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Resolution of Unexpected IDEXX ELISA Positive Results for Antibodies Against Porcine Reproductive and Respiratory Syndrome Virus with IFA and A Blocking ELISA

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Introduction & Objectives

The IDEXX HerdChek® PRRS ELISA has become the industry standard for monitoring the Porcine Reproductive and Respiratory Syndrome (PRRS) status of swine herds. IDEXX reports a sensitivity of 100% and a specificity of 99.5%.[1] Some veterinarians and researchers feel that the realized % false positive is higher than 0.5% in certain herds.[2]

When monitoring a PRRSV negative herd, "unexpected positive" IDEXX results are investigated using several different strategies. Virus isolation, polymerase chain reaction (PCR) or indirect fluorescent antibody (IFA) assays are used to help determine if an "unexpected positive" is a true positive result or a false positive. At some diagnostic labs, the IFA is commonly performed when unexpected positive results are obtained with the IDEXX ELISA. The IFA can be labor intensive, subjectively read, and influenced by the virus isolate used. To address the disadvantages of the IFA, a blocking ELISA (BLK) for the detection of antibodies against PRRSV was developed and evaluated using a group of unexpected positive samples.

Materials & Methods

The blocking ELISA was validated using serum samples from 748 animals of known PRRS status.[3] Receiver operating characteristic (ROC) analysis was used to determine an optimized cutoff for the blocking ELISA of 17 percent inhibition. At that cutoff, a sensitivity of 97.3 and a specificity of 100 % were determined. The positive predictive and the negative predictive value at 50 % prevalence were calculated to be 1.0 and 0.97, respectively. ROC analysis calculated an area under the curve (AUC) of 0.993. An AUC of greater than 0.900 indicates a highly accurate test.[4]

To evaluate the ability of the IFA and the blocking ELISA to identify the PRRS status of the unexpected positives, 4142 samples from 78 cases submitted from expected PRRSV negative sites were analyzed using the IDEXX ELISA at the University of Minnesota Veterinary Diagnostic Laboratory (MVDL). These samples were split for follow-up investigation. The first sample set was analyzed at the MVDL using the IDEXX ELISA for a second time. IFAs were also performed. The second set was sent to the South Dakota State University Animal Disease Research and Diagnostic Lab (ADRDL) for PRRS blocking ELISA. The true PRRS status of the herds was determined retrospectively and based on subsequent monthly serological testing according to established protocols.

Results & Discussion

3596 serum samples were analyzed using the old antigen format of the IDEXX ELISA at the MVDL. A total of 118 serum samples yielded unexpected positive results, or 3.3% of the total samples. 546 serum samples were analyzed using the new 2XR antigen format of the IDEXX ELISA at the MVDL. A total of 29 serum samples yielded unexpected positive results, or 5.3% of the total samples. The combined results of the two kit formats are displayed in Table 1.

Table 1. Unexpected IDEXX Positive Follow-up Testing

Old IDEXX PRRS format	Group 1	1 IDEXX POS	IFA Neg	BLK Neg	17
	Group 2	>1 IDEXX POS	IFA Neg	BLK Neg	92
	Group 3	>1 IDEXX POS	IFA Neg	BLK POS	9
				TOTAL	118
2XR IDEXX format	Group 4	1 IDEXX POS	IFA Neg	BLK Neg	8
	Group 5	>1 IDEXX POS	IFA Neg	BLK Neg	21
				TOTAL	29

17 samples had only one positive IDEXX result using the old format and were IFA and BLK negative (Group 1). The mean S/P of the IDEXX ELISA was 0.333 with an SD of 0.194. This could be the variation of the assay around the cutoff of 0.4 or one-time procedural errors.

92 samples had repeatable positive results on the old format of the IDEXX ELISA but were IFA and BLK negative (Group 2). The mean IDEXX S/P was 0.886 with an SD of 0.616. 83 of these 92 samples were from

herds that continue to test PRRS negative. This demonstrates that IDEXX S/P values can be quite high and still be false positive results. The remaining 9 samples were from herds that had PRRSV infections greater than 12 months prior to testing. Although samples were taken from presumed uninfected animals, it is possible that PRRSV specific antibodies may have been present if errors in the sampling population were made.

There were 9 samples where IFA and BLK results disagreed on the old IDEXX format (Group 3). The mean IDEXX S/P was 1.069 with an SD of 0.539. For 8 of the samples, the true PRRS infection status of the individual animals could not be determined as they were unable to be retested. However, these animals were from herds that had been infected with PRRSV greater than 12 months prior to testing and numerous samples from herdmates continued to test PRRS negative. These samples may represent false positive BLK results or other unknown immune responses in the animals. One sample is from a pig that was retested negative by all tests one month after the positive BLK and IDEXX test.

The new IDEXX format (PRRS 2XR) had 8 samples that had only one positive result and were IFA and BLK negative (Group 4). The mean IDEXX S/P was 0.402 with an SD of 0.252.

The PRRS 2XR testing results included 21 samples that repeatably tested IDEXX positive and were IFA and BLK negative (Group 5). The mean IDEXX S/P was 0.893 with an SD of 0.358.

The PRRS status of the herds was assessed retrospectively to calculate the specificity of the combination of the tests. 133 samples were from negative herds. All 133 samples had test results of IDEXX positive / IFA negative. The specificity of the IFA in a group of unexpected IDEXX positive samples was 100%. 129 of the 133 samples from the negative herds were IDEXX positive / BLK negative for a specificity of 97.0%.

Conclusion

Unexpected positive results with very high IDEXX S/P ratios can be demonstrated in a negative herd at higher than reported percentages. In this study, the old format IDEXX ELISA had 3.3% unexpected positives while the IDEXX 2XR ELISA had 5.3 % unexpected positives in these particular herds. Both the IFA and the BLK can be used as follow-up tests to investigate an unexpected positive IDEXX ELISA result in a negative herd. The specificity of the IFA in a group of unexpected positives was 100% while the specificity of the BLK in a group of unexpected positives was 97.0%. While serial testing of unexpected positive pigs is likely the most accurate determination of serostatus, using additional PRRSV serology tests can provide useful information.

References

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