Bovine Leukemia Virus Antibody Test Kit (ELISA)

Bovichek® BLV

Product code: TRM-506 (5 plates)
TRM-509 (2 plates)

- ✓ **Highly sensitive ELISA kit**
- ✓ **Can detect antibodies in serum and milk**
- ✓ **Approved by the Canadian Food Inspection Agency**
- ✓ **Available in two different formats**

INTRODUCTION

BLV is a retrovirus which causes enzootic bovine leukosis (EBL). Most infections with BLV are subclinical, but a proportion of adult cattle develop persistent lymphocytosis, and eventually lymphosarcomas (tumours) in various organs. Clinical signs, if present, depend on the organs affected.

BLV is present in blood lymphocytes and in tumour cells as provirus integrated into the DNA of the cell. It is also found in the cellular fraction of various body fluids (e.g. saliva, milk). Natural transmission depends on the transfer of infected cells (e.g. during parturition). Some blood-sucking insects may also transmit the virus mechanically. Artificial transmission occurs, especially by blood-contaminated needles, surgical equipment, gloves used for rectal examinations, etc.

Infection with BLV in cattle gives rise to a persistent antibody response. Antibodies can first be detected 3-16 weeks after infection. They are present in both serum and milk. Maternally derived antibodies may take up to 6 or 7 months to disappear. The antibodies most readily detected are those directed towards the envelope glycoprotein gp51 of the virus.

Routine diagnosis of BLV infection is based on the detection of specific antibodies. Serological testing is used in certification programs, eradication programs and for commercial exchanges.

The "Bovine Leukemia Virus Antibody Test Kit (ELISA)" (commercial name: Bovichek® BLV) has been approved by the Canadian Food Inspection Agency (CCVB file number 810DR/B7.2/D10) in 2010.

INTENDED USE

This kit is based on an immunoenzymatic assay for the detection of antibodies against bovine leukemia virus (BLV) in bovine serum and milk samples.

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PRINCIPLE OF THE TEST

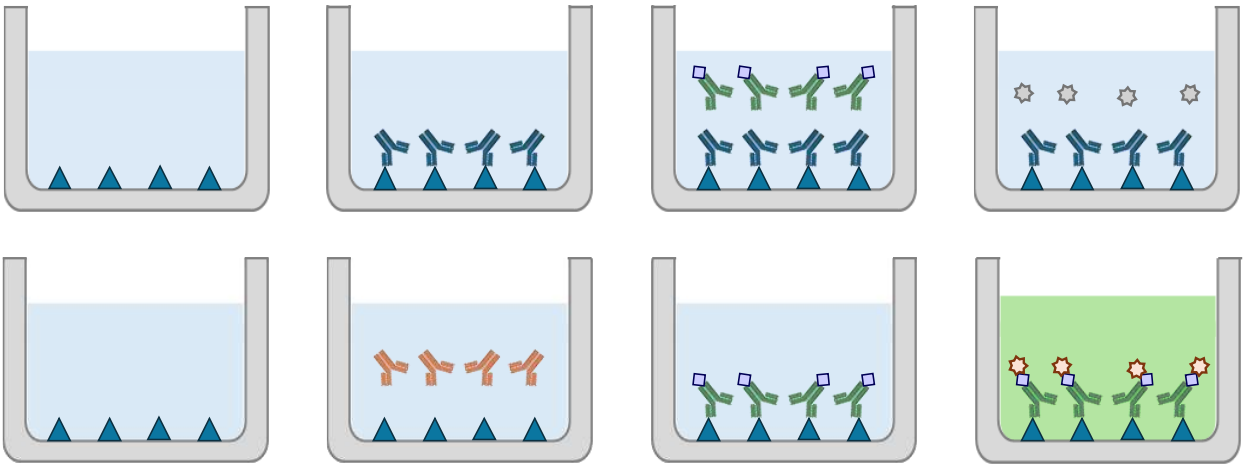
a. Assay description

1. The wells are coated with a purified antigen (gp51) from the envelope of the bovine leukemia virus (BLV).

2. Samples are added and incubated for 1 hour at 37 ± 1°C. Each well is then washed carefully to remove unbound material.

3. An HRPO-conjugated monoclonal antibody, specific to BLV gp51 antigen, is added and incubated for 30 min at 37 ± 1°C. Washing steps follow.

4. The substrate is added and incubated for 10 min at 23 ± 2°C. It will react with HRPO if present. The reaction is stopped, and OD values are measured.



Low OD values resulting in a high PI (%).

High OD values resulting in a low PI (%).

BLV gp51 antigen | Negative Serum Antibodies | Positive Serum Antibodies | Monoclonal Ab coupled with HRPO | Reacting Substrate

b. Results interpretation

OD values are used to calculate the percentage of Inhibition (PI) as follow:

$$PI\ (\%) = [\ 1 - (\ \frac{OD_{sample}}{mOD_{neg}}\ )\ ] \times 100$$

The following cut off values were chosen:

Result	PI (%) for Serum	PI (%) for Milk
Negative	0.00 – 34.99%	0.00 – 19.99%
Suspect*	35.00 – 44.99%	20.00 – 29.99%
Positive	45.00 – 100.00%	30.00 – 100.00%

* A second serum sample should be collected 2 weeks later and retested

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

- The mean OD of the Negative Control must be more than 5 times the mean OD value of the Positive Control.
- The mean OD of the Negative Control must be higher than 0.8.

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TECHNICAL DATA

a. Sensitivity & Specificity

A panel of 200 serum samples were used for evaluating the test sensitivity & specificity (A). The negative samples (n = 100) originated from herds certified to be bovine leukosis-free by the Canada Health Accredited Herd Program of the Canadian Food Inspection Agency (CFIA). The positive samples (n = 100) were collected from herds which had demonstrated several BLV-antibody positive animals using a reference test. The SVANOVIR® BLV-gp51-Ab kit (the only ELISA kit approved by the CFIA at the time of the study validation) was used as gold standard.

A field study was also conducted by the Canadian Food Inspection Agency, St-Hyacinthe Laboratory (CFIA) with milk samples (n = 411), using the HerdChekTM Bovine Leukemia Virus Antibody Test Kit (manufactured by Idexx) as gold standard (B). In addition, paired serum and milk samples from 180 cows were tested with Bovichek BLV kit to assess the agreement between the milk and serum results (C).

A	Reference +	Reference -	Total
Biovet +	99	1	**100**
Biovet -	0	100	**100**
Total	**99**	**101**	**200**

Statistic	Value	95% CI
Relative sensitivity	100.0%	96.34 - 100.0%
Relative specificity	99.01%	94.61 - 99.97%
Agreement	99.50%	

B	Reference +	Reference -	Total
Biovet +	79	0	**79**
Biovet -	1	331	**332**
Total	**80**	**331**	**411**

Statistic	Value	95% CI
Relative sensitivity	98.75%	93.23 - 99.97%
Relative specificity	100.0%	98.89 - 100.0%
Agreement	99.80%	

C	Serum +	Serum -	Total
Milk +	84	0	**84**
Milk -	1	95	**96**
Total	**85**	**95**	**180**

Statistic	Value
Agreement	99.40%

The study, with both serum and milk samples, showed an excellent agreement between Biovet kit and the gold standard. Detection of antibodies in paired serum and milk samples were also very similar.

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b. Repeatability

The intra-plate (A), inter-plate (B) and inter-lot (C) repeatability was evaluated using 6 samples. 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 OD values were used to calculate the Coefficient of Variation (CV).

A

Sample	Well 1	Well 2	Well 3	Mean ± SD	CV (%)
Positive	0,060	0,063	0,075	0,066 ± 0,008	**12,0**
Positive	0,058	0,062	0,054	0,058 ± 0,004	**6,9**
Weak positive	0,653	0,638	0,634	0,642 ± 0,010	**1,6**
Weak positive	0,834	0,877	0,770	0,827 ± 0,054	**6,5**
Negative	2,376	2,569	2,324	2,423 ± 0,129	**5,3**
Negative	2,330	2,559	2,206	2,365 ± 0,179	**7,6**

B

Sample	Well 1	Well 2	Well 3	Mean ± SD	CV (%)
Positive	0,060	0,067	0,057	0,061 ± 0,005	**8,4**
Positive	0,058	0,063	0,064	0,062 ± 0,003	**5,2**
Weak positive	0,653	0,617	0,588	0,619 ± 0,033	**5,3**
Weak positive	0,834	0,761	0,690	0,762 ± 0,072	**9,5**
Negative	2,376	2,331	2,037	2,248 ± 0,184	**8,2**
Negative	2,330	2,359	2,328	2,339 ± 0,017	**0,7**

C

Sample	Serial A	Serial B	Serial C	Mean ± SD	CV (%)
Positive	0,060	0,063	0,065	0,063 ± 0,003	**4,0**
Positive	0,058	0,069	0,059	0,062 ± 0,006	**9,8**
Weak positive	0,653	0,707	0,583	0,648 ± 0,062	**9,6**
Weak positive	0,834	0,854	0,706	0,798 ± 0,080	**10,1**
Negative	2,376	2,309	2,328	2,338 ± 0,035	**1,5**
Negative	2,330	2,416	2,232	2,326 ± 0,092	**4,0**

The results demonstrate the Bovichek® BLV kit's high repeatability. No sample had a CV greater than 15% in each study.

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c. Stability

Eleven (11) samples, with a reactivity ranging from negative to strongly positive were selected. Each sample was tested with 3 distinct lots of the BLV kit at different time points (0 and 13 months) postproduction. The kits used for the stability tests were stored at 2-8°C until final testing.

Sample ID	Reactivity	Serial A		Serial B		Serial C	
		0 mo.	13 mo.	0 mo.	13 mo.	0 mo.	13 mo.
Positive ctrl	NA	95,82	96,12	95,19	96,33	95,23	96,30
CQ#70	Negative	9,25	4,19	12,61	1,04	11,46	9,69
CQ#71	Negative	0,00	0,00	0,00	0,00	0,00	0,00
CQ#72	Negative	0,00	0,00	0,00	0,00	0,00	0,00
CQ#247	Weak positive	65,83	62,49	69,82	65,55	67,04	68,62
CQ#262	Positive	76,80	80,03	77,24	81,50	75,99	81,42
CQ#249	Positive	77,68	66,37	81,62	71,32	78,50	74,57
CQ#251	Positive	84,33	80,39	85,43	81,29	84,02	82,13
CQ#265	Positive	85,54	85,31	87,00	86,72	85,43	86,85
CQ#266	Positive	84,52	84,74	87,89	86,01	86,07	85,55
CQ#74	Strong positive	94,93	96,79	95,77	96,99	96,50	96,73
CQ#344	Strong positive	96,42	96,53	96,58	96,53	96,32	97,09

The PI of each sample remained stable over time for all three serials. No different results in terms of positivity were observed throughout the study. Tested samples stayed well within range of expected results at each time point.

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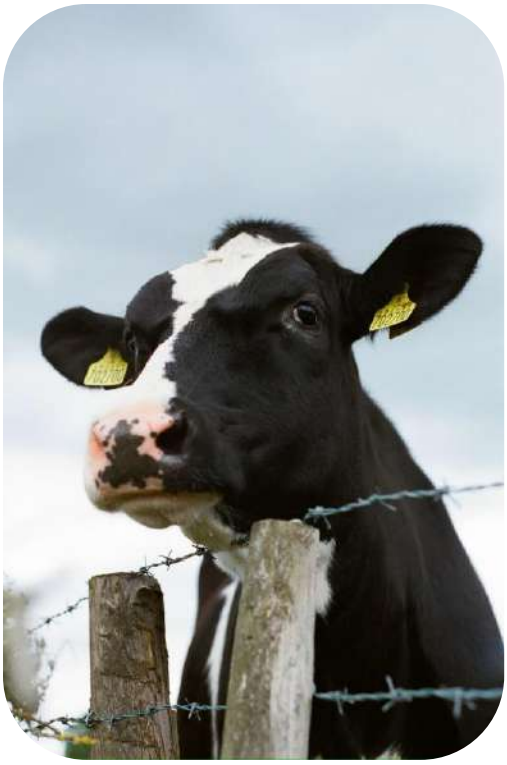
CONCLUSION

The Bovichek BLV kit has demonstrated excellent performances in terms of stability, repeatability, reproducibility, relative sensitivity, relative specificity and agreement when compared to the “gold standard”. This kit is licensed by the Canadian Centre for Veterinary Biologics.

KIT COMPOSITION

Components	Quantity TRM-500	Quantity TRM-506
12 strips of 8 wells coated with gp51 of BLV	2	5
Ready-to-use Positive Control	2 mL	4 mL
Ready-to-use Negative Control	2 mL	4 mL
Ready-to-use Dilution Buffer	50 mL	125 mL
Concentrated Conjugate	Variable	Variable
Concentrated Wash Solution (20X)	2 x 125 mL	2 x 125 mL
Ready-to-use Substrate	25 mL	60 mL
Ready-to-use Stop Solution	25 mL	60 mL



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