Experimental and field trial of TEGO™ animal blood collection kit for PRRS testing

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Introduction

Accurate and timely diagnosis of viral infections is necessary for effectively monitoring infection status of population and controlling disease. Serum has been the standard antemortem sample for virus and antibody testing. As venipuncture can impose safety issues for both animal and collector, less invasive and more convenient sampling methods (e.g., ear vein bleeding, tail docking, air sampling, and oral fluid sampling) have been evaluated for diagnostic use.[3,5,6,7] Specifically, use of dried blood spots on filter paper has been getting attention as it allows blood collection from the ear and provides convenient transport of biological materials without loss of analyte integrity.[2,4] The following experimental and field studies were conducted to evaluate the utility of the TEGO™ Animal Blood Collection (ABC) kit for blood sample collection and PRRS virus diagnostic testing.

Materials and methods

Experimental study

Twenty six, 4-week-old, crossbred pigs were purchased from a commercial herd historically known to be free of PRRS and housed in the Large Animal Infectious Disease Research Facility at Iowa State University. PRRS-negative status of all animals was reconfirmed by a real-time RT-PCR (Ambion® AgPath-ID™ PRRSV One-Step RT-PCR kit, Applied Biosystems) and ELISA (HerdCheck® PRRS 2XR, IDEXX) during acclimation period. Pigs were randomly assigned to one of 3 groups: a) North American PRRSV inoculated (n = 10); b) European PRRSV inoculated (n = 10); and c) sham control (n = 6). Pigs of the virus-inoculated groups received the respective PRRS virus at the rate of 10^3 $TCID_{50}$/ml via intranasal route. Control group pigs received virus-free cell culture media. Each group was housed separately. All pigs were kept for 5 weeks post inoculation with daily monitoring for clinical signs. During the observation period, pigs were bled at 0, 3, 7, 14, 21, and 35 days post inoculation (DPI). At each sampling, blood was collected on a filter membrane using TEGO™ ABC kit from ear vein or into a Vacutainer® serum separation tube (SST) from jugular vein. Serum was harvested from clotted blood in each Vacutainer® tube after centrifugation. From each blood stained filter paper, 3 discs (5mm in diameter) were punched out and put into a microfuge tube with 0.5ml of phosphate-buffered saline solution for elution. The elution procedure and number of discs used for the elution was optimized so that both PCR and ELISA testing could be performed on the same elution. After 1-hour elution at room temperature with gentle shaking, tubes were centrifuged at 10,000 × g for 5 min and supernatants saved for testing. All samples (sera and elutes) were stored at -80°C until being tested and then submitted together to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) for PRRS PCR and ELISA. Test results on elutes were compared to those on sera. Test agreement between the 2 sample types was assessed.

Field study

A total of 240 pigs from commercial operations (finishers or adult swine) in Midwest or East coast regions were sampled by 2 independent swine practitioners from 3 different populations: a) positive; b) negative; and c) unknown. The PRRS status of each herd was determined by the submitting veterinarian based on historical diagnostic data and was not disclosed to the laboratory personnel until after all tests were done. Sample collection from each herd was limited to 20 or 30 pigs. Both sera and blood stained filter papers were submitted to the ISUVDL for PRRS testing (PCR and ELISA) in a blind fashion. Filter papers were processed in the identical manner as described above prior to testing. Test agreement and performance between the 2 sample matrices were determined by *Chi-square* test[1] and Fisher's exact test.

Results and discussion

Experimental study

The control group remained negative for PRRS virus virologically and serologically throughout the study period. After experimental inoculation with PRRS virus, pigs demonstrated typical kinetics of viremia and antibody response. All pigs developed detectable viremia by 3 DPI and remained viremic until 14 DPI regardless of the PRRS virus strain given. No cross contamination was observed between the virus-inoculated groups. Serologically, all inoculated pigs seroconverted to PRRS virus by 14 DPI and continued to be seropositive until the end of the study (35 DPI). When the same pigs were tested using samples collected with TEGO™ ABC kit, comparable PCR and ELISA results were obtained (Table 1 and 2), although filter paper elutes had higher Ct values (i.e., less number of virus) and lower S/P ratios (i.e., lower amount of antibody) than the corresponding sera, implying a dilution effect. It should be noted that elute from one disc was sufficient to generate the comparable PCR result.

Field study

All samples (sera and filter papers) collected from historically PRRS-negative herds (boar stud or sow unit) were negative for both PRRS viral genome and virus-specific antibody, suggesting that cross contamination did not occur with filter papers during collections, sample handling and shipping. On samples from herds with positive or unknown PRRS status, both sample matrices yielded comparable PCR results. Overall the agreement of test results between the 2 sample matrices was 98.3% (Table 3). Filter paper samples showed 99.5% and 93.8% diagnostic specificity and sensitivity, respectively, in comparison to serum samples. A valid interpretation

Table 1: PCR detection of PRRS virus in serum and filter paper elute collected from pigs after experimental inoculation

Virus	Sample type	Days post inoculation				
		0	3	7	14	21
NA PRRSV	Serum	0/10[a]	10/10	10/10	10/10	4/10
	Filter paper	0/10	10/10	10/10	10/10	2/10
EU PRRSV	Serum	0/10	10/10	9/9[b]	9/9	3/9
	Filter paper	0/10	10/10	9/9	9/9	2/9
Control	Serum	0/6	0/6	0/6	0/6	0/6
	Filter paper	0/6	0/6	0/6	0/6	0/6

[a] Number of positive animal/number of animals tested
[b] One pig was removed from the study due to health issue.

Table 2: Detection of PRRS virus-specific ELISA antibody in serum and filter paper elute collected from pigs after experimental inoculation

Virus	Sample type	Days post inoculation				
		0	7	14	21	35
NA PRRSV	Serum	0/10[a]	6/10	10/10	10/10	10/10
	Filter paper	0/10	0/10	9/10	10/10	10/10
EU PRRSV	Serum	0/10	0/9[b]	9/9	9/9	9/9
	Filter paper	0/10	0/9	8/9	8/9	9/9
Control	Serum	0/6	0/6	0/6	0/6	0/6
	Filter paper	0/6	0/6	0/6	0/6	0/6

[a] Number of positive animal/number of animals tested
[b] One pig was removed from the study due to health issue.

Table 3: Agreement of PCR results for PRRS virus between sera and filter paper elutes collected from commercial swine with various historical PRRS status

		Serum	
		Positive	Negative
Filter paper	Positive	45	1
	Negative	3	191

on the performance of filter paper samples for ELISA could not be made because too few pigs were seropositive for PRRS virus at the time of sampling. Further study is underway to continue the assessment of the utility of the kit for serology under field conditions.

Conclusion

TEGO™ ABC kit combines a unique collection device and a filter paper media, which can be used for PCR and ELISA testing. The kit provides a safer, more convenient blood collection solution for sample collection in the field. The kit also provides an easy way to ship samples with cost saving.

Acknowledgement

The authors thank Raquel Eshelman, Scott Kostohryz and Beverly Fowls at the Iowa State University Veterinary Diagnostic Laboratory and farm staff for their assistance and help in the study. The study was supported in part by funding or in-kind from ITL Corporation, Iowa Healthy Livestock Initiative Research Grant and Cooperative Research Agreement with National Veterinary Research and Quarantine Service.

References

1. Greenwood, P.E. and M.S. Nikulin. 1996. A guide to chi-squared testing. Wiley, New York.

2. Hansen, T.V., M.K. Simonsen, F.C. Nielsen, and Y.A. Hundrup. 2007. Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. *Cancer Epidemiol Biomarkers Prev* 16:2072–2076.

3. Hermann, J.R., S.L. Brockmeier, K.-J. Yoon, and J.J. Zimmerman. 2008. Detection of respiratory pathogens in air samples from acutely infected animals. *Can J Vet Res* 72:367–370.

4. Moscoso, H., I. Alvarado, and C.L. Hofacre. 2006. Molecular analysis of infectious bursal disease virus from bursal tissues collected on FTA filter paper. *Avian Dis* 50:391–396.

5. Patterson, A.R., L.A. Karriker, R.B. Evans, and K.-J. Yoon. 2007. Evaluation of alternative antemortem diagnostic samples for porcine reproductive and respiratory syndrome virus (PRRSV). *J Swine Health Prod* 15:339–345.

6. Prickett, J., W.-I. Kim, R. Simmer , K.-J. Yoon, and J.J. Zimmerman. 2008. Surveillance of commercial growing pigs for PRRSV and PCV2 infections using pen-based oral fluid sample: a pilot study. *J Swine Health Prod* 16:86–91.

7. Rovira, A., T. Clement, J. Christopher-Hennings, B. Thompson, M. Engle, D. Reicks, and C. Muñoz-Zanzi. 2007. Evaluation of the sensitivity of reverse-transcription polymerase chain reaction to detect porcine reproductive and respiratory syndrome virus on individual and pooled samples from boars. *J Vet Diagn Invest* 19:502–509.