

Validation of PrioCHECK Porcine PRRSV Ab Strip Kit

Summary

In our validation study, the ELISA-based Applied Biosystems™ PrioCHECK™ Porcine PRRSV Ab Strip Kit:

- scored a high sensitivity (100%) on 52 samples derived from vaccinated pigs
- demonstrated a high specificity (99.7%) on 1,000 Swiss swine sera
- showed high agreement (kappa value = 0.976) with the results obtained with a frequently used porcine reproductive and respiratory syndrome (PRRS) ELISA from another supplier
- correctly identified samples of the 2013, 2014, and 2015 GD Ring Trials*
- delivered excellent repeatability and reproducibility data

The PrioCHECK Porcine PRRSV Ab Strip Kit is a highly reliable test for the detection of antibodies in PRRS virus (PRRSV)-infected pig herds.

Introduction

PRRS is caused by PRRSV, and is characterized by reproductive failure of sows and gilts and respiratory distress of piglets. The PrioCHECK Porcine PRRSV Ab Strip Kit was developed for the detection of antibodies directed against PRRSV in porcine serum samples. The test follows a four-step protocol, consisting of sample preparation, sample incubation, conjugate incubation, and detection. The antigen coated onto the test plate is a chimeric protein of the PRRSV Type 1 and Type 2 strain nucleocapsid protein (ORF 7), enabling detection of antibodies against both virus strains.

This application note describes the evaluation of the PrioCHECK Porcine PRRSV Ab Strip Kit for diagnostic specificity and sensitivity, repeatability, and reproducibility.

Results

Determination of the diagnostic specificity and sensitivity

The diagnostic specificity of the PrioCHECK Porcine PRRSV Ab Strip Kit was evaluated on 204 serum samples derived from different slaughterhouses in Switzerland. To estimate the diagnostic sensitivity of the test, 52 samples from PRRSV-vaccinated animals from Germany were analysed. 20 samples were collected from animals vaccinated with Ingelvac™ PRRS MLV (PRRS Type 2, US strain) and 32 samples from animals vaccinated with Porcilis™ PRRS (PRRS Type 1, EU strain).

The status of all samples was confirmed using an ELISA from another supplier as a reference test (Table 1).

Table 1. Diagnostic specificity and sensitivity.

		Alternative supplier		Total
		Negative	Positive	
PrioCHECK Porcine PRRSV Ab Strip Kit	Positive	52	2	54
	Negative	0	202	202
Total		52	204	256

* GD Ring Trial: GD Animal Health Service, The Netherlands, Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) antibody detection in serum ring trial.

All positive samples were identified correctly, resulting in a sensitivity of 100% (95% confidence interval, 94.4–100.0%). 202 out of 204 negative samples were identified correctly, resulting in a specificity of 99% (95% confidence interval, 97.7–100%). The kappa value of 0.976 calculated from these results shows an almost perfect agreement between the PrioCHECK Porcine PRRSV Ab Strip Kit and the PRRS ELISA from another supplier.

The diagnostic specificity of the PrioCHECK Porcine PRRSV Ab Strip Kit was also tested on 1,000 Swiss pig serum samples derived from 41 herds of fatteners. The samples were collected on five different days at the slaughterhouse in Zurich, Switzerland. As Switzerland has PRRSV-free status, all serum samples were considered PRRSV negative. The samples were processed to generate

Table 2. Diagnostic specificity.

PrioCHECK Porcine PRRSV Ab Strip Kit	Status, PRRSV-free		Diagnostic specificity	95% CI
	Positive	3		
	Negative	997	99.7%	99.4–100%
	Total	1,000		

Table 3. International PRRSV Antibody PTS (2013).

Sample	Description	Status of the sample	PrioCHECK Porcine PRRSV Ab Strip Kit status (S/P ratio)*	Alternative supplier test status (S/P ratio)*
1	Specific pathogen free (SPF) pig EU-type and US-type PRRS antibody-positive, 1:4 dilution	+	+ (1.02)	+ (0.93)
2	SPF pig EU-type and US-type PRRS antibody-positive, 1:8 dilution	+	+ (0.78)	+ (0.53)
3	EU- and US-type PRRSV-positive	+	+ (1.55)	+ (2.10)
4	7-week-old pig US-type PRRSV-positive, acute phase, weak positive on Abs	+	+ (0.40)	+ (1.28)
5	Strong PRRSV-positive	+	+ (1.51)	+ (1.71)
6	EU-type PRRSV-positive	+	+ (1.81)	+ (2.03)
7	EU- and US-type PRRSV-positive, 1:2 dilution	+	+ (1.34)	+ (1.41)
8	US-type PRRSV-vaccinated, blood collection 42 days post-vaccination	+	+ (1.09)	+ (2.00)
9	Positive	+	+ (1.37)	+ (1.02)
10	EU- and US-type-positive, 1:16 dilution	+	+ (0.66)	- (0.36)
11	PRRSV-negative	-	- (0.07)	- (0.01)
12	US-type PRRS-vaccinated, Ab-positive	+	+ (0.88)	+ (1.20)

Result: All samples were identified correctly by the PrioCHECK Porcine PRRSV Ab Strip Kit. Sample 10 is a positive sample that was detected as such with the PrioCHECK Porcine PRRSV Ab Strip Kit, but was tested just below cutoff with the test from another supplier and thus interpreted as negative.

* S/P ratio: sample to positive control ratio. Cutoff value for the PrioCHECK Porcine PRRSV Ab Strip Kit is an S/P ratio of 0.3; cutoff value is 0.4 for the alternative supplier.

the serum the same day and analysed with the PrioCHECK Porcine PRRSV Ab Strip Kit the following day. Two batches of the PrioCHECK Porcine PRRSV Ab Strip Kit were used for testing.

From 1,000 serum samples with a PRRSV-negative status, 997 tested negative with the PrioCHECK Porcine PRRSV Ab Strip Kit, leading to a specificity of 99.7% with a 95% confidence interval of 99.4–100% (Table 2).

GD Ring Trial of the PrioCHECK Porcine PRRSV Ab Strip Kit

Thermo Fisher Scientific participated in the 2013, 2014, and 2015 International PRRSV Antibody Proficiency Testing Schemes (PTS) organized by the Dutch Animal Health Service (GD Deventer). Each PTS was organized and performed according to the requirements of guideline ISO 17043 for providers of proficiency testing schemes. The goal of the PTS is to determine the performance of individual laboratories for specific tests.

Our laboratory participated in the PTS with the PrioCHECK Porcine PRRSV Ab Strip Kit and tested the samples in parallel with a test from another supplier (Table 3).

Table 4. International PRRSV Antibody PTS (2014).

Sample	Description	Status of the sample	PrioCHECK PRRSV Ab Strip Kit status (S/P ratio)*	Alternative supplier test status (S/P ratio)*
1	Specific pathogen free (SPF) pig EU-type and US-type PRRSV antibody-positive, 1:4 dilution	+	+ (0.74)	+ (0.91)
2	SPF pig EU-type and US-type PRRSV antibodies	+	+ (1.06)	+ (1.40)
3	EU-type PRRSV antibody-positive pig	+	+ (1.12)	+ (1.43)
4	Pig from sow vaccinated with US-type PRRSV vaccine. US-type PRRSV antibodies (PRRSV PCR-negative)	+	+ (0.58)	+ (1.11)
5	SPF pig EU-type and US-type PRRSV antibody-positive, 1:16 dilution	+	+ (0.52)	+ (0.46)
6	PRRSV antibody-positive pig: positive	+	+ (1)	+ (1.13)
7	SPF pig EU-type and US-type PRRSV antibody-positive, 1:2 dilution	+	+ (0.93)	+ (1.19)
8	Blood collected from a SPF pig (no PRRSV)	-	- (0.07)	- (0.01)
9	PRRSV antibody-positive pig: strong positive	+	+ (1.09)	+ (1.50)
10	7-week-old pig US-type PRRSV-positive herd (acute phase PRRSV infection); US-type PRRSV-antibodies: weakly positive; US-type PRRSV: PCR-positive	+	- (0.22)	+ (1.03)
11	SPF pig EU-type and US-type PRRSV antibody-positive, 1:8 dilution	+	+ (0.51)	+ (0.48)
12	SPF pig vaccinated with US-type strain VR-2332 PRRSV vaccine; blood collected day 42 post-vaccination.	+	+ (0.74)	+ (1.32)

Result: All samples were identified correctly by the test from the alternative supplier. Sample 10 is a weak positive sample derived from animals in the acute phase of infection and was tested negative with the PrioCHECK PRRSV Ab Strip Kit.

* S/P ratio: sample to positive control ratio. Cutoff value for the PrioCHECK Porcine PRRSV Ab Strip Kit is an S/P ratio of 0.3; cutoff value is 0.4 for the alternative supplier.

Table 5. International PRRSV Antibody PTS (2015).

Sample	Description	Status of the sample	PrioCHECK Porcine PRRSV Ab Strip Kit status (S/P ratio)*	Alternative supplier test status (S/P ratio)*
1	PRRSV antibody-positive pig: positive	+	+ (0.95)	+ (1.09)
2	Field sample EU-type-positive in PCR	-	- (0.05)	- (0.02)
3	SPF pig EU-type and US-type PRRSV antibodies	+	+ (1.19)	+ (1.75)
4	EU-type PRRSV antibody-positive pig, blind duplo	+	+ (1.34)	+ (1.74)
5	SPF pig EU-type and US-type PRRSV antibodies, 1:32 dilution	+	+ (0.4)	- (0.14)
6	Sample from sow vaccinated with US-type PRRSV vaccine: US-type-positive	+	+ (0.63)	+ (0.97)
7	9-week-old pig US-type, positive-herd (acute phase) US-type AB: weak positive	+	+ (0.39)	+ (0.55)
8	SPF pig infected with PRRSV EU, booster on 14 d.p.i., blood sample 28 days past booster	+	+ (1.1)	+ (1.79)
9	PRRSV antibody pig: positive	+	+ (0.79)	+ (1.02)
10	SPF pig: vaccinated with MLV V2332, booster on 21 d.p.v., blood sample 33 days past booster	+	+ (0.96)	+ (0.51)

Result: All samples were identified correctly by the PrioCHECK Porcine PRRSV Ab Strip Kit. Sample 5 is a diluted positive sample that was detected positive with the PrioCHECK Porcine PRRSV Ab Strip Kit but tested negative with the test from another supplier.

* S/P ratio: sample to positive control ratio. Cutoff value for the PrioCHECK Porcine PRRSV Ab Strip Kit is an S/P ratio of 0.3; cutoff value is 0.4 for the alternative supplier.

The PrioCHECK Porcine PRRSV Ab Strip Kit and the test from another supplier deliver comparable results on the PTS sample panels.

Repeatability and reproducibility of the PrioCHECK Porcine PRRSV Ab Strip Kit

Repeatability

The repeatability of the PrioCHECK Porcine PRRSV Ab Strip Kit was assessed by evaluating both the intra-plate and the inter-plate variation.

For the intra-plate variation, the weak positive control was tested 32 times on the same ELISA plate and the coefficient of variation (CV) was calculated (Table 6).

Table 6. Intra-plate variation.

Mean	Standard deviation	CV (%)*
1.092	0.035	3.21

* Intra-plate variance is 3.21% for the weak positive control sample.

The inter-plate variation was calculated by testing 13 negative and 8 positive samples on two different plates on the same day. The PrioCHECK PRRSV Ab Strip Kit demonstrated a mean CV of 3.05% for both negative and positive samples.

Both the intra-plate and inter-plate variation values show excellent repeatability of the test results.

Reproducibility

To assess the reproducibility, the PrioCHECK Porcine PRRSV Ab Strip Kit was run on three different days with 34 PRRSV Type 1–positive and 20 PRRSV Type 2–positive samples (Table 7). Additionally, positive control samples were included in the assay.

Table 7. Reproducible data.

Samples	Number	CV (%)*
Positive control	8	3.5
Weak positive control	8	6.7
PRRSV Type I–positive (EU)	34	5.2
PRRSV Type II–positive (US)	20	5.7

* The data indicate a CV of the positive serum samples of below 7%. The demonstrated reproducibility is excellent.

Conclusion

The PrioCHECK Porcine PRRSV Ab Strip Kit demonstrated a high sensitivity and specificity. The test also shows good repeatability and reproducibility, and the data show that the PrioCHECK Porcine PRRSV Ab Strip Kit is a robust ELISA. The results obtained with the test show a good agreement with the results of the PRRS ELISA from another supplier.

The PrioCHECK Porcine PRRSV Ab Strip Kit reliably detects antibodies in PRRSV-infected pigs and is a powerful tool for PRRSV serology testing in pig herds.

Ordering information

Product	Quantity	Cat. No.
PrioCHECK Porcine PRRSV Ab Strip Kit	5 strip-plate kit (450 samples)	7610880

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