The performance of ELISA test kit (Biochek®) on different PRRS-status farms

Introduction

PRRSv causes many impacts in swine industry worldwide including reproductive problems and severe respiratory problems [1]. Sows show clinical signs increased abortions, stillborn, mummified fetuses, and number of weak piglets. The percentage of mortality in nursery and fattening pigs also increases. Many cases of PRRS show co-infection with other diseases such as *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Sreptococcus suis*, *Haemophillus parasuis* or *Actinobacillus pleuroneumoniae* [2-3]. To prevent and control of the disease, PRRS-status of a farm needs to be known beforehand. Serological profile using ELISA method is the useful tool to confirm the stage of PRRS infection in herd level. Several commercial ELISA test-kits are used to monitor PRRS-status in a farm. However, little information on the performance of these test-kits in farms is provided. This study was performed to determine the use of two commercial ELISA test-kits, "Biochek®" and IDEXX PRRS X3 Ab test, in negative, PRRS-stable and PRRS-active herds.

Materials and Methods

Farms

Fourteen swine farms with different PRRS status was classified into 3 groups according to Holtkamp et al., 2010 [4]

- Group 1 PRRS-negative farm (Category IV); 2 farms
- Group 2 PRRS-unstable farm (Category I); 9 farms
- Group 3 PRRS-stable farm Category II; 3 farms

Previous history and some production parameters involved with PRRS clinical signs in farms together with PRRS-PCR results were used for classification of PRRS status of farms.

Sample collections and Laboratory testing

Blood samples of sows and pigs in each farm will be collected as described below; 5 samples at each stage.

- Gilt
- Sow at 3 week after farrowing
- Piglets at 2 weeks of age
- Piglets at 4 weeks of age
- Piglets at 8 weeks of age
- Piglets at 12 weeks of age

- Piglets at 16 weeks of age

Pooled blood sample at each stage of pigs PCR technique will be assayed for PRRS-nucleic acid using real-time PCR technique. Samples were kept at -20ºC until testing for antibodies against PRRS using Biochek® and IDEXX PRRS X3 Ab ELISA test kits. The cut-off value at s/p ratio ≥ 0.4 was considered positive for both ELISAs.

Statistical analysis

R program version 3.1.3 was used to perform statistical analysis [5]. Pearson's correlation between s/p ratio of Biocheck® and IDEXX ELISAs and kappa's value between PRRS results were calculated. The significant level was determined at p value of 0.05.

Results

The mean s/p ratios of Biochek® and IDEXX ELISAs in 3 groups were shown in Figure 1. The s/p ratio of both ELISAs were in similar pattern either in 3 PRRS-status groups or in individual farm (Figure 2). In group 1, 11% of samples of Biochek® ELISA showed s/p ratio slightly greater than 0.4 except one sample with a high value of 3.60 whereas all of samples of IDEXX® ELISA showed s/p ratio lower than 0.4. The mean s/p ratios and their standard error of mean of Biochek® ELISA also showed higher values than of IDEXX ELISA.

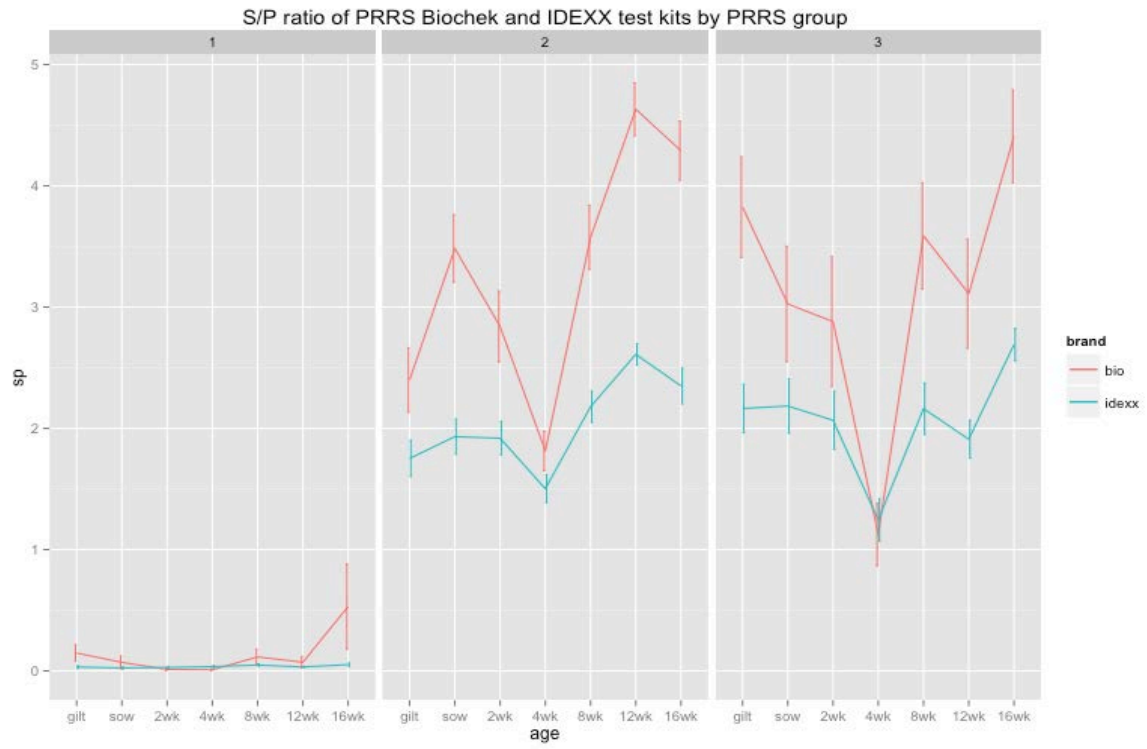

Figure 1: Graphs showed mean s/p ratios of each group. Standard error of mean was shown

as vertical lines. The s/p ratios of Biochek® and and IDEXX ELISAs were in red and green lines, respectively. X-axis indicated pigs in each stage whereas y-axis indicated s/p ratio.

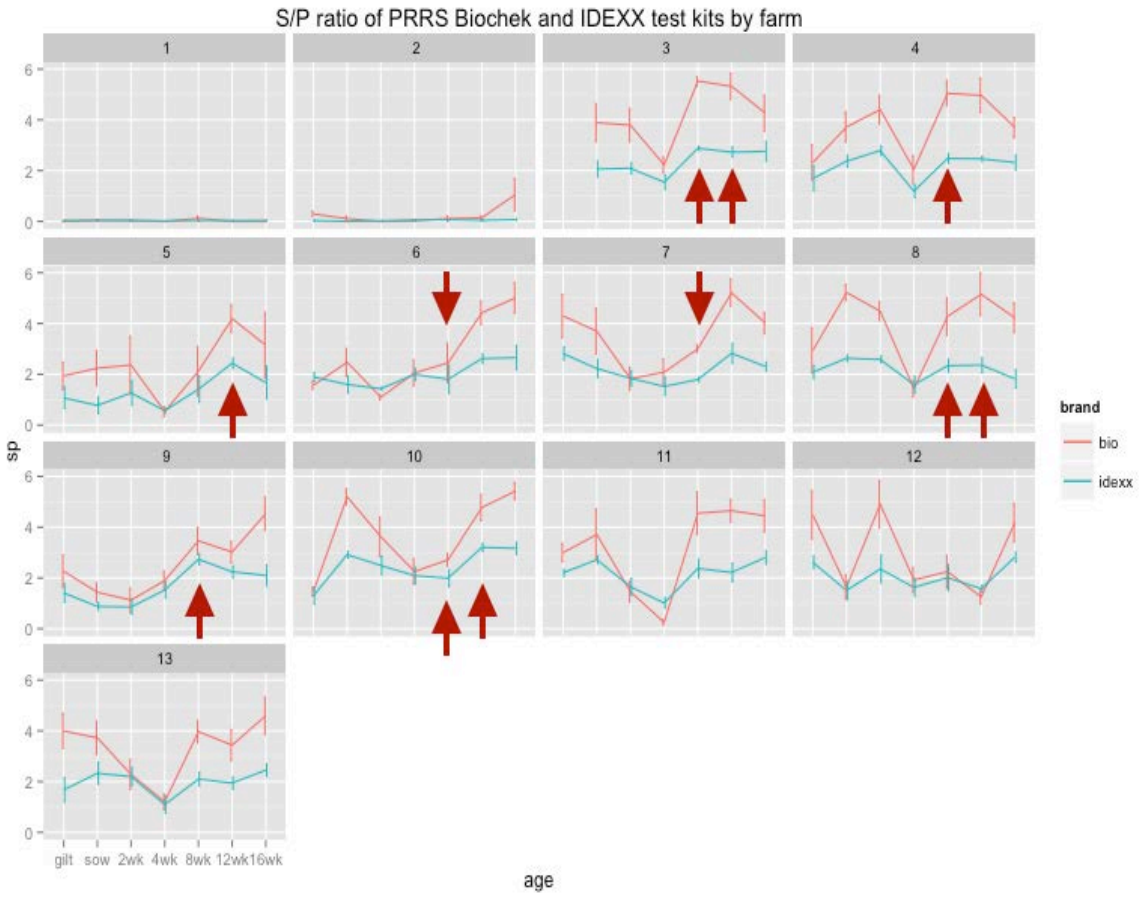

Figure 2: Graphs showed mean s/p ratios of each farm. Farm 1-2, 3-10 and 11-13 were in group 1, 2 and 3, respectively. Standard error of mean was shown as vertical lines. The s/p ratios of Biochek® and and IDEXX ELISAs were in red and green lines, respectively. X-axis indicated pigs in each stage whereas y-axis indicated s/p ratio. Red arrows on graph 3-10 indicated stage of pig with positive PCR against PRRS in pooled serum.

Table 1: Results of Biochek and IDEXX ELISAs for PRRS-antibody detection in each group

Group			IDEXX® ELISA		Kappa's value
			Positive	Negative	
1		Positive	0	8	
		Negative	0	62	
2	Biochek® ELISA	Positive	259	6	0.85 (p-value <0.001)
		Negative	3	7	
3		Positive	100	0	
		Negative	3	2	

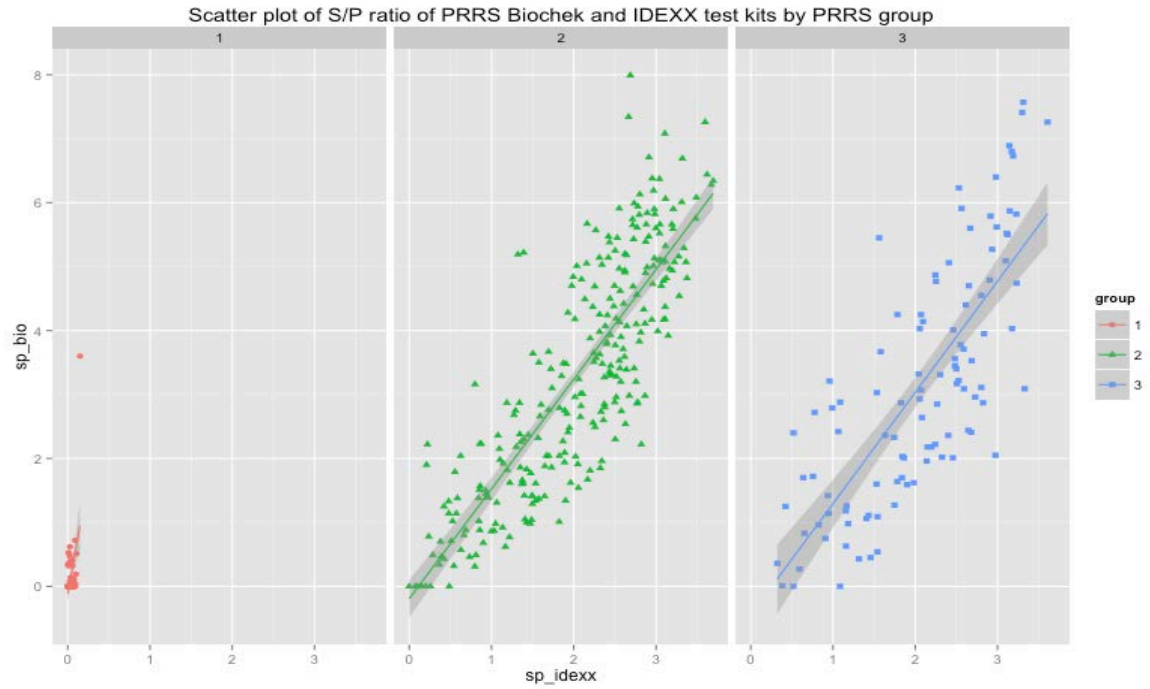

Figure 3: Scatter plots showed correlations between s/p ratios of Biochek® and and IDEXX ELISAs. S/p ratios in Group 1, 2 and 3 were shown in red green and blue points, respectively. X-axis indicated s/p ratio of IDEXX ELISA whereas y-axis indicated s/p ratio of Biochek ELISA.

Figure 4: Scatter plots showed correlations between s/p ratios of Biochek® and and IDEXX ELISAs. S/p ratios in individual farm. Farm 1-2, 3-10 and 11-13 were in group 1, 2 and 3, respectively. X-axis indicated s/p ratio of IDEXX ELISA whereas y-axis indicated s/p ratio of Biochek ELISA.

Table 1 showed results of two ELISAs in each group and a kappa's value. Correlations between s/p ratios of two ELISAs in each group and in individual farm were shown in Figure 3 and 4, respectively. Pearson's correlation coefficient of 0.88 (p-value <0.001) was calculated.

Discussion

The very strong positive correlation between s/p ratio of Biocheck® and IDEXX ELISAs was supported by Pearson's correlation coefficient of 0.88 (p-value <0.001) [6]. Moreover the s/p ratios of samples in group 2 increased at the same time that viremia was detected (Figure 2). This showed that Biochek® ELISA can be used to monitor the PRRS status in a farm, as same as IDEXX ELISA. The kappa's value of 0.85 also indicated an almost perfect agreement (k > 0.80) between both ELISAs [7]. However, some false positive results were found in samples in group 1 (PRRS-negative farm) using Biochek® ELISA. According to manufacturer, IDEXX PRRS X3 Ab ELISA claim to have sensitivity of 98.8% and specificity of 99.9% whereas Biocheck® ELISA has sensitivity of 90% and specificity 98%. The lower specificity of Biochek® ELISA may cause false positive result when compare with specificity of 99.9% of IDEXX ELISA. The lower sensitivity and lower specificity of Biocheck® ELISA may also cause higher variation in s/p ratios of Biochek® ELISA. However, the s/p ratios of both ELISAs were in similar pattern either in all groups or all farms (Figure 1 and 2).

In conclusion, Biochek® ELISA showed very strong correlation and agreement of test with IDEXX ELISA. It can be used as a monitoring tool to detect PRRS status in a swine farm.

References

1. Zimmerman JJ, Benfield DA, Dee SA, Murtaugh MP, Stadejek T, Stevenson GW, Torremorell M. Porcine reproductive and respiratory syndrome virus (Porcine arterivirus). In Diseases of swine. 10th edition. Edited by Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW; Ames, Iowa: Wiley-Blackwell; 2012:461-486.
2. Thacker EL, Halbur PG, Ross RF, Thanawongnuwech R, Thacker BJ. *Mycoplasma hyopneumoniae* potentiation of porcine reproductive and respiratory syndrome virus-induced pneumonia. J Clin Microbiol 1999; 37:620-627.
3. Thanawongnuwech R, Brown GB, Halbur PG, Roth JA, Royer RL, Thacker BJ Pathogenesis of porcine reproductive and respiratory syndrome virus-induced increase in susceptibility to *Streptococcus suis* infection. Vet Pathol 2000; 37:143–152
4. Holtkamp DJ, Polson DD, Torremorell M, Morrison B, Classen DM, Becton L, Henry S, Rodibaugh MT, Rowland RR, Snelson H, Straw B, Yeske P, Zimmerman J: Terminology for classifying swine herds by porcine reproductive and respiratory syndrome virus status. J Swine Health Prod 2011, 19:44-56.
5. R Core Team. R: A language and environment for statistical computing. 2015. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

6. Altman DG. Practical statistics for medical research.Chapman&Hall/CRC; 1991.
7. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb) 2012, 22: 276-282