUTIONS

- For *in vitro* veterinary use only.
- The materials used in this kit must be considered as infectious. Therefore, all waste must be decontaminated before being discarded.
- Do not use the kit after the expiry date indicated on the package.
- Do not mix the reagents or instructions from different serial numbers.
- The sensitivity and specificity of this test are guaranteed only if the procedures are strictly observed.
- Do not expose substrate to either light or oxidizing agent. Always keep the substrate in a plastic container. This solution might cause skin or eye irritation.
- Keep all reagents at 2-7°C and bring to room temperature before use.
- The wash solution contains thimerosal (0.01%).
- Dispose of the wash solution, the substrate and the stop solution according to local regulations for chemicals.

EXECUTION

A. Preparation of Wash Solution

After homogenizing the concentrated wash solution (no evidence of crystals), dilute concentrated wash solution in purified water at 1/10 (e.g.: 100 mL of 10X concentrated wash solution in 900 mL of purified water). Once diluted, the solution (1X) is stable for 1 week at 2-7°C.

B. Samples Preparation

Dilute bovine serum samples in 1X wash solution (see section A) at 1/200 (e.g., 4 µL sample in 796 µL 1X wash solution) or skimmed milk samples at ½ (e.g., 100 µL skimmed milk sample in 100 µL 1X wash solution). It is important to use a new tip for each sample. Make sure each dilution is properly mixed before being distributed into the wells.

C. Conjugate Preparation

Dilute the conjugate with 1X wash solution (see section A) according to the dilution indicated on the Final Control Sheet. Dilute conjugate a few minutes prior to its use and always prepare a fresh solution.

D. Test Procedure

Bring all reagents to room temperature and mix well manually before use.

1. Make a schematic representation of the plate and the distribution of controls and samples.
2. Dispense 100 µL of ready-to-use positive control, ready-to-use negative control, diluted wash solution (see section A) or diluted samples (see section B) into appropriate wells (it is recommended to run control sera and wash solution in duplicates).
3. Cover the wells and incubate at 37 ± 1°C for 45 minutes.
4. Wash each well 3 times with 300 µL 1X wash solution (see section A). Throw away all liquid contained in the plate after each wash. After the last wash, dry the plate by tapping it onto absorbent paper.
5. Dispense 100 µL of diluted conjugate (see section C) into each well.
6. Cover the wells and incubate at 37 ± 1°C for 30 minutes.
7. Wash each well 4 times following the procedure described at step 4.
8. Dispense 100 µL of ready-to-use substrate into each well.
9. Cover the wells and incubate, away from light, at 23 ± 2°C for 15 minutes.
10. Dispense 100 µL of ready-to-use stop solution into each well.
11. Measure optical densities (OD) at 405 nm. If the microplate reader is equipped with a reference filter, set it at 490 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.
12. Calculate the results.