



Bovine Viral Diarrhea Milk ELISA

Highlights

- AntelBio Bovine Viral Diarrhea (BVD) Milk ELISA detects BVD viral antigens present in fresh or DHI-preserved milk samples.
- Test sensitivity, specificity and the overall agreement are 100% relative to BVD ear notch or serum ELISA.
- The presence of milk preservative and milk fat does not affect the sensitivity of the assay. Testing for BVD can be conducted on samples routinely submitted for DHI testing.
- BVDV I and BVDV II isolates of the virus can be detected.

Introduction

The AntelBio BVD Milk ELISA is a highly effective tool for producers and veterinarians to positively identify cows persistently infected (PI) with BVD in their dairy operations. The present test is an excellent follow-up tool after a positive bulk tank test indicates the presence of PI animal(s) in the milking herd. The viral antigen ELISA can be conducted on fresh or DHI-preserved milk samples and eliminates individual ear notching or blood sampling required with traditional analysis. Coupled with existing DHI services, this alternative approach can effortlessly detect PI animals without investing additional resources in a BVD screening program. Results from the Milk ELISA are available within five business days following sample submission to the testing center.

Test Description

The AntelBio BVD Milk ELISA is an immunodiagnostic assay designed to detect BVD virus and viral antigens in mixed, whole milk samples. The test is a sandwich ELISA in 96-well format using an antigen-specific monoclonal antibody as the capture reagent and polyclonal antibodies for detection. Detected antigens are subsequently quantified with a horseradish peroxidase (HRP) conjugate.

Assay Validity

Individual milk samples, collected through the DHI collection process or hand-stripped samples were analyzed using the AntelBio BVD Milk ELISA and evaluated relative to serum or ear notch analysis by commercial ELISA. Individual milk samples submitted through DHIs contain varying amounts of milk fat and are often preserved with bronopol and natamycin. Therefore, the effect of preservative and milk fat on the performance of the milk ELISA was assessed. Furthermore, serial dilution experiments were conducted using milk from PI cows to demonstrate the analytical sensitivity of the Milk ELISA.

BVD Milk ELISA Sensitivity and Specificity

To determine the relative sensitivity and specificity, test results from the AntelBio BVD Milk ELISA were compared to results from serum and ear notch samples tested by a commercially available ELISA (IDEXX, BVD Antigen Test Kit, Westbrook, Maine). Table 1 compares the results obtained from 398 dairy cows that were tested individually by both methods.

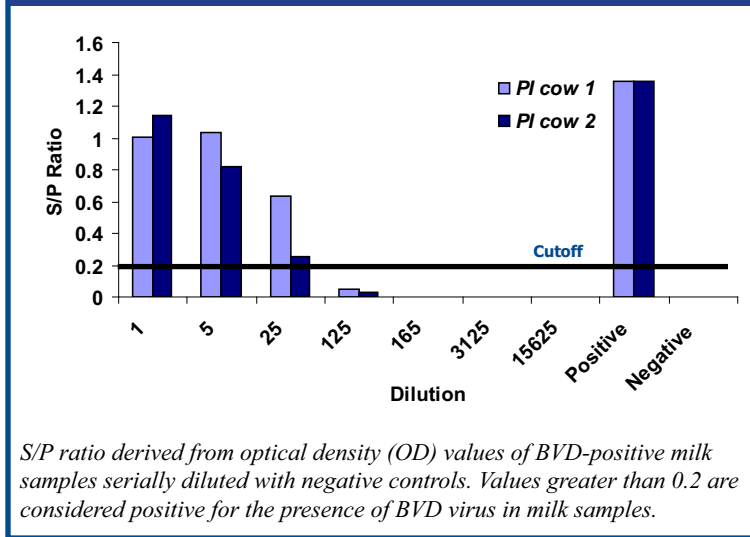
Table 1.
Milk ELISA vs. Ear Notch or Serum ELISAs

BVD Status*	AntelBio BVD Milk ELISA		
	Positive	Negative	Total
Positive	12	0	12
Negative	0	386	386
Total	12	386	398

* Tested by Commercial ELISA

Sensitivity of a test is defined as the ability to correctly identify a diseased animal as positive, whereas, specificity is defined as the ability of a test to correctly identify a non-diseased animal as negative. Data from this experiment clearly indicate that the AntelBio BVD Milk ELISA was able to correctly identify all of the PI cows as well as all of the negative cows. Therefore, relative to the serum and ear notch ELISAs, the sensitivity, specificity and overall agreement of the AntelBio BVD Milk ELISA is 100%.

Figure 1:
Analytical Sensitivity of AntelBio BVD Milk ELISA



Additional experiments were conducted on serial dilutions of milk samples from PI cows to measure the effective analytical range of the assay. Figure 1 illustrates the remarkable range of detection for the Milk ELISA; even at a 1:25 dilution with negative milk the BVD Milk ELISA can positively identify samples from diseased animals. These results clearly demonstrate that even during peak milk production, PI cows can be readily detected by the Milk ELISA. Therefore, the sensitivity and reliability of this screening assay make it an excellent choice to detect individual PI cows at any stage of lactation.

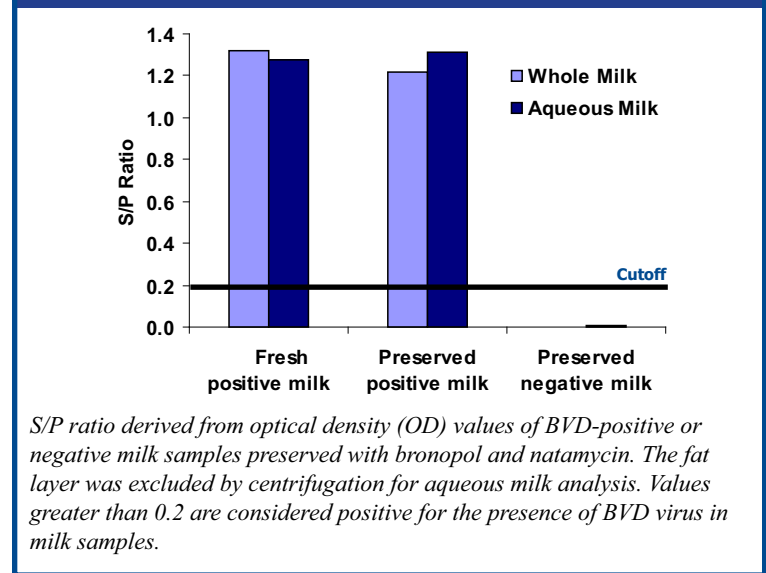
Use of DHI Preserved Milk

At regular intervals DHI collects and analyzes individual milk samples from dairy farms. If the DHI milk samples can also be used to test for BVD, it greatly reduces the cost associated with taking blood or ear notch samples and shipping them to traditional laboratories for analysis. During the collection process, the DHI milk samples are routinely preserved with bronopol and natamycin for easy storage and transportation. Milk samples from a PI cow were analyzed with or without preservative to assure preservative in milk samples would not interfere with the BVD ELISA. Similarly, tests were conducted to investigate the effect of milk fat on the efficacy of the assay.

Figure 2 shows the ELISA results of milk samples with and without preservative and milk fat. Neither milk preservative nor milk fat interfered with the performance of the assay.

Furthermore, preserved milk samples were analyzed following long-term storage (4 weeks) at room temperature with similar results (data not shown). In conclusion, these studies indicate that preserved, whole milk samples, particularly those collected through DHI can be successfully analyzed for the presence of BVD without compromising the sensitivity or specificity of the Milk ELISA.

Figure 2: Effect of preservative and milk fat on performance of BVD Milk ELISA



Conclusions

The AntelBio BVD Milk ELISA is a valuable tool for identifying PI cows in suspect dairy herds. The high sensitivity and specificity of this assay allows accurate detection of diseased animals, and the robustness of the assay allows flexibility in sample composition, collection and storage. Utilizing DHI milk samples for analysis makes screening herds for PI cows more cost effective without compromising test results. Testing individual animals using the AntelBio Milk ELISA is not only a rapid, reliable and economic way to screen existing herds, but can also be used to screen herds prior to purchase to prevent the introduction of BVD in negative herds during expansion.



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