

AntelBio[®] Leukosis Milk ELISA

Highlights

- Detects antibody titers to Bovine Leukosis Virus (BLV) in fresh, frozen and DHI-preserved milk samples.
- Test sensitivity is 98.1%, specificity is 100% and overall agreement is 98.9% compared to the serum ELISA.
- The effect of cross contamination in a small percentage of samples (<5%) collected during the DHI process is eliminated by the designation of a **suspect** category and subsequent analysis of hand-stripped samples on the next test date.
- Can detect the presence of one positive cow in a 125 cow dairy using a bulk tank sample for analysis.

Introduction

The AntelBio Leukosis Milk ELISA is an extremely sensitive assay that detects antibody titers to Bovine Leukosis Virus (BLV) in fresh, frozen and DHI-preserved milk samples. The extreme sensitivity of the milk ELISA coupled with the persistent production of antibody in infected animals allows the test to be used on single milk samples for the diagnosis of individual animals, and on bulk tank samples to monitor negative herds. The high overall agreement between the milk ELISA and a USDA-licensed serum ELISA suggests that milk testing can be effectively substituted for serum testing in BLV control programs. When incorporated with routine DHI sampling, BLV testing with the milk ELISA eliminates the additional labor and expense required to obtain and ship blood samples in traditional BLV control programs. Results are available five business days after the samples are received in the testing center.

Milk ELISA Validation

The AntelBio Leukosis Milk ELISA was evaluated against the serum ELISA on hand-stripped milk samples and milk samples obtained via the DHI collection process. Serial dilution experiments were also conducted to determine the limit of detection for use as a bulk tank screen in negative herds.

Comparisons between milk and serum ELISA were first conducted on hand-stripped milk samples. Table 1. shows the comparison between serum and milk analysis using the respective ELISA on samples drawn on the same day from 89 cows in two herds with a high prevalence of leukosis.

		Positive	Negative	Total
Milk ELISA	Positive	51	0	51
	Negative	1	37	38
Total		52	37	89

Compared to the serum ELISA, the AntelBio Leukosis Milk ELISA had an overall agreement of 98.9%, sensitivity of 98.1% and specificity of 100%. It is of interest to note that the value of the milk ELISA in the single mismatch (serum positive, milk negative) was high enough to be considered suspect (see below) and would have indicated subsequent retesting.

Figure 1. shows the correlation between the two assays expressed on the correlation scale of -1 (complete negative correlation) to 0 (no correlation) to +1 (complete positive correlation). An R^2 of .90 demonstrates the direct relationship between antibody titers in milk and those found in the blood. Such a relationship is consistent with the process of passive immunization whereby antibodies are transferred from serum to milk to provide immediate disease protection for suckling calves.

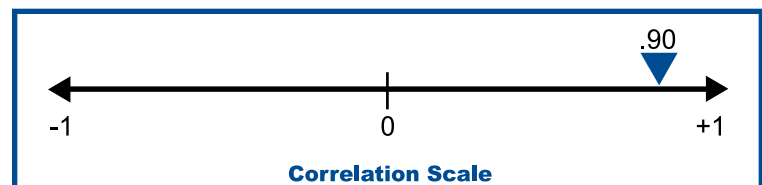


Figure 1. Correlation between AntelBio Leukosis Milk ELISA and a commercial serum ELISA.

Serial dilutions of positive milk samples were analyzed with the milk ELISA for a crude measure of analytical sensitivity. Figure 2. shows that positive milk samples can be diluted by as much as 1:125 and still generate a positive signal. This data indicates the assay can readily

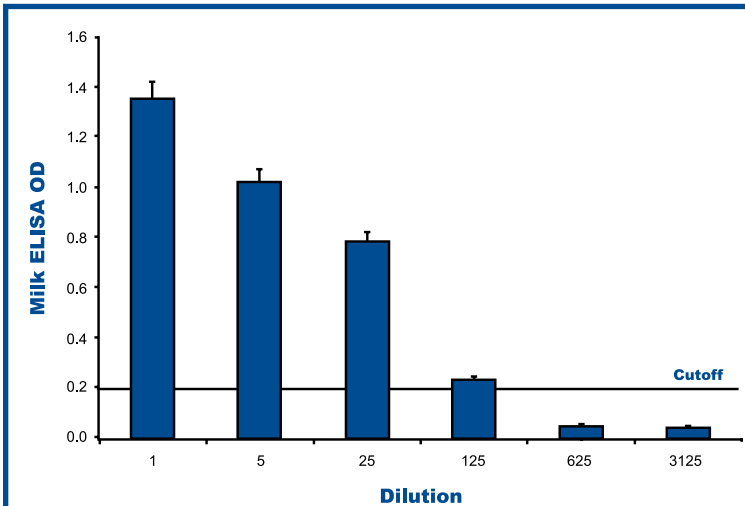


Figure 2. Optical density (OD) values of leukosis-positive milk samples serially diluted with negative controls. Values greater than 0.2 are considered positive for the presence of leukosis

detect the presence of one positive cow in a 125 cow dairy using a sample from the bulk tank for analysis. In leukosis-free herds, monthly bulk tank screens offer an economical surveillance system to quickly detect the introduction of leukosis.

While this extraordinary level of sensitivity is fortuitous for the accurate detection of leukosis in individually collected milk samples, it potentially presents a problem for the analysis of DHI milk samples due to the carry-over or cross contamination that occurs in the DHI metering process. Assuming an average carry-over of 1% (1:100 dilution)² in the milk meters, the milk ELISA was also validated with an additional 1:10 dilution against the

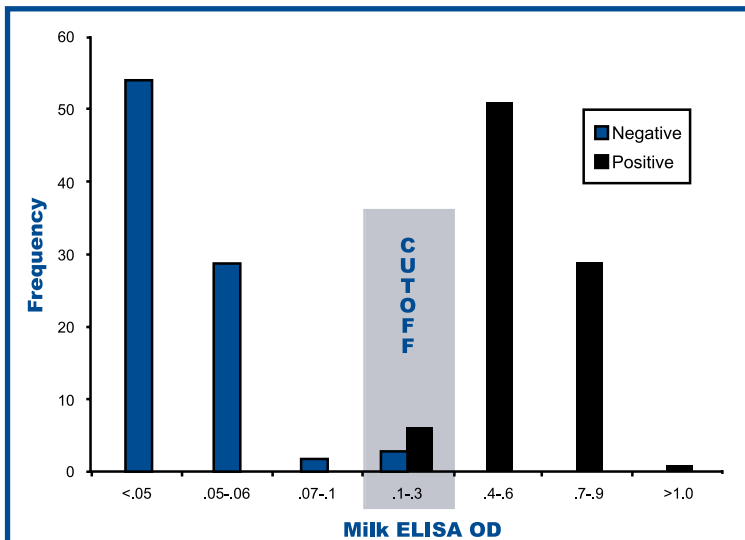


Figure 3. Frequency of milk ELISA optical density values for DHI milk samples from leukosis positive and negative cows.

serum ELISA in 176 animals comprising two whole herds with a high prevalence of leukosis.

Figure 3. shows the frequency of optical density values in seronegative and seropositive animals in relation to the cutoff score for positivity. While positive and negative animals were clearly differentiated in 95% of the animals tested, 5% of results fell into the gray area surrounding the cutoff. Milk samples (**suspects**) in the gray area must be either negative samples that have been contaminated from milk of positive animals preceding negative cows on the same milker, or positive samples from cows with low circulating antibodies. To differentiate suspects, blood or hand-stripped milk samples can be resubmitted for analysis at no additional charge.

In the present study, hand-stripped milk samples from suspect animals were analyzed, the combined results are presented in Table 2.

		Positive	Negative	Total
Milk ELISA	Positive	89	0	89
	Negative	0	87	87
Total		89	87	176

With sensitivity, specificity and overall agreement of 100% compared to serum analysis, the inclusion of a **suspect** category and subsequent reanalysis using hand-stripped milk or serum samples eliminate the effect of cross-contamination occurring in the DHI metering system.

Conclusion

With a high sensitivity and specificity, the AntelBio Leukosis Milk ELISA directly substitutes for the serum ELISA in the diagnosis of leukosis in individual milking cows. On DHI collected milk samples, the elimination of blood collection and shipping greatly reduces the overall cost of leukosis control programs in dairy herds. Therefore, the milk ELISA is an exceptional tool not only for diagnosing leukosis in infected herds, but also for prescreening prospective purchases in expanding herds.

On bulk tank samples, the high analytical sensitivity of the milk ELISA is able to detect the presence of one infected cow in herds up to 125 milking animals. Thus, periodic screening of bulk tank samples is an outstanding means of detecting the earliest introduction of leukosis in leukosis-free herds.

¹IDEXX Laboratories, Portland, Maine.

²Tru-Test, Mineral Wells, Texas.