applied biosystems

Earlier and easier diagnostic tools for PRRSV herd management: Comparison of sampling and prevalence under field conditions

Calvin GUNTER[1], Nardy ROBBEN[2], Sandrine MOINE[3] and Anne QUIJADA[3]
[1]Thermo Fisher Scientific, Austin, TX, USA; [2]Thermo Fisher Scientific, Bleiswijk, Netherlands; [3]Thermo Fisher Scientific, Lissieu, France

ABSTRACT

The main goal of several studies was to validate oral fluid against blood/serum and tissue and also establish a sampling recommendation for oral fluids under field conditions for an earlier diagnostic of PRRSV with an easier sampling method. Thermo Fisher Scientific requested several laboratories and research institutes throughout the world to evaluate the rtRT-PCR tools on over 800 field samples from different genotypes. A field study in European or North-American allowed evaluation of the performance of the kit on oral fluids samples. Based on these results, a biostatistical study was conducted to calculate the probability of genotype 1 and 2 virus detection in a pen using oral fluid samples taking into account the prevalence of PRRSV in serum as an independent variable.

The LSI VetMAX™ PRRSV EU/NA kit has shown a sensitivity of 97.2% on 383 positive field samples and a specificity of 100% on 421 negative field samples. With regards to the oral fluids samples, the kit showed an excellent correlation at pen level (oral fluid sample) compared to animal level (blood/serum sample) with a difference of +/-1C_T. The number of oral fluids that need to be sampled on herd level in order to find at least 1 PRRSV oral fluid positive was, for example, 3 for a serum prevalence of 50%.

INTRODUCTION

Diagnostic tests are often used to assess the PRRSV infection status of pig herds. For routine settings, ELISA test methods and reversed transcriptase PCR (RT-PCR) are used to determine antibody titers and detect antigen. Until now in Europe, RT-PCR on serum/blood and tissue samples is the most used technique to detect PRRSV. For several years, the detection of many pig pathogens in orals fluids was reported as an alternative technique being more economical, easier and less invasive. One of the main goal of this study was to establish clear recommendations in herd management for swine practitioners, when oral fluids are used as a diagnosis procedure.

MATERIALS AND METHODS

Part 1 : Field studies

Thermo Fisher Scientific requested several laboratories and research institutes to evaluate the LSI VetMAX™ PRRSV EU/NA Kit. Over 800 fields samples panels (serum, semen, tissues, organs, fluids, swabs, etc) were selected from different swine herds containing either European or North-American PRRSV subtype, or containing both, or without any PRRSV contamination. The disease status of test samples had been previously determined using another method (sequencing, alternative real-time RT-PCR, ELISA).

Figure 1. Partnerships for Part 1 –Field studies

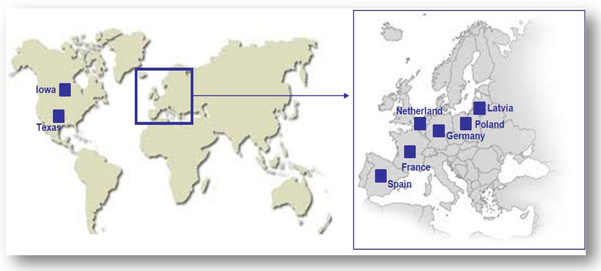


Table 1. Panel presentation for Part 1 –Field studies

		Sample status		
		Positive	Negative	Total
Samples	Blood/Serum	103	100	203
	Tiissues / Organs(1)	51	9	60
	Oropharyngeal area(2)	63	187	250
	Cell culture Strain/Vaccine	44	11	55
	Various field samples(3)	122	114	236
	Total	383	421	804

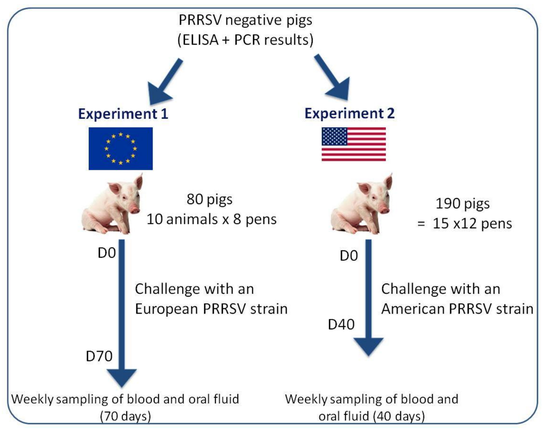
(1)Tonsil. Lung. Placenta. Liver. Etc.
(2) Bronchoalveolar washings. Oral fluids. Swab
(3)Panel of samples (organ. serum. tissue)

Part 2: Experimental challenge

In both experiments, sample extraction is carried out using magnetic beads extraction (MagMAX™ Viral RNA Isolation Kit or MagVet™ Universal Isolation Kit). RT-PCR is carried out using LSI VetMAX™ PRRS EU/NA or TaqMan® NA and EU PRRSV Reagents. A C_T value over 40 is considered negative.

A logistic regression analysis was carried out in SPSS 15.0 (SPSS Inc., 1989-2006) on the data from both challenges. With this probability, basic epidemiologic information (Thrusfield, 1997) was used to correctly estimate the number of pens necessary to be sampled in order to detect a disease.

Figure 2. Part 2 – Experimental challenge design



RESULTS

Table 2. Sensitivity/Specificity for Part 1 – Field studies

		Other methods sequencing, alternative rtRT-PCR, ELISA		
		Positive	Negative	Total
LSI VetMAX™ PRRS EU/NA Kit	Positive	372	10*	382
	Negative	11	411	422
	Total	383	421	804

*10 EU PRRSV positive samples expected negative have been confirmed by sequencing.

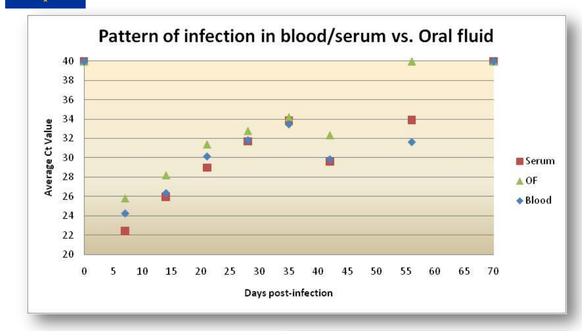
LSI VetMAX™ PRRSV EU/NA kit has shown a sensitivity of 97.2% on 383 positive field samples and a specificity of 100% on 421 negative field samples.

Figure 3. Sensitivity/Specificity for Part 2 - Experimental challenge

Diagnostic agreement of PRRSV infectious level on blood/serum and oral fluid samples over several days post-infection (dpi) by RT-PCR.

One pen is highly positive to PRRSV if, at least, one animal is positive in serum. This result will be compared with the result obtained from the oral fluid analysis.

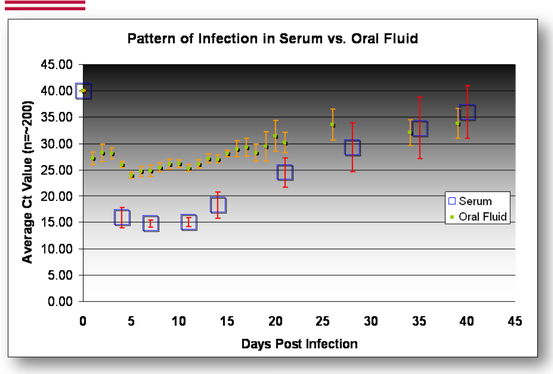
Figure 3.a Experiment 1



		At least 1 PRRS positive animal per pen (Serum)	
		Diseased	Healthy
Oral Fluid	Positive	49	0
	Negative	7	16

Sensitivity : 87.5% (78.8%, 96.2%)
Specificity : 100.0%

Figure 3.b Experiment 2



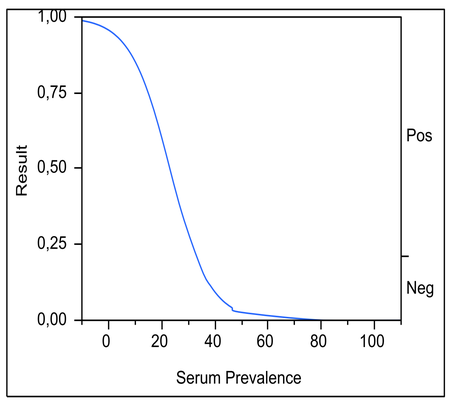
		At least 1 PRRS positive animal per pen (Serum)	
		Diseased	Healthy
Oral Fluid	Positive	96	0
	Negative	0	12

Sensitivity : 100.0%
Specificity : 100.0%

Results obtained per pen (oral fluids analysis) demonstrate an excellent correlation with results on individual samples (blood/serum analysis). The presence of PRRSV EU/NA RNA was identified in an early infectious stage.

Figure 4. Determination of the probability to detect PRRSV virus in oral fluid taking into account the prevalence of PRRSV in serum

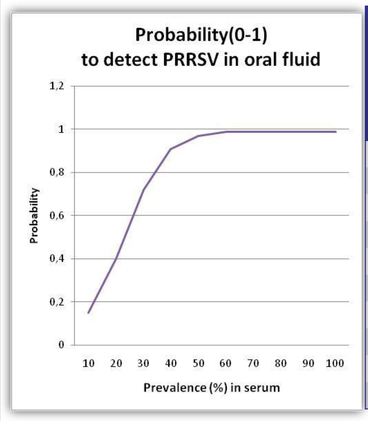
Figure 4a. Logic regression analysis merging data from the North American and European studies



The probability to detect PRRSV in oral fluid is significantly associated to the PRRSV prevalence in serum (p=0.0001) and the determination coefficient of the logistic regression analysis is 82%.

Thus, much of this probability to detect PRRSV in oral fluid can be explained only by the PRRSV prevalence in serum.

Figure 4b. Relation between the prevalence of PRRSV in serum and the detection in oral fluid



Prevalence of PRRSV (determined in serum)	Probability (0-1) to detect PRRSV in oral fluid
10	0,15
20	0,40
30	0,72
40	0,91
50	0,97
60	0,99
70	0,99
80	0,99
90	0,99
100	0,99

If the serum prevalence is higher than 50%, the probability to detect this virus in oral fluid samples is close to 1.

Table 3. Estimation of the number of pens to be sampled in order to detect at least one oral fluid positive taking into account the prevalence present in serum

Number of pens to be sampled in order to detect at least one positive pen, taking into account the prevalence present in serum and the information obtained previously in the logistic regression analysis.

Oral fluids sampling recommendation			
Prevalence of PRRSV (determined in serum)	Confidence level of 95%	Confidence level of 99%	Confidence level of 99.99%
10	19	29	58
20	6	9	19
30	3	4	8
40	1	2	4
50	1	2	3
60	1	1	2
70	1	1	2
80	1	1	2
90	1	1	1
100	1	1	1

If the estimated prevalence of PRRSV in serum is higher than 50%, only one oral fluid sample per pen would be needed to detect a positive sample for a confidence level of 95 %.

CONCLUSIONS

LSI VetMAX™ PRRSV EU/NA kit showed a sensitivity of 97.2% and a specificity of 100% over 800 fields samples (serum, semen, tissues, organs, fluids, swabs, etc)

LSI VetMAX™ PRRSV EU/NA kit showed an excellent correlation at pen level (oral fluid sample) compared to animal level (blood/serum sample) with a difference of +/-1C_T.

Oral fluids samples are a good tool to gain information about PRRS status on pen level. Based on epidemiology and prevalence of the virus in the herd, an oral fluid sample is able to get same information as blood samples. Whereas, the percentage of oral fluid positive samples depends of the number of infected animals per pen.

Oral fluids allow the swine industry to generate a more accurate and reliable monitoring system, where early detection can result in faster response time. It is an opportunity to increase the number of pigs tested while decreasing the cost of analysis.

Oral fluids sampling is easy to use for farmers and veterinarians. Animals are less stressed than blood sampling and have a natural attraction to the rope and start chewing. It could be an early warning system for monitoring of herds, on pen level, for example to estimate the circulation of different swine pathogens (PRRSV, PCV2 and SIV) as the data generated in this study clearly support.

REFERENCES

▪J. Brouwer, K. Frankena, M.F. De Jong, R. Voets, A. Dijkhuizen, J.H.M. Verheijden, R.E. Komijn. PRRS: effect on herd performance after initial infection and risk analysis Vet. Q., 2 (1994), pp. 95–100
▪E. Mattheu, M. Tello, A. Coll, J. Casal, M. Martin. Comparison of three ELISAs for the diagnosis of porcine reproductive and respiratory syndrome. Vet. Rec., 159 (2006), pp. 717–718
▪E.J. Neumann, J.B. Kliebenstein, C.D. Johnson, J.W. Mabry, E.J. Bush, A.H. Seitzinger, A.L. Green, J.J. Zimmermann. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States JAVMA, 227 (2005), pp. 385–392
▪Thrusfield, M. (2007). Diagnostic testing. In: “Veterinary Epidemiology”, 3rd Ed. Blackwell publishing, Hong Kong.
▪G.J. Wellenberg. Review: diagnostic methods for the detection of porcine reproductive and respiratory syndrome virus (PRRSV) infections. Tijdschr. Diergeneeskd., 131 (16) (2006), pp. 566–572

ACKNOWLEDGEMENTS

▪GSP laboratory, Lleida (Spain)
▪Lorenzo José FRAILE SAUCE, University of Lleida (Spain)
▪The veterinary team of the cooperating farm in Spain
▪Dr Bob Rowland, Kansas State University

TRADEMARKS/LICENSING

▪LSI VetMAX™ PRRSV EU/NA (Ref.: PRRSEUNA)
▪TaqMan® NA and EU PRRSV Reagents
▪MagMAX™ -96 Viral RNA Isolation Kit (Ref.: 4462359)
▪MagVet™ Universal Isolation kit (Ref.: MV384)
▪TEGO™ Swine Oral Fluid Collection Kit (Ref.: 12329)

ThermoFisher SCIENTIFIC