Neospora caninum Antibody Test Kit (ELISA)

Bovichek® Neospora

Product code: TRM-500 (2 plates)

- ✓ **Highly sensitive ELISA kit**
- ✓ **A shelf life of 18 months**
- ✓ **Approved by the Canadian Food Inspection Agency**

INTRODUCTION

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.

The "*Neospora caninum* Antibody Test Kit, ELISA" (commercial name: Bovichek® Neospora) has been approved by the Canadian Food Inspection Agency (CCVB file number 810DR/N5.0D/10) in 2018.

INTENDED USE

This kit is based on an immunoenzymatic assay for the detection of antibodies against *Neospora caninum* in bovine serum.

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PRINCIPLE OF THE TEST

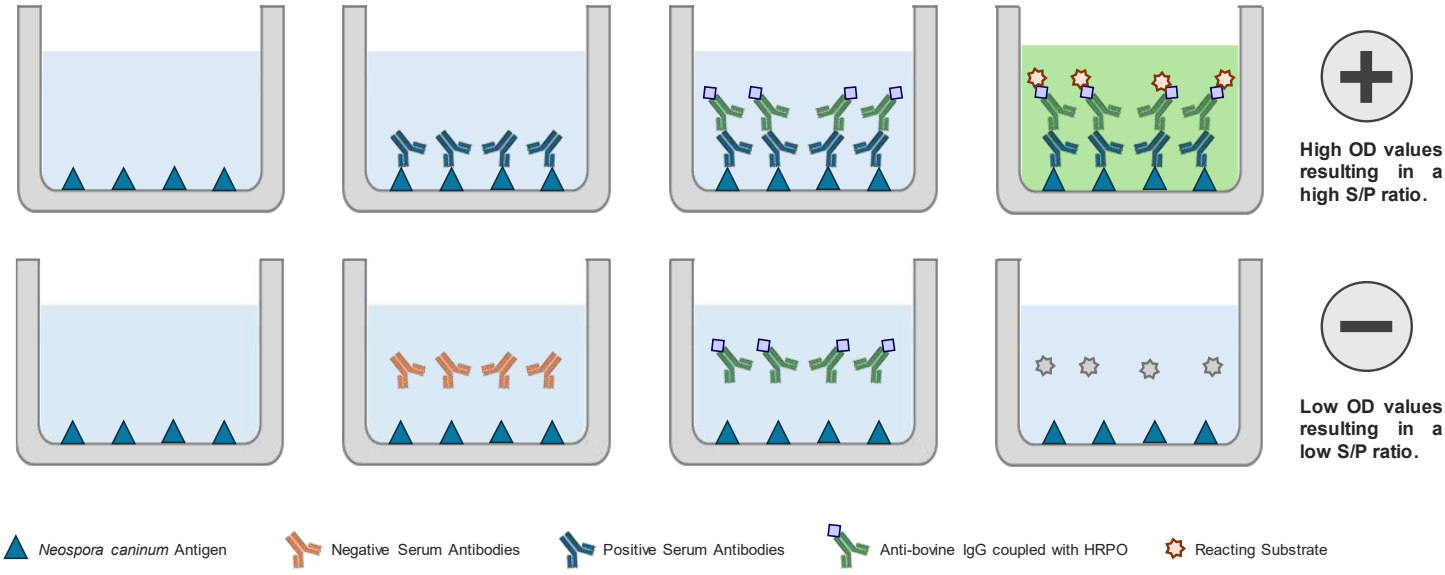
a. Assay description

1. The wells are coated with a purified *Neospora caninum* antigen.

2. Samples are added and incubated for 45 min at 37 ± 2°C. Each well is then washed carefully to remove unbound material.

3. Peroxidase-conjugated anti-bovine immunoglobulins are added and incubated for 30 min at 37 ± 2°C. Washing steps follow.

4. The substrate is added and incubated for 15 min at 23 ± 2°C. It will react with peroxidase if present. The reaction is then stopped, and OD values are measured.



b. Results interpretation

Corrected OD values are used to calculate sample / positive (S/P) ratio as follow:

$$\frac{[\ OD_{sample} - \text{mean } OD_{buffer}\ ]}{[\ \text{mean } OD_{pos} - \text{mean } OD_{buffer}\ ]} = \text{Ratio}$$

The status of a test sample is determined with the SP ratio.

Result	S/P ratio
Negative	Ratio < 0.45
Suspect	0.45 ≤ Ratio < 0.60
Positive	Ratio ≥ 0.60

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 0.750.

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TECHNICAL DATA

a. Sensitivity & Specificity

A panel of 200 samples were tested with Bovichek Nespora kit and Idexx Neospora X2 ab test (ELISA) as a reference test. Results are shown with suspect results as negative (A) and suspect results as positive (B).

A	Idexx +	Idexx -	Total
Biovet +	94	0	**94**
Biovet -	6	100	**106**
Total	**100**	**100**	**200**

Statistic	Value	95% CI
Relative sensitivity	94.00%	87.40 - 97.77%
Relative specificity	100.0%	96.38 - 100.00%
Kappa value	0.95	

B	Idexx +	Idexx -	Total
Biovet +	100	0	**100**
Biovet -	0	100	**100**
Total	**100**	**100**	**200**

Statistic	Value	95% CI
Relative sensitivity	100.0%	96.38 - 100.00%
Relative specificity	100.0%	96.38 - 100.00%
Kappa value	1.00	

The Bovichek® Neospora kit showed excellent relative sensitivity & specificity when compared with the reference test.

b. Stability

The stability of the kit was evaluated with a panel of 10 samples, including 4 negatives, 1 weak positive and 5 positives. Samples were tested with 3 serials of the Bovichek® Neospora kit that had been stored at 4-8°C for up to 19 months. S/P ratios are presented in the following table.

Sample ID	Serial 1			Serial 2			Serial 3		
	0 mo.	13 mo.	19 mo.	0 mo.	13 mo.	19 mo.	0 mo.	13 mo.	19 mo.
CQ# 181	0,24	0,18	0,23	0,31	0,30	0,30	0,28	0,33	0,32
CQ# 180	0,12	0,13	0,14	0,19	0,16	0,22	0,11	0,21	0,20
CQ# 190	0,22	0,24	0,26	0,32	0,30	0,33	0,22	0,33	0,39
CQ# 189	0,17	0,14	0,18	0,22	0,20	0,20	0,18	0,25	0,27
CQ# 200	0,51	0,49	0,50	0,61	0,52	0,61	0,51	0,50	0,52
CQ# 179	0,84	0,87	0,81	0,96	0,93	0,91	0,82	1,00	1,08
CQ# 176	0,91	0,84	0,83	1,03	0,88	0,79	0,73	0,83	1,08
CQ# 202	0,78	0,81	0,80	0,88	0,84	0,83	0,87	0,86	0,86
CQ# 192	1,24	1,18	1,08	1,46	1,40	1,22	1,16	1,42	1,34
CQ# 185	1,21	1,14	1,12	1,34	1,33	1,18	1,18	1,23	1,36

The ratios remained stable throughout the study, and no changes in result outcomes were observed highlighting the kit's great stability.

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c. Repeatability

The intra-plate (A), inter-plate (B) and inter-lot (C) repeatability was evaluated using 8 samples with a reactivity ranging from negative to strongly positive. Each sample was tested 3 times in one plate of a serial, 3 times in 3 plates of a serial and 3 times in 3 distinct serials. Mean and standard deviation of S/P ratios were used to calculate the Coefficient of Variation (CV).

A

Sample ID	Well 1	Well 2	Well 3	Mean ± SD	CV (%)
Ctl Neg	0,10	0,09	0,10	0,10 ± 0,00	**3,6**
Ctl Pos	1,00	1,00	1,00	1,00 ± 0,00	**0,0**
CQ no.3	0,41	0,39	0,37	0,39 ± 0,02	**5,4**
CQ no.4	0,22	0,22	0,19	0,21 ± 0,01	**6,8**
CQ no.5	0,40	0,38	0,37	0,38 ± 0,02	**4,0**
CQ no.7	0,30	0,30	0,27	0,29 ± 0,02	**7,1**
CQ no.13	1,04	1,01	0,85	0,97 ± 0,10	**10,5**
CQ no.14	0,95	0,95	0,94	0,95 ± 0,01	**0,9**
CQ no.16	0,96	0,93	0,91	0,93 ± 0,03	**2,7**
CQ no.18	1,25	1,17	1,15	1,19 ± 0,06	**4,7**

B

Sample ID	Plate 1	Plate 2	Plate 3	Mean ± SD	CV (%)
Ctl Neg	0,10	0,11	0,10	0,10 ± 0,01	**6,0**
Ctl Pos	1,00	1,00	1,00	1,00 ± 0,00	**0,0**
CQ no.3	0,41	0,40	0,39	0,40 ± 0,01	**2,4**
CQ no.4	0,22	0,21	0,21	0,21 ± 0,01	**3,3**
CQ no.5	0,40	0,39	0,38	0,39 ± 0,01	**2,6**
CQ no.7	0,30	0,29	0,29	0,30 ± 0,01	**2,4**
CQ no.13	1,04	0,99	0,92	0,99 ± 0,06	**6,3**
CQ no.14	0,95	0,95	0,93	0,94 ± 0,01	**1,4**
CQ no.16	0,96	0,95	0,93	0,94 ± 0,02	**1,7**
CQ no.18	1,25	1,20	1,14	1,20 ± 0,06	**4,7**

C

Sample ID	Serial A	Serial B	Serial C	Mean ± SD	CV (%)
Ctl Neg	0,10	0,13	0,13	0,12 ± 0,02	**15,1**
Ctl Pos	1,00	1,00	1,00	1,00 ± 0,00	**0,0**
CQ no.3	0,41	0,32	0,35	0,36 ± 0,05	**12,9**
CQ no.4	0,22	0,18	0,16	0,19 ± 0,03	**16,3**
CQ no.5	0,40	0,32	0,30	0,34 ± 0,05	**14,9**
CQ no.7	0,30	0,25	0,25	0,27 ± 0,03	**11,8**
CQ no.13	1,04	1,04	0,81	0,96 ± 0,13	**13,8**
CQ no.14	0,95	0,94	0,85	0,91 ± 0,06	**6,2**
CQ no.16	0,96	0,85	0,82	0,88 ± 0,07	**8,4**
CQ no.18	1,25	1,34	1,29	1,29 ± 0,04	**3,3**

The results demonstrate the Bovichek® Neospora kit's high repeatability. No sample had a CV greater than 15% in the intra- and inter-plate studies (A & B). The variation was slightly higher in the inter-lot study, especially in negative samples, but the standard deviation remained low.

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CONCLUSION

The "*Neospora caninum* Antibody Test Kit, ELISA – Bovichek Neospora" demonstrates satisfactory diagnostic performances (sensitivity, specificity, repeatability) and has a shelf life of 18 months at 2-8°C. This kit is licensed by the Canadian Centre for Veterinary Biologics.

KIT COMPOSITION

Components	Quantity TRM-500
12 strips of 8 wells coated with *Neospora* 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

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For more info, contact us at: 1-888-824-6838, option 3
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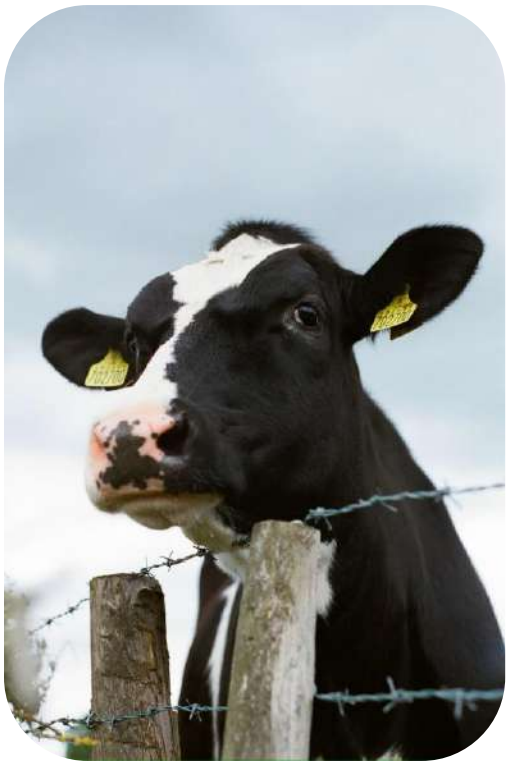

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