

4. Buschmann A, Biacabe A-G, Ziegler U, et al.: 2004, Atypical scrapie cases in Germany and France are identified by discrepant reaction patterns in BSE rapid tests. *J Virol Methods* 117: 27–36.
5. González L, Martin S, Begera-Mc Gorum I, et al.: 2002, Effects of agent strain and host genotype on PrP accumulation in the brain of sheep naturally and experimentally affected with scrapie. *J Comp Pathol* 126:17–29.
6. Jeffrey M, Begera-McGorum I, Clark S, et al.: 2002, Occurrence and distribution of infection-specific PrP in tissues of clinical scrapie cases and cull sheep from scrapie-affected farms in shetland. *J Comp Pathol* 127:264–273.
7. Monleón E, Monzón M, Hortells P, et al.: 2004, Detection of PrP<sup>sc</sup> on lymphoid tissues from naturally affected scrapie animals: comparison of three visualization systems. *J Histochem Cytochem* 52:145–151.
8. Official Journal of The European Union: 2005, Commission Regulation (EC) No 36/2005 of 12 January 2005 amending Annexes III and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards epidemiological surveillance for transmissible spongiform encephalopathies in bovine, ovine and caprine animals. L010/2005. Publications Office (European Union).
9. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: 2004, Scrapie. 5th ed., part 2, section 2.4, chapter 2.4.8, pp. 642–653. Office of International Epizootics, Paris, France.
10. Schaller O, Fatzer R, Stack M, et al.: 1999, Validation of a Western immunoblotting procedure for bovine PrP<sup>sc</sup> detection and its use as a rapid surveillance method for the diagnosis of bovine spongiform encephalopathy (BSE). *Acta Neuropathol* 98:437–443.
11. Valdez RA, Rock MJ, Anderson AK, O'Rourke KI: 2003, Immunohistochemical detection and distribution of prion protein in a goat with natural scrapie. *J Vet Diagn Invest* 15:157–162.
12. Van Keulen LJ, Schreuder BE, Vromans ME, et al.: 2000, Pathogenesis of natural scrapie in sheep. *Arch Virol* 16:57–71.

*J Vet Diagn Invest* 17:469–473 (2005)

## A simple and rapid chromatographic strip test for detection of antibody to porcine reproductive and respiratory syndrome virus

Y. S. Lyoo, S. B. Kleiboeker, K.-Y. Jang, N. K. Shin, J.-M. Kang, C.-H. Kim, S.-J. Lee, J.-H. Sur<sup>1</sup>

**Abstract.** Porcine reproductive and respiratory syndrome virus (PRRSV) continues to be a major economic problem for swine industries worldwide despite several disease-reduction strategies such as age-segregated early weaning and all-in-all-out pig movement. Routine diagnosis of PRRSV is carried out by the combined use of an antibody-detecting enzyme-linked immunosorbent assay (ELISA), immunofluorescence, reverse transcription–polymerase chain reaction, and virus isolation. These assays require specialized laboratory equipment in addition to multistep sample handling and sample preparation. The objective of this study was to evaluate a simple pen-side assay (BioSign<sup>®</sup> PRRSV) for rapid detection of PRRSV antibody based on a lateral flow chromatographic strip immunoassay system. This assay uses *Escherichia coli*-expressed viral nucleocapsid protein antigen for detecting antibodies against PRRSV in swine sera. In this report, the authors describe the evaluation of this assay using sera from both clinical samples and experimentally infected piglets. The results were compared with those of a standard, commercially available antibody ELISA (HerdChek<sup>®</sup> PRRS ELISA) and an indirect immunofluorescence assay using the same serum samples. The BioSign<sup>®</sup> PRRSV assay was capable of detecting antibodies in sera known to contain antibodies to PRRSV, resulting in 93.2% sensitivity for samples from experimentally infected pigs and 98.7% sensitivity for clinical serum samples. For sera that did not contain antibodies to PRRSV, the specificity was found to be 98.5% and 99.2% for clinical and experimental serum samples, respectively.

**Key words:** ELISA; porcine reproductive and respiratory syndrome virus; serology.

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent of 1 of the most economi-

---

From the Department of Veterinary Pathology, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea (Lyoo, Lee, Sur), Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri-Columbia, Columbia, MO (Kleiboeker), Princeton BioMeditech Corp., Princeton, NJ (Jang, Shin, Kang), and the Princeton BioMeditech East Corp., Kyeonggi-do, 440, Korea (Kim), MO 65211.

<sup>1</sup>Corresponding Author: Jung-Hyang Sur, Department of Veterinary Pathology, College of Veterinary Medicine, Konkuk University, #1 Hwayang-dong Kwangjin-gu, Seoul 143-701, Korea, NJ 08543.

cally significant infectious diseases of swine in many parts of world.<sup>3,10</sup> Infection of domestic swine by PRRSV is characterized by respiratory dysfunction in suckling, weaned, and growing pigs.<sup>3,18</sup> In addition, PRRSV infection causes late-term abortions and a high incidence of stillborn, mummified, and debilitated newborn piglets. Various assays have been developed for detection of PRRSV-specific antibodies in swine sera. These tests include an immunoperoxidase monolayer assay, indirect immunofluorescent antibody (IFA) test, and an enzyme-linked immunosorbent assay (ELISA).<sup>1,7,13,14,19,20</sup> Although these assays provide accurate, sensitive detection of anti-PRRSV antibodies, each can only be used in laboratories with significant technical expertise

**Table 1.** Comparison of Biosign<sup>™</sup> PRRSV to HerdChek<sup>®</sup> PRRS ELISA using swine sera submitted for routine diagnostic evaluation.

		HerdChek <sup>®</sup> ELISA	
		Positive	Negative
Biosign <sup>™</sup>	Positive	470	5
	Negative*	6	329

\* Ambiguous results were counted as negative.

and specialized equipment. The purpose of this study was to develop an antibody detection assay for use in a field setting and to compare it with standard diagnostic tests such as ELISA and IFA for detecting anti-PRRSV antibody.

To obtain the PRRSV nucleocapsid (N) gene for expression of recombinant N protein in *Escherichia coli*, reverse transcriptase–polymerase chain reaction (RT-PCR) was performed on viral genomic RNA from the Korean PRRSV field isolate PL 96-1. This isolate was made from a clinical case of PRRSV and confirmed to be of the North American genotype by nucleotide sequencing. To generate complementary DNA (cDNA), Superscript One-step RT-PCR was used with the PLATINUM Taq system.<sup>a</sup> The following oligonucleotide primers<sup>8</sup> were used: forward primer, 5'-ATC GGA TCC ATG CCA AAT AAC AAC GGC AAG-3'; reverse primer, 5'-GGT GAATTC TTA TGC TGA GGG TGA TGC TGT GAC-3'. Synthesis of cDNA was performed with 1 cycle of 50°C for 30 minutes, followed by predenaturation at 95°C for 5 minutes. Amplification by PCR was then performed for 35 cycles at 94°C for 15 seconds, 50°C for 30 seconds, and 72°C for 2 minutes. The amplified gene was digested with the restriction endonucleases *Bam*HI and *Eco*RI and then ligated into the modified pET-5a vector.<sup>b</sup> The ligated plasmid was used to transform XL-1 Blue competent cells.<sup>c</sup> Transformed cells were plated on Luria-Bertaini (LB) plates supplemented with 100 µg/ml ampicillin. Individual colonies were selected and tested for the presence of the desired insert and then grown in LB broth supplemented with 100 µg/ml ampicillin at 37°C with vigorous shaking until the cultures reached midlogarithmic growth. Isopropylthiogalactoside was then added at a final concentration of 1 mM, and the cultures were incubated for an additional 3 hours to induce the expression of recombinant protein. After the induction period, cells were harvested and lysed by sonication.<sup>d</sup> The antigen was purified from the soluble crude lysate by fractionation through 17–30% ammonium sulfate.

The details of the chromatographic strip test assembly are described elsewhere.<sup>2,11,16</sup> The major component, the PRRSV capture antigen (recombinant N protein) was applied to a nitrocellulose membrane.<sup>e</sup> Proteins G and A<sup>f</sup> were conjugated with colloidal gold and used as the indicator. The chromatographic strip assay<sup>g</sup> was performed using 10 µl of serum applied directly onto the sample well. After 1 minute, 100 µl of developer solution was applied to the developer well. As proteins G– and A–gold conjugates migrated along the membrane, they were immobilized by forming complexes with PRRSV N protein–specific antibodies at the test line, resulting in a red color. The assay was completed within 15 minutes after sample application and scored “negative”

**Table 2.** Comparison of Biosign<sup>™</sup> PRRSV to HerdChek<sup>®</sup> PRRS ELISA using swine sera collected from experimentally infected pigs.

		HerdChek <sup>®</sup> ELISA	
		Positive	Negative
Biosign <sup>™</sup>	Positive	139	1
	Negative*	10	120

\* Ambiguous results were counted as negative.

when no staining of the test line was observed or “positive” when a distinct staining of the test line was observed (data not shown). Undetermined results represented by weak (+/–) staining were considered negative. The test results were stable after drying, although drying resulted in a slightly increased intensity of positive staining as well as background staining. For assay development, 10 positive control sera were obtained at 10 days postinoculation (p.i.) from pigs experimentally infected with PRRSV. Infection of pigs was confirmed by positive virus isolation and RT-PCR for PRRSV from sera and nasal swabs of inoculated swine (data not shown). For the positive control samples, the presence of 2 colored bands, 1 each at the test position and at the control position, indicated that antibodies against N protein of PRRSV were detected. For negative control sera collected from uninfected pigs, only a single colored line was noted at the control position.

Sera were collected from swine in production systems in both the United States and South Korea for routine diagnostic assessment for the presence of antibody to PRRSV. Of these, 250 were of US origin and submitted to the Veterinary Medical Diagnostic Laboratory, University of Missouri–Columbia, where they were tested using a commercially available standard indirect ELISA<sup>h</sup> for antibody to PRRSV in the course of routine diagnosis of PRRSV. The commercially available ELISA<sup>h</sup> used has reported sensitivity and specificity values 97.4% and 99.6%, respectively (HerdChek<sup>®</sup> PRRS ELISA<sup>h</sup> package insert). Another 560 sera were obtained from several swine herds in South Korea for diagnostic purposes. They were tested using the same commercially available ELISA<sup>h</sup> for the presence of antibodies against PRRSV. Serum samples were tested within 1–3 days postcollection or stored at –20°C and then subsequently thawed and tested by the chromatographic strip assay.<sup>g</sup> A clear positive or negative result was observed in 99.1% of the 810 clinical samples tested; however, some results were ambiguous. For all samples in which ambiguous results were obtained, a second aliquot of serum was retested and the second result recorded. A sample yielding an ambiguous result on 2 successive tests was scored negative. The results of this analysis were compared with the results of testing using a standard ELISA<sup>h</sup> (Table 1). For the 810 sera tested, the sensitivity, specificity, and accuracy (defined as the sum of true-positive and true-negative samples divided by the number of samples tested) of the chromatographic strip assay<sup>g</sup> was found to be 98.7%, 98.5%, and 98.6%, respectively, compared with the standard ELISA.<sup>h</sup> As a general observation, positive samples with high sample to positive (S:P) ratios detected by the com-

**Table 3.** Comparison of HerdChek® PRRS ELISA, IFA, and Biosign® PRRSV serological results obtained for experimentally infected sera.\*

Pig no.	Day 4			Day 5			Day 7			Day 10			Day 12			Day 16		
	ELISA†	Biosign		ELISA	Biosign		ELISA	IFA	Biosign	ELISA	IFA	Biosign	ELISA	IFA	Biosign	ELISA	IFA	Biosign
1	- (0.14)	-		- (0.11)	-		+ (0.59)	-	+	+ (1.70)	+	+	+ (1.20)	+	+	+ (0.80)	-	+
2	- (0.04)	-		- (0.09)	-		+ (0.64)	±	+	+ (0.87)	+	+	+ (1.56)	+	+	+ (1.06)	+	+
3	- (0.09)	-		- (0.38)	±‡		+ (0.44)	-	+	+ (0.44)	+	+	+ (0.52)	+	+	+ (0.53)	+	+
4	- (0.03)	-		- (0.04)	-		- (0.13)	-	-	- (0.11)	-	-	+ (0.87)	±	±	+ (0.59)	±	±
5	- (0)	-		+ (0.48)	+		+ (0.48)	±	+	+ (0.80)	±	+	+ (0.40)	-	±	- (0.36)	±	±
6	- (0.10)	-		- (0.08)	-		+ (0.42)	-	±	+ (0.52)	+	+	+ (0.52)	±	±	+ (0.43)	±	±
7	- (0.21)	-		- (0.15)	-		+ (0.80)	+	+	+ (1.06)	+	+	+ (1.32)	+	+	+ (1.87)	+	+
8	- (0.12)	-		- (0.15)	-		- (0.15)	-	-	+ (1.30)	+	+	+ (0.91)	+	+	+ (1.20)	+	+
9	- (0.05)	-		+ (0.44)	+		+ (0.89)	+	+	+ (0.91)	+	+	+ (1.87)	+	+	+ (1.32)	+	+
10	- (0.15)	-		- (0.09)	-		+ (0.47)	-	+	+ (0.74)	+	+	+ (1.80)	+	+	+ (1.34)	+	+
11	- (0.01)	-		- (0.04)	-		+ (0.50)	-	+	+ (0.79)	+	+	+ (1.20)	+	+	+ (0.91)	+	+
12	- (0)	-		- (0.04)	-		- (0.10)	-	-	- (0.33)	-	-	- (0.05)	-	-	- (0.39)	±	±
13	- (0.05)	-		- (0.07)	-		+ (0.70)	±	+	+ (0.59)	±	+	+ (0.91)	+	+	+ (0.87)	+	+
14	- (0.04)	-		- (0.15)	-		+ (0.52)	-	+	+ (1.06)	+	+	+ (0.73)	+	+	+ (1.04)	+	+
15	- (0.02)	-		- (0.09)	-		+ (0.56)	-	+	+ (1.32)	+	+	+ (1.34)	+	+	+ (0.95)	+	+
16	- (0)	-		- (0.14)	-		+ (0.63)	+	+	+ (1.87)	+	+	+ (2.45)	+	+	+ (1.07)	+	+
17	- (0.09)	-		- (0.07)	-		+ (0.76)	+	+	+ (0.52)	-	+	+ (0.75)	+	+	+ (0.92)	+	+
18	- (0.04)	-		- (0.12)	-		+ (0.59)	±	+	+ (1.20)	+	+	+ (1.63)	+	+	+ (0.80)	+	+
19§	- (0.10)	-		- (0.06)	-		- (0.02)	-	-	- (0.06)	-	-	- (0.14)	-	-	- (0.09)	-	-
20§	- (0.07)	-		- (0.10)	-		- (0.04)	-	-	- (0.15)	-	-	- (0.02)	-	-	- (0.14)	-	-

\* -, negative; +, positive; ±, ambiguous.  
 † S:P ratio shown in parentheses; samples with S:P ratios > 0.4 were considered positive.  
 ‡ Ambiguous sera samples were counted on negative.  
 § Uninfected animal.

**Table 4.** Comparison of Biosign<sup>™</sup> PRRSV to HerdChek<sup>®</sup> PRRS ELISA and IFA using swine sera collected from experimentally infected pigs.

	IFA	HerdChek <sup>®</sup> ELISA	Biosign <sup>™</sup> PRRSV
Positive	44	64	62
Negative*	36	16	18

\* Ambiguous results were counted as negative.

mercial ELISA gave a strong positive result with intense positive staining of the chromatographic strip device.

To obtain sera at known times p.i., experimental inoculation of 3- to 4-week-old piglets obtained from a PRRSV-free, specific pathogen-free herd was performed. Sera were obtained from all pigs before challenge and were determined to be negative for the presence of antibodies against PRRSV. The virus used for inoculation was PRRSV isolate PL 96-1, which was isolated in December 1996 from a case of respiratory failure in Kyeonggi-do, South Korea. The inoculum was prepared after 3 passages in MARC-145 cells. Eighteen pigs were inoculated intranasally with PRRSV (median tissue culture infective dose, 10<sup>7</sup>/ml), which was delivered in volumes of 0.5 ml per nostril. Two uninfected pigs were sham inoculated in a similar manner using MARC-145 cell culture supernatant. Blood samples were collected at 0, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, 23, and 26 days p.i. The sera were tested by chromatographic strip assay<sup>§</sup> and the commercial ELISA.<sup>h</sup> A comparison of results from the 2 tests using this set of 270 samples is presented in Table 2. The sensitivity, specificity, and accuracy of the chromatographic strip assay were found to be 93.2%, 99.2%, and 95.9%, respectively, compared with a standard ELISA. Both the chromatographic strip test and the standard ELISA test detected seroconversion as early as 5 days p.i. after infection for 2 animals (pigs nos. 5 and 9). Using the chromatographic strip assay, antibody was consistently detected in inoculated piglets beginning at 7 days and continuing through 26 days p.i.

Sera collected at 7, 10, 12, and 16 days p.i. from experimentally inoculated pigs were also tested by indirect immunofluorescence (IFA), which was performed essentially as described previously.<sup>21</sup> Briefly, the test sera were heat inactivated at 56°C for 30 minutes and then diluted 1:10 in phosphate-buffered saline (PBS). MARC-145 cells were cultured in 96-well cell culture plates. The PRRSV PL 96-1 strain was inoculated at multiplicity of infection of 0.01 onto each well. The infected plates were incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> for 24 hours and then each well was washed 3 times with 300 µl of PBS. Diluted serum samples were added to duplicate wells and held at 37°C in a humidified chamber for 30 minutes, and then each well was washed 3 times with 300 µl of PBS. Subsequently, fluorescein isothiocyanate anti-swine conjugate<sup>i</sup> was applied and incubated for 30 minutes at 37°C and then washed 3 times with PBS. The plate was observed under a fluorescent microscope, and positive wells were those for which distinct staining of individual cells could be observed. Indirect immunofluorescent antibody test results were not well corre-

lated with the disease status or the results of the chromatographic strip assay<sup>§</sup> or the standard ELISA<sup>h</sup> (Tables 3, 4).

In this study, the authors report the rapid detection of anti-PRRSV antibody using a chromatographic strip device in sera from pigs experimentally infected with virus and in sera collected from swine in production systems. The results of this analysis were compared with the results of a commercial indirect ELISA and an in-house IFA test using the same sera. Importantly, the individuals performing all analyses were blinded to the results of the other tests, including retests of chromatographic strip assays that yielded an ambiguous result upon initial testing. All 3 tests used in this study detect antibodies to N protein. Previous studies investigating the kinetics of the humoral response after PRRSV infection found that the majority of antibodies produced during PRRSV infection are specific for the N protein, the latter being abundantly expressed in vivo by PRRSV-infected cells.<sup>6,9,12,15,21</sup> The N protein of PRRSV is also encoded by a region of the viral genome that is relatively well conserved among North American and European strains.<sup>4,9,13,17</sup> Therefore, all these properties of the PRRSV N gene make it a suitable candidate for the detection of PRRSV antibodies.

Currently, field diagnosis of PRRSV was based on a combination of clinical signs and gross lesions with laboratory confirmation performed using ELISA, RT-PCR, immunohistochemistry (IHC), or virus isolation (or all).<sup>1,5,14</sup> A rapid and specific on-site test for PRRSV for use in swine industries would provide the benefit of an early presumptive diagnosis. An early, rapid diagnosis would allow implementation of efforts to block further spread to other swine. Importantly, the chromatographic strip assay investigated in this study was capable of detecting antibody against PRRSV at early times p.i., with results comparable with the “gold-standard” standard ELISA test for PRRSV antibody. For the 1,080 swine serum samples analyzed (Tables 1, 2), discordant results between the chromatographic strip assay<sup>§</sup> and the standard ELISA<sup>h</sup> were predominantly because of the occurrence of ambiguous results (which were scored as negative) for the chromatographic strip assay.<sup>§</sup> It is interesting to note that the standard ELISA<sup>h</sup> S:P ratios for 88% of the samples yielding ambiguous results were between 0.35 and 0.45, which is close to the cutoff for the standard ELISA.<sup>h</sup>

In summary, the chromatographic strip device is a simple, sensitive, and specific test for screening swine sera for presence of anti-PRRSV antibodies. In addition to speed, accuracy, and sensitivity, the field test should be easy to carry out by unskilled personnel and should be also relatively inexpensive, costing less than US \$4 per sample tested. Moreover, the assay components are stable when stored without refrigeration until the expiration date shown on the sealed package. The test was able to detect anti-PRRSV antibodies in both naturally infected swine sera and experimentally infected swine sera with sensitivity, specificity, and accuracy typically greater than 95% compared with the standard ELISA. This assay would reduce the delay in diagnosis and could be performed without the need for sophisticated laboratory equipment.

**Acknowledgements.** This research was supported by a grant from PBM East, Seoul, Korea, and in part by grant from Konkuk University Faculty Research Fund 2003.

### Sources and manufacturers

- a. Gibco BRL, Life Technologies Inc., Gaithersburg, MD.
- b. New England Biolabs, Beverly, MA.
- c. Stratagene Co., Cedar Creek, TX.
- d. Branson, model S-125, Golden, CO.
- e. Schleicher & Schnell Bioscience Inc., Keene, NH.
- f. Sigma Chemical Co., St. Louis, MO.
- g. Biosign<sup>®</sup> PRRSV, PrincetonBioMeditech, Princeton, NJ.
- h. HerdChek<sup>®</sup> PRRS ELISA, IDEXX Laboratories Inc., Westbrook, ME.
- i. KPL Inc., Gaithersburg, MD.

### References

1. Albina E, Lafordan Y, Barron T, et al.: 1992, An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to the porcine reproductive and respiratory syndrome (PRRS) virus. *Ann Rech Vet* 23:167–176.
2. Bruning A, Bellamy K, Talbot D, et al.: 1999, A rapid chromatographic strip test for the pen-side diagnosis of rinderpest virus. *J Virol Methods* 81:143–154.
3. Christianson WT: 1992, Experimental reproduction of swine infertility and respiratory syndrome in pregnant sows. *Am J Vet Res* 53:485–488.
4. Dea S, Gagnon CA, Mardassi H, et al.: 1996, Antigenic variability among North-American and European strains of PRRSV as defined by monoclonal antibodies to the matrix protein. *J Clin Microbiol* 34:1488–1493.
5. Denac H, Moser C, Tratschin JD, et al.: 1997, An indirect ELISA for the detection of antibodies against porcine reproductive and respiratory syndrome virus using recombinant nucleocapsid protein as antigen. *J Virol Methods* 65:169–181.
6. Loemba HD, Mounir R, Dea S, et al.: 1996, Kinetics of humoral response to the major structural proteins of the porcine reproductive and respiratory syndrome virus. *Arch Virol* 141:751–761.
7. Magar R, Larochelle R, Dea S, et al.: 1995, Antigenic comparison of Canadian and US isolates of porcine reproductive and respiratory syndrome virus using monoclonal antibodies to the nucleocapsid protein. *Can J Vet Res* 59:232–234.
8. Mardassi H, Athanassious R, Mounir S, et al.: 1994, Porcine reproductive and respiratory syndrome virus: morphological, biological and serological characteristics of Quebec isolates associated with acute and chronic outbreaks of porcine reproductive and respiratory syndrome. *Can J Vet Res* 58:55–64.
9. Meng XJ, Paul PS, Halbur PG, et al.: 1995, Phylogenetic analyses of the putative M (ORF6) and N (ORF7) genes of porcine reproductive and respiratory syndrome virus (PRRSV): implication for the existence of two genotypes of PRRSV in the USA and Europe. *Arch Virol* 140:745–755.
10. Meulenbergh JJM, Hulst MM, de Meijer EJ, et al.: 1993, Leystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. *Virology* 192:62–72.
11. Mueller MJ, Blomster M, Oppermann Udo CT, et al.: 1996, Leukotriene A4 hydrolase: protection from mechanism-based inactivation by mutation of tyrosine-378. *Proc Natl Acad Sci USA* 93:5931–5935.
12. Nelson EA, Christopher-Hennings J, Benfield DA: 1994, Serum immune responses to the proteins of porcine reproductive and respiratory syndrome (PRRS) virus. *J Vet Diagn Invest* 6:410–415.
13. Nelson EA, Christopher-Hennings J, Drew T, et al.: 1993, Differentiation of U.S. and European isolated of porcine reproductive and respiratory syndrome virus by monoclonal antibodies. *J Clin Microbiol* 31:3184–3189.
14. Nodelijk G, Wensvoort G, Kroese B, et al.: 1996, Comparison of a commercial ELISA and an immunoperoxidase monolayer assay to detect antibodies directed against porcine respiratory and reproductive syndrome virus. *Vet Microbiol* 49:285–295.
15. Osorio FA, Galeota JA, Nelson E, et al.: 2002, Passive transfer of virus-specific antibodies confers protection against reproductive failure induced by a virulent strain of porcine reproductive and respiratory syndrome virus and establishes sterilizing immunity. *Virology* 302:9–20.
16. Reid SC, Ferris NP, Bruning A, et al.: 2001, Development of a rapid chromatographic strip test for the pen-side detection of foot-and-mouth disease virus antigen. *J Virol Methods* 96:189–202.
17. Suarez P, Zardoya R, Martin MJ, et al.: 1996, Phylogenetic relationships of European strains of porcine reproductive and respiratory syndrome virus (PRRSV) inferred from DNA sequences of putative ORF-5 and ORF-7 genes. *Virus Res* 42:159–165.
18. Sur JH, Doster AR, Christian JS, et al.: 1997, Porcine reproductive and respiratory syndrome virus replicates in testicular germ cells, alter spermatogenesis, and induces germ cell death by apoptosis. *J Virol* 71:9170–9179.
19. Wensvoort G, Terpstra C, Pol JMA, et al.: 1991, Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Vet Q* 13:121–130.
20. Yoon IJ, Joo HS: 1992, An indirect fluorescent antibody test for the detection of antibody to swine infertility and respiratory syndrome virus in swine sera. *J Vet Diagn Invest* 4:144–147.
21. Yoon KJ, Wu LL, Zimmerman JJ, et al.: 1995, Characterization of the humoral immune response to porcine reproductive and respiratory syndrome virus (PRRSV) infection. *J Vet Diagn Invest* 7:305–312.