Internal validation report

ID Screen® BLV Competition

Competitive ELISA kit for the detection of antibodies against the gp51 envelope protein of the Enzootic Bovine Leukosis Virus.

- **Excellent specificity and sensitivity**
- **Calibrated to detect the French substandard of the international E05 serum standard**
- **Straightforward, versatile test,** with short or overnight incubations for individual or pooled serum or plasma.

Introduction

Bovine leukemia virus (BLV), an oncogenic retrovirus, is widely distributed and endemic in many cattle herds. The most important disease caused by BLV is enzootic bovine leukosis, essentially a form of malignant lymphoma (ML). ML develops in only a small percentage of cattle infected with BLV, but it is a fatal disease characterized by lymphomatous involvement of multiple organs. Horizontal transmission of BLV is probably the primary means by which cattle become infected, although both vertical and vector transmission can also occur.

Most cattle infected with BLV do not exhibit clinical signs. BLV infection is life-long in cattle, so demonstration of serum antibodies to BLV indicates persistent infection.

The **ID Screen® BLV Competition** ELISA may be used as part of disease surveillance and eradication programs to detect anti-gP51 antibodies in bovine serum and plasma.

Test Principle

Wells are coated with gp51 antigen.

Samples to be tested and the controls are added to microwells. Anti-gp51 antibodies, if present, form an antigen-antibody complex which masks the gp51 epitopes.

A gp51-peroxidase (HRP) conjugate is added to the wells. It fixes to the remaining free gp51 epitopes, forming an antigen-conjugate-HRP complex.

After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested. In the absence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution. In the presence of antibodies, no coloration appears after addition of the Stop Solution

The microplate is read at 450 nm.

Result interpretation:

For each sample, the competition percentage (S/N%) is calculated: $[OD_{sample} / OD_{NC}] \times 100$.

Samples presenting a S/N% less than or equal to 50% are considered positive, greater than 50% and less than 60% are considered doubtful, and greater than or equal to 60 % are considered negative.

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Analytical sensitivity

The test is calibrated with respect to the French substandard of the international E05 serum standard, which must be found positive diluted 1:10.

Results (Figure 1):

- The French substandard serum diluted 1/10 was found positive regardless of the protocol used.

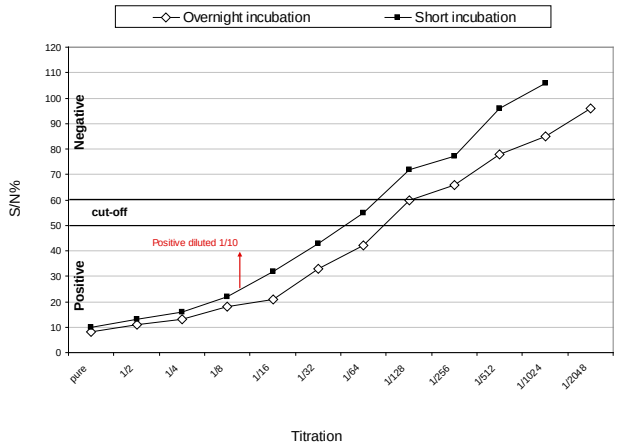


Figure 1: Titration of the French substandard serum.

Sensitivity

100 individual sera were tested. These sera gave positive or weak-positive results with the AGID (Agar Gel Immunodiffusion) method.

These sera were then tested diluted 1/10 in negative sera.

Results (Figure 2):

- All sera gave positive results.
- Individual samples**: measured sensitivity = 100% ($CI_{95\%}$: 96.3-100%), n=100.**
- Sera diluted 1/10**: measured sensitivity = 100% ($CI_{95\%}$: 96.3-100%), n=100.**

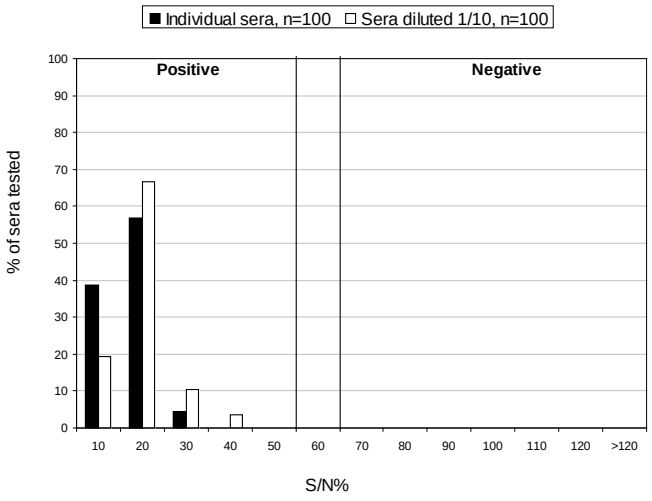


Figure 2: S/N% distribution for positive sample population

Specificity

Disease-free herds

The following sera from disease-free certified herds from France (Hérault and Brittany) were tested:

- 1150 individual sera
- 300 pools of 10 sera

Results (Figure 3):

- All sera were found negative.
- Individual samples**: measured specificity = 100% ($CI_{95\%}$: 99.67-100%), n=1150.**
- Pooled samples**: measured specificity = 100% ($CI_{95\%}$: 98.74-100%), n=300.**

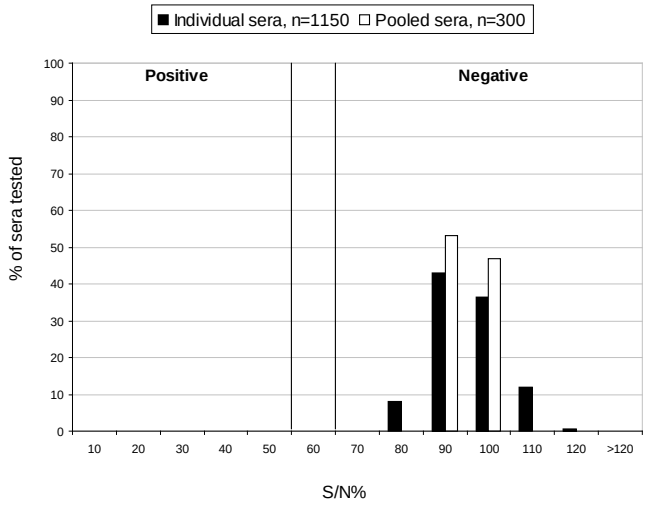


Figure 3: S/N% distribution for negative sample population.

Vaccinated animals

50 sera from cattle vaccinated with the Mucobovin BVD inactivated Merial vaccine were tested. These sera gave high background or false positive results when tested on commercial indirect BLV ELISAs.

Results:

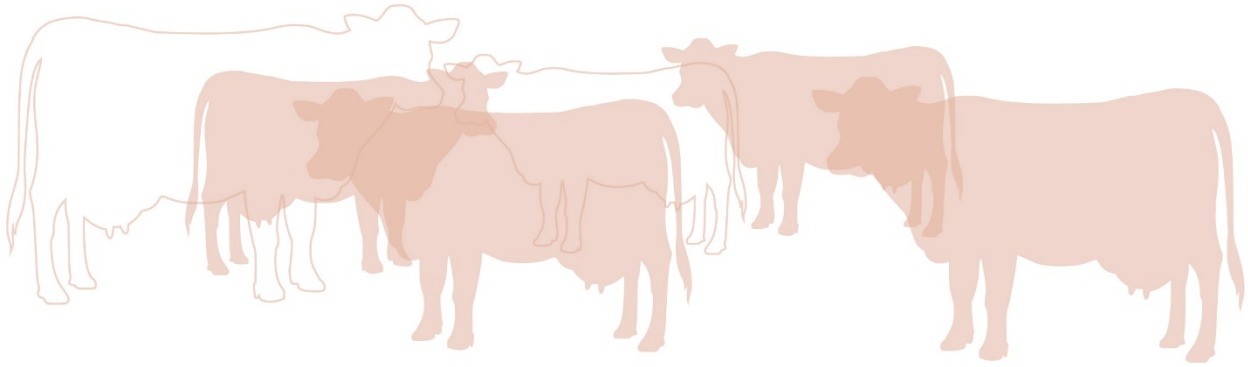
- None of these sera gave non-specific results with the ID Screen® ELISA.

Conclusion

The **ID Screen® BLV Competition** ELISA demonstrated high specificity and sensitivity. It is calibrated to detect the French substandard serum of the International E05 serum standard. It is a reliable tool for the surveillance of BLV infection. In addition, the test is easy-to-use with results in 90 minutes.

References

(1) OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2012. Chapter 2.4.11: Enzootic Bovine Leukosis.



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