



Bovine Viral Diarrhea Milk PCR Assay

Highlights

- AntelBio Bovine Viral Diarrhea (BVD) Milk PCR detects BVD viral RNA present in fresh or DHI-preserved milk samples.
- Assay based on viral RNA isolation and one-step RT-PCR. High test sensitivity allows bulk milk sample screening for routine surveillance.
- BVD testing can be conducted on samples routinely submitted for DHI testing.
- Test detects either BVD I or II isolates of the virus.

Introduction

The AntelBio BVD Milk PCR assay is a valuable testing tool for producers to routinely screen their bulk tanks for the presence of persistently infected (PI) animals. The PCR assay, based on BVD viral nucleic acid isolation, amplification and detection, has been shown to be highly sensitive and can consistently detect a single PI cow in a group of 250 cows.

Milk preservative does not affect the performance of the assay; therefore, preserved milk obtained through DHIs can be successfully utilized for screening. The BVD Milk PCR assay is an economical way to diagnose the presence of PI cow(s) in the milking herd before investing in individual animal testing in BVD control programs.

Test results are available within two weeks after receipt of samples at the testing center. Expedited analytical services are also available upon request.

Test Description

The AntelBio BVD Milk PCR, based on Kim and Dubovi (2003)¹, is designed to detect BVD viral nucleic acid (RNA) in milk samples. The PCR assay has 100 percent sensitivity and 96.2 percent specificity as compared to the gold standard, virus isolation¹. After isolating nucleic acid from milk somatic cells by a specialized protocol, the presence of BVD viral RNA is determined using one-step reverse transcription and DNA amplification by Polymerase Chain Reaction (RT-PCR) followed by gel analysis and quantification.

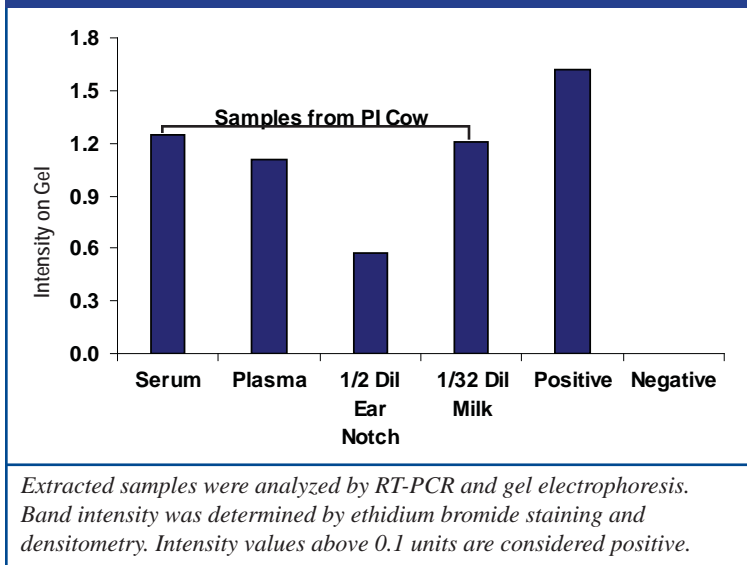
Each reaction also includes the components for the simultaneous amplification and detection of bovine-actin RNA, an internal control used to validate the performance of each individual assay. The internal control detects any problems with RNA yield or PCR during the analytical process, thus reducing the occurrence of false negatives. The assay parameters are designed to detect both BVD type I and II viral isolates.

BVD Milk PCR Validation

Bulk milk samples were simulated by serially diluting milk samples from known PI cows with BVD-free milk samples obtained from bulk tanks or through the DHI collection process. These samples were analyzed using the AntelBio BVD Milk PCR assay to determine analytical sensitivity and specificity. The PCR assay was also conducted on blood and tissue extracts for comparison. Finally, milk samples, preserved with bronopol and natamycin were stored at 4°C and tested at regular intervals to determine the stability of viral RNA in DHI samples.

Figure 1 shows the AntelBio BVD Milk PCR assay tested on different sample matrices from a known PI cow. The newly developed isolation and extraction process is acceptable for a variety of sample matrices submitted for BVD diagnosis including serum, plasma, tissue extracts and milk.

Figure 1: AntelBio BVD Milk PCR Assay



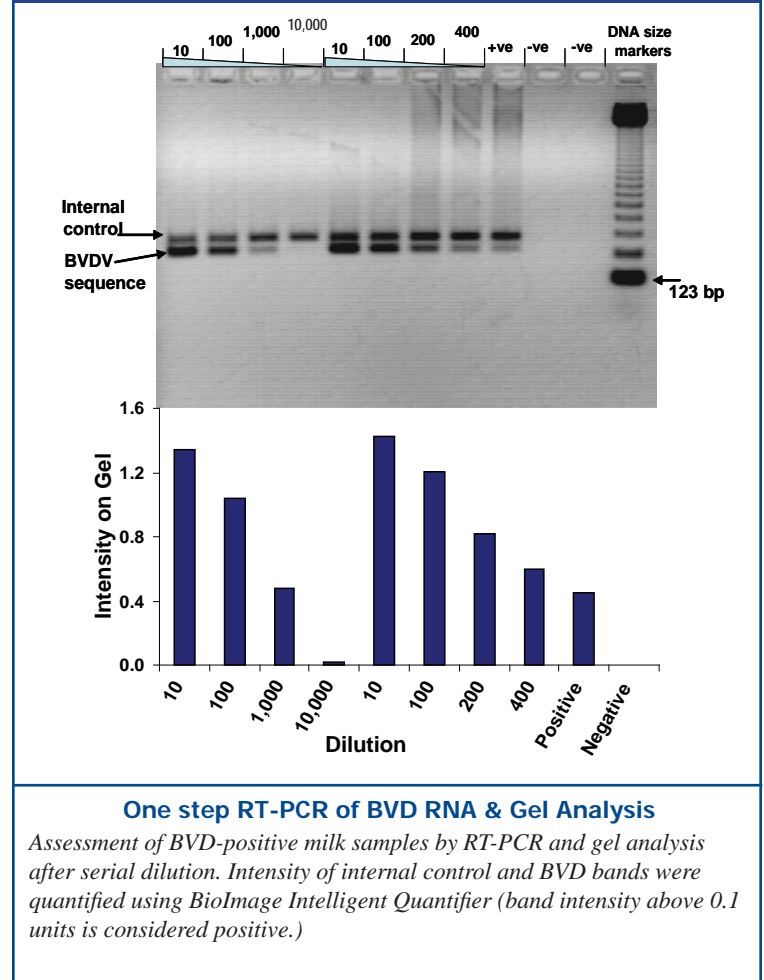
In order to establish the analytical sensitivity of the assay, sets of serial milk dilutions were prepared from PI cows and tested with the AntelBio Milk PCR assay. Results from two sets of these dilutions are shown in Figure 2, which contains an actual picture of the analysis and an accompanying bar graph that displays the band intensities of the BVD sequences. It is evident that the RT-PCR assay is capable of detecting BVD viral RNA with very high sensitivity, yielding quantifiable signals in dilutions up to 1/1000 in negative milk samples. Conservatively, a 1/250 dilution is recommended as the limit of detection to ensure adequate sensitivity.

Figure 2 also shows the internal control bands, the presence of which indicate satisfactory sample processing and RT-PCR conditions for each sample. If the internal control band is absent in any diagnostic sample, the assay is considered invalid and would need to be repeated. Therefore, negative results are reported only when proper sample processing and RT-PCR conditions have been validated.

The samples submitted through the DHI collection process often contain the preservative bronopol and natamycin. The performance of the Milk PCR assay was also assessed in the

presence of milk preservative and did not show any significant affect (data not shown).

Figure 2: Dilution series with milk from Persistently Infected (PI) Cow



Conclusion

The AntelBio BVD Milk PCR can be used to economically screen for the presence of BVD in lactating cows. Either bulk tank or DHI samples can be employed in the assay. While the analytical sensitivity suggests lower detection limits, it is recommended that group size not exceed 250 cows.



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¹Kim SG. and Dubovi EJ., A novel simple one-step single-tube RT-duplex PCR method with an internal control for detection of bovine viral diarrhea virus in bulk milk, blood, and follicular fluid samples. *Biologicals* Vol. 31 (2003) pp103-106