



#### Revised 15 Nov. 2007

## For Veterinary Use Only

#### **INTRODUCTION**

Serological identification of cattle infected with Bovine Leukaemia Virus (BLV) can be performed by screening for antibodies against the major core protein of BLV, a 24,000 dalton polypeptide (p24) and against the major envelope antigen, a 51.000 dalton glycoprotein (gp51).

The agar gel precipitation test (AGPT), and conventional enzyme-linked immunosorbent assays (ELISA) have proved to be adequate for antibody detection in serum. However, more sensitive and specific ELISA's are required for detection of the low antibody titres usually found in milk samples. An indirect ELISA system has been developed for screening programs. This test enables BLV antibodies to be detected in individual or pooled milk and serum samples. The specifications of this test kit are in accordance with the guidelines provided by the European Community (88/406/EC). According to the same guidelines 10 to 20 samples can be pooled.

### **INTENDED USE OF THE TEST KIT**

This diagnostic test system for BLV infected cattle is intended to identify BLV p24 and gp51 antibodies in individual or pooled serum samples.

The sensitivity of the test is adjusted to the standards of the European Community with reference to the E4 serum and meets the requirements for testing pooled serum samples in the European enzootic bovine leukosis (EBL) screening program. In contrast to test systems which make use of polyclonal antibodies or only one gp51 monoclonal antibody, this two monoclonal antibodies mediated ELISA gives a minimum of non-specific reactions.

#### **STANDARDIZATION**

Standardisation against the E4 serum has been developed in such a way that a positive signal is obtained after a dilution of E4 serum (either in buffer or in negative serum) of at least 1:2500. (Hoff Jorgensen, R., et al., 1989)

#### PRINCIPLE OF THE TEST KIT

The test is based on the reaction of anti-BLV-p24 and anti-BLV-gp51 antibodies in test samples with BLV antigen. To this end, monoclonal anti-BLV-p24 and anti-BLV-gp51 antibodies have been coated to a 96 well microtiter plate. Inactivated BLV p24/gp51 antigens added to the wells are captured by the coated monoclonal antibodies.

After washing, test serum samples are added to the wells, and if present, anti-BLV-p24 and anti-BLV-gp51 antibodies from the samples will bind to the antigen.

The complex is detected by a horseradish peroxidase (HRPO) conjugated monoclonal antibody directed against bovine IgG1.

Colour reaction in the wells is directly related to the presence of BLV-p24 and BLV-gp51 antibodies in the sample.

#### **CONTENTS**

- 1 x 96 well **microtiter plate** coated with BLV antigen.
- 1 x 11 ml HRPO-conjugated anti-bovine IgG monoclonal antibody.
- 1 x 1 ml inactivated **positive control** serum (freeze-dried).
- 1 x 1 ml inactivated **negative control** serum (freeze-dried).
- 1 x 20 ml wash solution 200 x concentrated, must be diluted in deionized water before use!
- 1 x 12 ml conjugate diluent

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- 1 x 12 ml ELISA buffer
- 1 x 11 ml substrate mix (ready to use)
- 1 x 8 ml stop solution.
- 1 x plastic cover seal.

Each kit contains sufficient reagents to perform 96 tests.

## HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at +4°C.

After reconstitution the lyophilised reagents should be used immediately.

Positive and negative controls may be stored after reconstitution in aliquots at -25°C and used until the expiry date. Repeated freezing and thawing must be avoided as this increases non-specific reactivity and decreases specific reactivity. Fresh samples can be used. However addition of 0.1% sodium azide to the samples has no influence on the results of the test.

## WASHING PROTOCOL

In ELISA's uncomplexed components must be removed efficiently between each immunological incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is advised to carefully follow the washing procedures outlined below.

Both manual washing and washing with automatic equipment can be performed. (Automatic washing equipment usually gives better results).

### Manual washing

- 1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical movement to remove the buffer.
- 2. Fill all the wells with 250  $\mu$ l washing solution.
- 3. This washing cycle (1 and 2) should be carried out <u>at least 4 times</u>.
- 4. Turn the plate upside down and empty the wells by a firm short vertical movement.
- 5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual washing solution in the wells.
- 6. Take care that none of the wells dries out before the next reagent is dispensed.

### Washing with automatic equipment

When using automatic plate wash equipment, check that all wells can be aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute <u>at least 4 washing cycles</u>.

### **PREPARATION OF SPECIMENS**

Individual or pooled serum samples (up to 15 samples can be pooled) should be diluted 1:25 in ELISA buffer. Milk samples should be centrifuged 15 min 2500 g to remove the lipid layer.





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### **TEST PROTOCOL**

1. Allow samples to come to room temperature. <u>Samples and controls should preferably not be diluted in the coated</u> <u>microtiter plate</u>, but in a separate round bottomed microtiter plate.

Reconstitute negative and positive controls in 1 ml Demi water (not provided). Add 100  $\mu$ l negative control (diluted 1:25) and 100  $\mu$ l positive control (diluted 1:25) to 3 wells each. Add 100  $\mu$ l of the diluted (1:25) serum samples (preferable in duplo) to the 90 remaining wells of the coated microtiter plate. The centrifuged milk samples should be used undiluted (100  $\mu$ l per sample) milk samples should be tested pre

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- 2. Seal the microtiter plate and incubate for 60 minutes at 37°C.
- 3. Wash the microtiter plate with wash solution, according to wash protocol. The wash solution provided must be diluted 200 x in deionized water!
- 4. Reconstitute the freeze dried vial with 11 ml of conjugate diluent. Add 100 µl conjugated antibody to all wells.
- 5. Seal and incubate 1 hour at 37°C.
- 6. Wash as in 3.
- 7. Add 100 µl substrate solution to each well. Incubate for 15 min. at room temperature (21°C.).
- 8. Add 50 µl stop solution to each well.
- 9. Read the absorbency value immediately, within 10 min. at 450 nm. Use 620 nm as reference wave length.

#### **PRECAUTIONS**

- Handle all biological materials as though capable of transmitting BLV.
- Do not pipette by mouth.
- Do not eat, drink, smoke, prepare foods, or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past expiry date and do not intermingle components from different serial lots.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing
- throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore do not touch the under-surface of the microtitre plate and prevent it form damage and dirt.

### VALIDATION OF THE TEST

In order to confirm appropriate test conditions, the mean absorbency value of the negative control should be lower than 0.200 OD units and the mean OD value of the positive control provided should be higher than 1.0 OD units.

#### **INTERPRETATION OF TEST RESULTS**

A sample is scored BLV negative if the OD value is below or equal to the average OD value of the negative control plus 0.150 OD units.

**Negative:** OD samples  $\leq$  OD negative control plus 0.150 OD units.





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In case of **milk samples** the following calculation should be used:

A sample is scored BLV negative if the OD value is below or equal to the average OD value of the negative control plus 0.100 OD units. Negative: OD samples  $\leq$  OD negative control plus 0.100 OD units.

**<u>NOTE</u>**: If a sample does not meet these criteria for being negative, the following protocol is advised: <u>Serum samples</u>: Confirmation of positive results can be achieved by testing individual serum samples in the Bovine Leukaemia Serum complex-trapping-blocking ELISA test or PCR.

#### **REFERENCES**

Hoff Jorgensen, R., 1989, An international comparison of different laboratory tests for the diagnosis of bovine leukosis: suggestions for international standardisation.

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