

## INTERPRETATION

### Calculation of the Inhibition Percentage

Calculate the mean of ODs obtained for the negative control ( $mOD_{neg}$ ). Divide the OD obtained for each sample by the  $mOD_{neg}$ . Then, subtract these results from 1 and multiply by 100 to obtain the inhibition percentage (%).

$$\text{Inhibition percentage (\%)} = \left[ 1 - \left( \frac{OD_{sample}}{mOD_{neg}} \right) \right] \times 100$$

### Validity Criteria

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.

### Interpretation

	Serum	Milk
BLV-Ab positive	inhibition % greater or equal to 45%	inhibition % greater or equal to 30%
BLV-Ab suspicious <sup>1</sup>	inhibition % greater or equal to 35% but less than 45%	inhibition % greater or equal to 20% but less than 30%
BLV-Ab negative	inhibition % less than 35%	inhibition % less than 20%

<sup>1</sup> A second serum or milk sample should be collected 2 weeks later and retested.

## Bovine Leukemia Virus Antibody Test Kit, ELISA Bovichek® BLV – 2 plates Insert

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This kit is based on an immunoenzymatic assay for the detection of antibodies against bovine leukemia virus (BLV) in bovine serum and milk. Samples can be tested individually or pooled (up to 10 serum samples or 2 milk samples per pool).

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.

## PRINCIPLE OF THE TEST

This kit is a blocking enzymatic immunoassay (blocking ELISA) based on the use of monoclonal antibodies against the viral gp51 protein. It allows the detection of antibodies against BLV in bovine serum or milk. Controls and serum or milk samples are incubated in wells coated with BLV gp51 antigen. After several washes to eliminate unbound substances, a monoclonal antibody (Mab) coupled to an enzyme (conjugate) and specific to BLV gp51 antigen is added. The Mabs bind to the antigenic sites that have not been bound by the antibodies present in positive samples. After incubation, the excess of Mabs is eliminated by a second wash and their attachment is revealed with a chromogenous substrate. Following this incubation, the enzymatic portion of the conjugate, if present, reacts with the substrate and a blue color develops. The reaction is then stopped (the color changes from blue to yellow) and the optical densities are read. The intensity of the color allows the determination of the status of sample tested. A strong positive will show a weak reaction (pale yellow), whereas a negative sample will show a strong reaction (dark yellow). All shades of yellow between dark and pale represent various degrees of positivity.



## MATERIAL

Components	Quantity
• 12 strips of 8 wells coated with BLV gp51 Ag	2
• Ready to use positive control	2 mL
• Ready to use negative control	2 mL
• Ready to use dilution buffer	50 mL
• Concentrated conjugate	250 - 350 µL**
• Concentrated wash solution (10X)*	125 mL
• Ready to use substrate	25 mL
• Ready to use stop solution	25 mL

\* Crystals may form when wash solution is kept at 2-7°C. This will not affect the efficiency of the product. However, crystals have to be dissolved by bringing the solution to room temperature and agitating.

\*\* Precise volume depends on the lot of conjugate and the recommended working dilution (see section C).

The materials provided are sufficient for testing up to 184 samples.

### Material Required but not Provided:

- Purified water
- Adjustable single- and multi-channel micropipettes (5 to 300 µL)
- Single-use micropipette tips
- ELISA microplate washer (optional)
- Containers for preparation of solutions (from 5 to 250 mL)
- ELISA 96-well microplate reader equipped with 450 nm filter
- Test tubes for sample dilution

### PRECAUTIONS

- For *in vitro* veterinary use only.
- Store all kit components at 2-7°C and bring all components to room temperature before use **except conjugate**. Reagents should be stored back at appropriate temperature as soon as possible after use.
- If using partial plates, remove only the wells required for the samples to be tested. Then put the remaining wells, along with the supplied desiccant, in a sealed bag and store them at 2-7°C.
- Do not use the kit after the expiry date indicated on the package.
- Do not intermix instructions or components from different serials.
- The material used in this kit must be considered as potentially infectious. Therefore, all waste must be decontaminated before being discarded.
- Do not expose the substrate to either light or oxidizing agent. Always keep the substrate in a plastic container. This solution may cause skin or eye irritation.
- The stop solution contains a strong acid and must be manipulated with caution to avoid contact with skin or mucous membranes.
- Dispose of 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 at 1/10 with purified water (e.g., 100 mL of 10X concentrated wash solution in 900 mL purified water). Once diluted, the solution (1X) is stable for 1 week at 2-7°C.

### B. Sample Preparation

Serum: dilute samples at 1/2 in the dilution buffer (e.g., 100 µL sample in 100 µL dilution buffer). It is important to use a new tip for each sample. Make sure that each solution is properly mixed before being distributed into the wells.

Milk: use skimmed milk. Do not dilute samples. Use a new tip for each sample.

### C. Conjugate Preparation

Dilute the conjugate with the dilution buffer according to the dilution indicated on the Final Control Sheet. Dilute conjugate a few minutes prior to its use and return the concentrated conjugate at 2-7°C immediately after dilution. Prepare only the quantity required every time.

### D. Test Procedure

Bring all reagents to room temperature **except conjugate** (see section C) and mix well manually before use. Once used, reagents have to be stored back at appropriate temperature as soon as possible.

1. Make a schematic representation of the distribution of samples and controls on the plate.
2. Dispense 100 µL of ready to use positive control, ready to use negative control or samples (see section B) into appropriate wells (it is recommended to run control sera in duplicates).
3. Cover the wells and incubate at  $37 \pm 1^\circ\text{C}$  for 1 hour.
4. Wash each well 3 times with 300 µL of 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 an absorbent paper. Do not keep the wells dry more time than strictly needed.
5. Dispense 100 µL of diluted conjugate (see section C) into each well.
6. Cover the wells and incubate at  $37 \pm 1^\circ\text{C}$  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 keep the plate at  $23 \pm 2^\circ\text{C}$ , in the dark, for 10 minutes.
10. Dispense 100 µL of ready to use stop solution into each well following the same order in which the substrate was added.
11. Measure optical densities (OD) at 450 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.

