Instructions for use
BIOK368-Abort-BNQSV_NO_(EN)_V02
05/06/2026

Multiscreen AbELISA bovine abortion

Reference : BIO K 368

ELISA kit for serodiagnosis of BoHV-4, Neosporose, Q fever, Salmonellose and BVDV

Biwell, indirect test

In vitro and strictly veterinary use

Sample / Dilution	Bovine
Serum - Plasma / 100X	✓

*Hereafter, we will refer to it as serum.

Presentation

Product reference	BIO K 368/2
Format	2 plates, strip of 8 wells
Reactions	32 tests

Composition of the kit

Provided material		Code	Type*	BIO K 368/2
Microplate	Microplates	D01623	1	2
Washing solution (20X)	Washing solution (20X)	D00695	A	1 X 100 mL
Dilution solution (1X)	Colored dilution solution (1X)	D01511	A	1 X 125 mL
TMB solution (1X)	Single component TMB (1X)	D01585	A	1 X 30 mL
Stop solution (1X)	Stop solution (1X)	D00680	A	1 X 30 mL
Conjugate (50X)	Conjugate (50X)	D01476	1	1 X 0,6 mL
CTL POS	Positive control	D01624	a	1 X 0,5 mL
CTL NEG	Negative control	D01123	a	1 X 0,5 mL
*: (1): dependent on kit and batch : (a): dependent on kit / (A): substitutable with components A / (B): substitutable with components B.				

Revision history

Date	Version	Modifications
2016	V01	Creation
05/06/2026	V02	Layout and simplification of the entire leaflet. Replacement of Leptospirose by BVDV. Adjustment of component volume. Distribution of stop solution modified from 50 µL to 100 µL.

Note : minor changes to typography, grammar and formatting are not included in the revision history.

A. Introduction

The task of determining the cause of an abortion in cattle is generally a rather difficult one because, most of the time, it is the consequence of an event which happened weeks to months earlier. Often also, the foetus is maintained in the uterus for hours and even days after its death, and, when it is finally evacuated, it has undergone autolysis, in such a way that it is difficult to do any type of analysis. Also, many causes of abortion in cattle are to this day still unknown. Moreover, many pathogens are rarely looked for because they are difficult to or dangerous to handle (*Coxiella burnetii*, *Chlamydia abortus*…). Pathogens directly or indirectly responsible for abortions are numerous and varied, which complicates the diagnosis.

The Bio-X abortion ELISA kit allows the humoral immune response of cattle to be evaluated against five agents frequently involved in abortions. These agents are BoHV-4 (bovine herpesvirus type 4), *Neospora caninum*, *Coxiella burnetii*, *Salmonella* and BVDV (bovine viral diarrhea virus).

B. Test principle

The test uses 96-well microtitration plates sensitised by a monoclonal antibody specific to *Neospora caninum*. This antibody is used to trap Neospora caninum as well as to purify it from cultures of this protozoan.
For *Salmonella Dublin*, the plate is sensitized by purified LipoPolySaccharide (LPS).
For BoHV-4 the plate is sensitized by purified virus.
For *Coxiella burnetii*, the plate is sensitized by phase I and phase II antigenic extract from *Coxiella burnetii* cells.
For BVDV, the plate is sensitized by a monoclonal antibody specific from a BVDV protein which ensures the capture and purification of this protein from a BVDV culture.

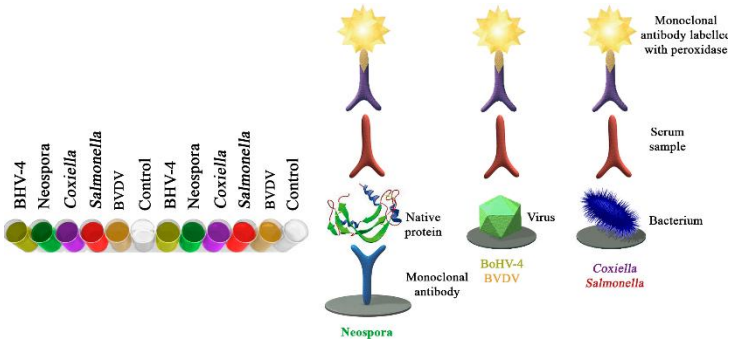
The distribution of these pathogens on the microtitration plate is as follows:

- Columns 1 & 7: BoHV-4
- Columns 2 & 8: *Neospora caninum*
- Columns 3 & 9: *Coxiella burnetii phase I + II*
- Columns 4 & 10: *Salmonella dublin*
- Columns 5 & 11: BVDV
- Columns 6 & 12: negative control

Columns 6 & 12 contain one monoclonal antibody. Using such a control reduces the number of false positives considerably. The test sera and plasma are diluted 1:100 in the dilution solution and incubated on the plate for one hour at 21±3°C. The plate is washed and the conjugate, a peroxidase-labelled anti-bovine IgG1 monoclonal antibody, is added to the wells. The plate is reincubated at 21±3°C for 1 hour. After this second incubation, the preparation is washed and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific immunoglobulins are present in the test sera the conjugate remains bound to the corresponding microwell and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of specific antibody in the sample. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells. It is possible to quantify the reactivity of an unknown sample on a scale ranging from 0 to +++++.

C. Additional material and required equipment (not provided)

- Distilled/demineralized water.
- Graduated mono- or multichannel pipettes (2-20 µL, 20-200 µL et 100-1000 µL range) and single-use tips.
- Microplate reader (450nm filter).
- Microplate washer (optional).
- Dilution microplate (optional).
- Incubator at 21±3°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid, …



D. Precautions for use

- The reagents must be kept between +2 et +8°C.
- Unused strips must be stored with the desiccant in the hermetically sealed aluminum envelope.
- Do not use reagents beyond shelf-life date.
- Make sure to use distilled/demineralized water.
- The stopping solution contains 1 M phosphoric acid. Handle it carefully.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- Keep the TMB solution away from light.

E. Preparation of solutions

- The solutions are to be prepared extemporaneously.
- The washing solution must be diluted 20-fold in distilled/demineralized water. The cold solution crystallizes spontaneously. Bring the vial to 21±3°C to make sure that all crystals have disappeared; mix the solution well and withdraw the necessary volume.
- The dilution solution is ready to use. The dilution solution is colored in yellow. It is used for the dilution of samples, kit controls (positive and negative) and conjugate.
- The conjugate is to be diluted 50-fold in the dilution solution.
- The stop solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of samples

- **Serum** samples as well as kit controls (positive and negative control) are to be diluted 100-fold in the dilution solution. Avoid using haemolysed samples or those containing coagula.

  Recommended dilution:
  990 µL dilution solution + 10 µL sample

G. Procedure

- Bring all the reagents to **21±3°C** before use.
- Carefully read through the previous points.

1. Distribute **100 µL per well** of **diluted** samples, and **diluted** kit controls, for example as follows: positive control: wells H1 to H6, negative control: wells G1 to G6, sample 1: wells A1 to A6, samples 2: wells B1 to B6.
   Cover and incubate the plate **at 21 ± 3°C** during **60 ± 5 min**.

2. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL** of washing solution per well. Avoid the formation of bubbles in the well between each wash.

3. Add **100 µL of diluted conjugate** per well.
   Cover and incubate the plate **at 21 ± 3°C** during **60 ± 5 min**.

4. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL** of washing solution per well. Avoid the formation of bubbles in the well between each wash.

5. Distribute **100 µL** of **TMB solution** per well.
Incubate for **10 ± 1 min** at **21 ± 3°C** away from the light, without covering.

6. Distribute the stop solution at rate of **100 µL** per well. The colour changes from blue to yellow.

7. Record the optical densities using a plate spectrophotometer with a **450 nm** filter **within 5 minutes** after adding the stop solution.

H. Validation of results

The test can only be **validated** if the positive control yields a difference in optical density at 10 minutes that is greater for each valence than:

BoHV-4	> 0,550
Neospora caninum	> 1,000
Coxiella burnetii	> 1,000
Salmonella dublin	> 1,000
BVDV	> 1,000

And the negative control yields a difference in optical density at 10 minutes that is lower than 0,300.

I. Interpretation of results

- Substract from each value recorded in columns 1, 2, 3, 4, 5, the signal of the corresponding negative control well 6 and write down the result (calculation of delta OD). Allow for any negative values that may exist in performing the calculation.
- Carry out the same operations for the columns corresponding to the positive and negative controls.

Divide the signal read for each sample well by the corresponding positive control signal and multiply this result by 100 to express it as a percentage.

$$Val(ue) = \frac{\text{Delta OD sample}}{\text{Delta OD positive control}} * 100$$

Using the table at the bottom of the page, determine each serum's degree of positivity.

A reliable diagnosis can be made only if frank seroconversion can be documents using two coupled serum samples taken at 2 to 3 week intervals. The first sample must be taken during the acute phase of the infection. A Frank seroconversion is considered to have occurred if the signal cincreases by two orders of magnitude (for example, ++ → ++++ or + → +++).

A sample must be considered positive if it yields a result that is greater than or equal to one plus sign (+).

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ANALYSISCREEN

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SCAN ME

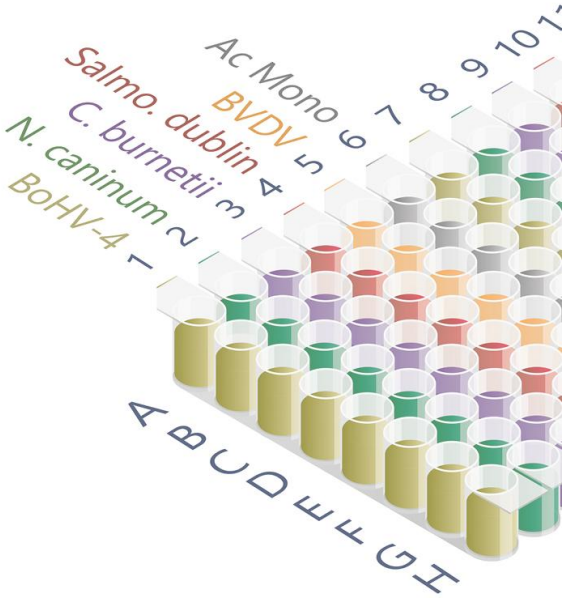
Symbols

Symbol	Meaning
REF	Catalog number
	Manufacturer
	Temperature limit
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	Keep away from light
	Keep dry
	Corrosive substance
	Hazardous/irritating product

	0		+		++		+++		++++		+++++
BoHV-4	Val ≤	55%	< Val ≤	91%	< Val ≤	128%	< Val ≤	164%	< Val ≤	200%	< Val
N. caninum	Val ≤	12%	< Val ≤	40%	< Val ≤	69%	< Val ≤	97%	< Val ≤	125%	< Val
C. burnetii	Val ≤	43%	< Val ≤	64%	< Val ≤	84%	< Val ≤	105%	< Val ≤	125%	< Val
Salmo. dublin	Val ≤	65%	< Val ≤	80%	< Val ≤	95%	< Val ≤	110%	< Val ≤	125%	< Val
BVDV	Val ≤	20%	< Val ≤	40%	< Val ≤	60%	< Val ≤	80%	< Val ≤	100%	< Val

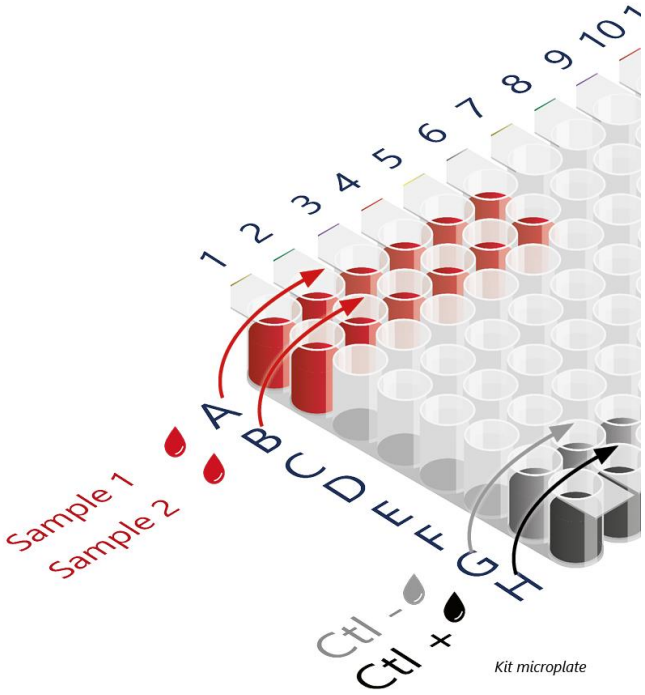
Notes*

1 Distribute 100 µL of diluted samples 1/100
& 100 µL of diluted kit controls (Ctl+ & Ctl-) 1/100



Kit microplate

2 Add 100 µL of conjugate 1/50

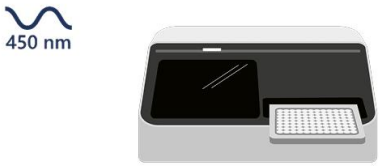


Kit microplate

3 Add 100 µL of TMB

4 Add 100 µL of stop solution

5 Record optical densities



*Notes do not replace the insructions for use of which they are synthesis.

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