

# Know The Facts About Pooling Understand the risks with pooling for BVD testing

**By: Jill J. Dunkel**

As cattlemen choose to test for PI-BVDV, it's important that best practice protocols are followed to insure the quality of the sample tested. Taking steps to insure samples are preserved appropriately and shipped quickly are all the responsibility of the person submitting the ear notches. And most cattlemen would assume their responsibilities end there.

Or do they? Research in the last few years shows that not all BVD testing is created equal. Different types of tests are available, and some tests are conducted on individual samples, while others are pooled together in groups, and the groups are then tested. This "pooling" is raising concerns among several in the industry.

## **What is pooling?**

Simply stated, pooling is where eight to 10 samples (for Antigen Capture Elisa or ACE testing) or up to 25 samples (for PCR testing) are put in one test well. If the test result is negative, then one would assume all samples in the well are negative. If the test is positive, then each sample is retested individually to identify which sample in the pool is positive.

Testing by pooling samples is less expensive because a single test is run on several samples, as opposed to a test run on each, individual sample. Pooling is effective in testing for diseases that have a high prevalence.

The logic behind pooling is simple, and one would assume that a single positive animal in a pool would cause the entire pool to test positive. However, Bill Hessman, DVM, with Central States Testing says that's not always the case. When testing for a disease where the prevalence rate is low, it may not be your best option.

"We explored pooling with ACE testing years ago. At that time, we accidentally came across a case where we pooled, and the test missed a sample that we knew was positive," he said. "We did a lot of work to see how that happened, how the positive sample was masked by a single negative sample in the group."

That led to further research by Hessman and others to identify the accuracy of pooling in BVD testing.

## **Pooling studies**

A peer-reviewed study released in *The Bovine Practitioner* evaluated outcomes between pooled PCR and single sample ACE testing. Authored by Brendan Kraus, DVM, et al., the test monitored 2,424 calves. Samples sent to "Lab A" were tested using the pooled PCR test. Four

samples (0.165%) were found to be positive. Duplicate samples were sent to “Lab B” which used single sample ACE testing. There, the same four calves tested positive, as did eight additional calves for a total of 12 positives (0.495%).

One to two months later, new specimens from the same calves – both serum and ear notches – were obtained from the surviving calves and sent to Oklahoma State University. There, OSU labs confirmed the specimens were positive for PI-BVDV.

“In this case, a pooled PCR test did not identify 67% of the PI-positive calves that were sampled,” Hessman said.

Another research summary written by Julia Ridpath, Research Microbiologist with the National Animal Disease Center, USDA, and others states “the concentration range of virus in ear notch extractions and the detection limits of real-time PCR suggest that pooling of samples in surveillance programs must be approached cautiously.”

The study further states, “Pooling of 10 samples, where a sample pool includes one positive and nine negative samples, could result in the failure to detect 10% of the samples in the study. Pooling of 100 samples, where the sample pool includes one positive and 99 negative samples, could result in failure to detect over 50% of the samples used in this study.”

Dr. Hessman said he is not sure what is causing the false negative results, but it is important cattlemen understand how pooling works and the risks with using pooling in BVD testing. “There have been several papers on clinical research saying we can pool samples together and find the positives. But there are also several papers that show pooling will produce false negatives.

“There are multiple indications that pooling, whether using the ACE or PCR testing, will decrease the accuracy of the test,” he said. “Cattlemen don’t want to have to understand the scientific reasoning behind it, and they shouldn’t have to. But they should know the risks you take when you elect to test by pooling.”

Pooling has gained in popularity due to the decrease in cost with pooling samples, compared to the cost of testing each sample individually. However, Hessman questions if saving a few dollars is worth it when pooling may not provide accurate results.

“Do you want a percentage of positives to fall through the cracks?” he asked. “With the idea of BVD testing to identify and remove all PI animals, leaving a single, positive PI calf in a herd negates the money spent on testing.”

“Either you identified and got rid of all of them, or you didn’t,” Hessman said. “It’s a risk benefit analysis that a producer has to consider. How much is that one remaining PI going to hurt you if you miss it?”