The Effect of Sample Handing on Test Results for Antigen Capturing ELISA for BVDV

M. C. Reed; A. M. O'Connor; K.J. Yoon; V Cooper
VDPAM, Iowa State University, Ames, IA

Introduction

Certain handling practices of diagnostic samples may affect the sensitivity and specificity of the antigen capturing ELISA (ACE) for bovine viral diarrhea virus (BVDV). Leaving samples at room temperature for an extended period of time may have a negative impact on test sensitivity, and failure to wash the ear notcher between subsequent skin sampling may lead to virus accumulation and may decrease test specificity. We tested these hypotheses in two studies by collecting known positive and negative samples and giving groups of samples different treatments.

Materials and Methods

Study 1: Assessment of the effect of storage at room temperature on sensitivity 120 skin samples were collected using a commercially available notcher from the carcass of an animal previously confirmed persistently infected with BVDV. The samples were assigned to be held at room temperature for 24, 48, 72 or 96 hours using a random number generation sequence (MS Excel®). When the samples had been at room temperature for the designated length of time, 1.0 ml of PBS was added to each tube, the samples vortexed and submitted for BVDV detection with antigen capturing ELISA (ACE). The laboratory staff were unaware of the handling of the samples. BVDV ACE was performed with a commercially available kit. Samples with an S/P ratio of less than 0.2 were considered negative for BVDV. Samples with an S/P ratio between 0.2-0.4 were retested with a modified ELISA as described by the manufacturer. Samples with an S/P ratio above 0.4 were considered positive for BVDV. Study 2: Positive samples included in the study were provided by the sample animal used in study 1. Negative samples were collected from a calf submitted to the Iowa State Veterinary Diagnostic Laboratory with a problem unrelated to BVDV. IHC and ACE were used to confirm BVDV negative status. The study assessed three methods of cleaning an ear notcher between sample collections: no cleaning between sample collection, tap water rinse between sample collection and Nolvasan solution rinse, wiped dry with a paper towel between each sample collection For each method of cleaning thirty samples were collected as follows: 1st to 9th samples from the BVDV positive calf, 10th sample from the BVDV negative calf, 11th to 19th samples from the BVDV positive calf, 20th sample from the BVDV negative calf, 21st to 29th sample from BVDV positive calf and the 30th sample from BVDV negative calf Once collected, all samples were held at room temperature for 24 hrs, then 1.0 mL of PBS added, the sample vortexed and submitted to the VDL for ACE as described in study 1. The laboratory staff was unaware of the study and the sample status. For each study, the results are reported as the count of samples that tested positive or negative.

Results

Study 1: The BVDV ACE was apparently not affected by storage at room temperature for up to 96 hours as all samples tested positive regardless of time stored at room temperature. Study 2: In each treatment, the three negative samples tested negative for BVDV based on ACE i.e. no false positives. Of the 27 positive samples collected with either no cleaning, tap water rinsing or Nolvasan rinse between notches all were correctly identified by ACE.

Significance

Producers frequently collect samples in less than ideal conditions; however the results of this small study indicate sample handling may have little effect on test sensitivity for ACE. Storage to room temperature for extended periods appears to have little impact on BVDV detection using ACE. Further, we found no evidence that BVDV accumulates on ear notchers leading to false positive test results. This is an important consideration for enterprises, such as feedlots, which are testing large number of animals, and do not wait 21- 28 days for confirmatory testing to remove the animal from the production system. Further, we tested 3 groups of 9 BVDV positive samples sequentially, a situation rarely likely to occur.