

PERFORMANCE CHARACTERISTICS OF A NEW PSEUDORABIES VIRUS PRVgB ANTIBODY ELISA TEST

Hardi Liauw¹, DVM, MSc, PhD; Liselotte Soderlund², BMA; Elisabeth Vauclore³, BSc; and Russ Shoberg¹, PhD
¹IDEXX Laboratories, Inc., Westbrook, Maine USA ²IDEXX Scandinavia AB., Osterbybruk, Sweden ³IDEXX S.A.R.L., Eragny, France

Introduction

Currently, IDEXX PRV antibody screening tools consist of the HerdChek* PRV Antibody Test Kit for Screening and PRV Antibody Test Kit for Verification. In order to simplify the task of screen-testing herds, a single PRVgB antibody test has been developed. The PRV highly conserved genes gB or gD have been recommended to be used for PRV testing in swine¹. This new PRVgB antibody test gives an option of either short (60 minutes) or long (overnight, ON) protocols for laboratory flexibility. The performance of the PRVgB antibody test compared to the current HerdChek PRV antibody test kits is reported in this study.

Materials and methods

The following PRV antibody ELISA test kits were used in this study: IDEXX HerdChek PRV Antibody (Screening) and IDEXX HerdChek PRV Antibody (Verification). Occasionally, the IDEXX HerdChek PRV gI (=g1) Antibody Test Kit was also used. Characterized swine sera were obtained from a variety of sources in the United States and in the European Union.

The PRVgB assay is a blocking format ELISA test. The biological reagents for the antibody test described in this manuscript consist of: PRV antigen immobilized on polystyrene microtiter plates, a PRVgB-specific antibody enzyme conjugate, and two control sera (positive and negative) for antibodies against the PRVgB antigen. The cutoff S/N values for the new PRVgB assay using the short protocol are: negative as greater than 0.70, positive as less than or equal to 0.60, and suspect as greater than 0.60 and less than or equal to 0.70. The cutoff S/N values for the new PRVgB assay using the overnight protocol are: negative as greater than 0.60, positive as less than or equal to 0.50, and suspect as greater than 0.50 and less than or equal to 0.60.

Sensitivity was assessed by testing temporal bleeds from nine pigs inoculated with PRV Shope strain and two pigs inoculated with a PRV modified live vaccine. Sensitivity was also tested using a PRV reference serum from the OIE ADV Reference Laboratory in Maisons-Alfort, France. Finally, the apparent sensitivity of the PRVgB test was measured using a set of 189 positive field sera.

Specificity was determined by testing several hyperimmune sera acquired from the National Veterinary Services Laboratory (NVSL), Ames, Iowa, which were produced monospecifically against other swine viruses. Negative population samples from the United States, France and Germany were also evaluated. And, during field trials held in three different laboratories in Germany, a total of 999 field negative sera were tested.

Results and discussion

Sensitivity

Both the PRVgB and the current HerdChek PRV Antibody Test Kits correlated very well in their detection of seroconversion time. Most of the temporal pigs were positive in both assays by Day 7. This demonstrates that

the PRVgB test has a comparable sensitivity for early detection of seroconversion following exposure to PRV. The European Union requirement for PRV screening assays states that the OIE SEAgB reference serum must be detected as positive at a dilution of greater than or equal to 1:2². Both the PRVgB (short protocol) and the current HerdChek PRV Antibody Test Kits detected the OIE SEAgB reference serum as positive at 1:16; and in the overnight protocol, the PRVgB detected 1:32 as positive (Table 1). This demonstrates that the PRVgB test has adequate sensitivity to be used as a screening tool for herd surveillance.

Table 1: PRVgB Sensitivity Study on OIE SEAgB reference sera

	Diluted in PBS	PRV Screen		PRV Verification		PRVgB (Short Protocol)		PRVgB (Overnight Protocol)		
		S/P	Result	S/P	S/NHC	Result	S/N	Result	S/N	Result
OIE	1:2	3.057	+	2.944	14.71	+	0.111	+	0.051	+
SEAgB	1:4	2.123	+	2.024	13.51	+	0.147	+	0.073	+
reference	1:8	0.877	+	1.046	9.12	+	0.243	+	0.090	+
	1:16	0.574	+	0.450	5.13	+	0.470	+	0.132	+
	1:32	0.238	-	-	-	-	0.702	-	0.198	+
	1:64	0.073	-	-	-	-	0.988	-	0.514	S
	1:128	0.010	-	-	-	-	1.070	-	0.719	-
Cutoff:										
Positive:		S/P >=0.40		S/P >=0.40; S/NHC >=1.80		S/N <=0.60			S/N <=0.50	
Suspect:						S/N >0.60-0.70			S/N >0.50-0.60	

For the 189 field positive samples, the current HerdChek PRV antibody and PRVgB tests showed 98.94% agreement (with both the PRVgB short and overnight protocols). Three different samples were observed to be discrepant between the two tests. The first discrepant sample was PRV screen- and verification-positive, suspect in the PRVgB short protocol (0.613 S/N), and positive in the PRVgB overnight protocol (S/N 0.171). The second discrepant sample was positive on both PRV screening and verification, negative on PRVgB short protocol (S/N 0.754), and suspect in the PRVgB overnight protocol (S/N 0.579). The third sample was positive on both PRV screen and verification, positive on PRVgB short protocol (S/N 0.586) and suspect on PRVgB overnight protocol (S/N 0.505).

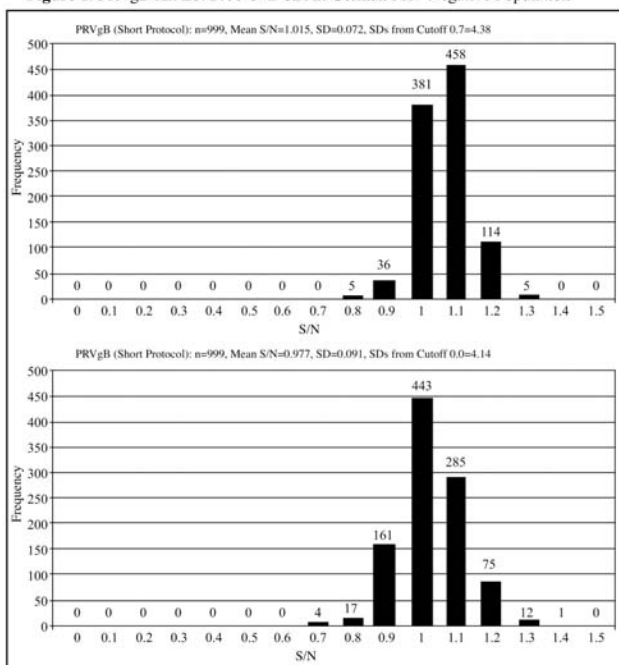
Specificity

Nine of the hyperimmune sera produced by NVSL against swine influenza virus (H1N1) and (H3N2), porcine adenovirus, porcine parvovirus, porcine reovirus, porcine rotavirus, encephalomyocarditis, hemagglutinating encephalomyelitis, and Transmissible Gastroenteritis virus did not cross-react on the PRVgB assay.

The PRVgB (short protocol) showed 99.38% agreement and 99.48% specificity compared to the current HerdChek PRV Antibody Test Kits performed on a total of 960 sera from negative field populations. Seven animals were observed to be discrepant among this set. The two discrepant animals from the U.S. sample set included: one sample that was positive on PRV screen (S/P 0.552) and negative on PRV verification (S/P 0.385 and S/NHC 1.868) was positive on PRVgB (S/N 0.398), and a second discrepant was negative on PRV screen (0.091) and suspect on PRVgB (S/N 0.684). From the European sample set, one discrepant animal tested as positive on PRV screen and verification, and as negative on PRVgB and PRVgI (S/N 0.817 and 1.044, respectively). The second EU discrepant sample was

positive on PRV screen and verification, positive on PRVgB (S/N 0.524) and negative on PRVgI (S/N 1.078), and is considered to be a potential PRVgI vaccinee. The fifth through seventh discrepant were all negative on PRV screen (S/P values of 0.336, 0.314 and 0.141), suspect on PRVgB (S/N values of 0.612, 0.660 and 0.690) and negative on PRVgI (S/N values of 1.083, 1.105 and 1.096). During field trials in eastern regions of Germany (considered to be free of PRV), the specificity of PRVgB test was determined to be 100% (short protocol) and 99.90% (overnight protocol). The single discrepant animal had an S/N of 0.569 (overnight protocol). The frequency distributions of all German PRV-negative populations tested during the field trials are presented in Figure 1. Therefore, in these studies, the PRVgB specificity ranged from 99.48 to 100%. The field testing data from Germany demonstrate that in PRV-free status herds, the specificity is expected to be very good, with our specificity results being between 99.90 and 100.0%.

Figure 1: PRVgB Kit Lot 2755-34B on All German PRV-Negative Population



Conclusions

The IDEXX PRVgB test described above demonstrated a high level of sensitivity and specificity. Further, it showed a good correlation to the current HerdChek PRV Antibody Test Kits (for screening and for verification). Therefore, this new PRV assay is validated as an effective tool for the testing of PRV antibodies in swine serum.

Acknowledgments

We appreciate the help we received in the form of swine serum samples from: the National Veterinary Services Laboratory, Ames, Iowa; and Dr. Howard Hill, Dr. Joseph Connor, Dr. Fernando Osorio and staff at the University of Nebraska at Lincoln, Nebraska.

Sera from the EU were provided by: Professor Bernard Toma at the OIE ADV Reference Laboratory at Maisons-Alfort, France; and the Wusterhausen ADV Reference Laboratory, Germany.

During our German field testing, we are indebted to the help of: Dr. Siegfried Lange and staff at Bad Langensalza Diagnostic Laboratory; Drs. Korber, Mewes, Gehrman and staff at Stendal Diagnostic Laboratory; and Drs. Volker Pohle, Hermann Nieper and staff at Leipzig Diagnostic Laboratory. These three facilities were gracious in providing laboratory space and serum samples.

References

- Muller T, Conraths FJ, Hahn EC. Pseudorabies virus infection (Aujeszky's disease) in wild swine. *Infect Dis Rev.* 2000;2(1):27-34.
- Official Journal of the European Communities. *Commission decision of 23 July 2001 on additional guarantees in intra-Community trade of pigs relating to Aujeszky's disease, criteria to provide information on this disease and repealing decisions 93/24/EEC and 93/244/EEC.* L 215/53. 2001.

* HerdChek is a trademark or registered trademark of IDEXX Laboratories, Inc. in the United States and/or other countries.

© 2004 IDEXX Laboratories, Inc. All rights reserved. 2792-00 (6)