applied biosystems

PRRS virus surveillance: Role of virus sequencing and virus detection by PCR

ESPHM-VVD-007

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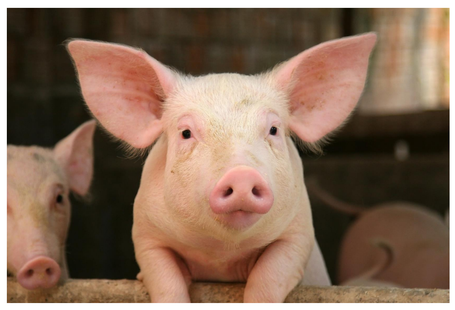
INTRODUCTION

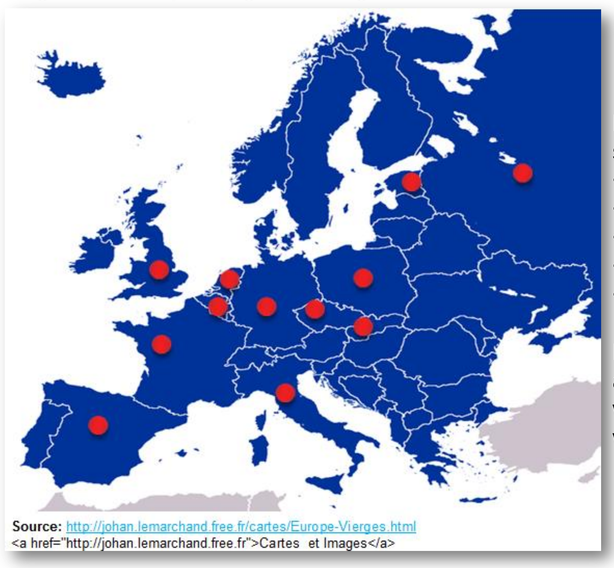
Porcine reproductive and respiratory syndrome (PRRS) is a highly infectious disease, endemic in pigs throughout the world. PRRS is caused by a single stranded positive-sense RNA enveloped virus with a high mutation rate leading to greater heterogeneity of the nucleotide sequence between individual strains.
The genetic diversity of the virus increases the risk of reduced sensitivity for diagnostic nucleotide detection methods. The aim of the present study was to monitor circulating PRRSV strains throughout Europe using sequencing technologies, in order to update our diagnostic test method.



MATERIALS AND METHODS

Thermo Fisher Scientific established different partnerships to collect more than 100 PRRSV positive samples in more than 10 different countries (Figure 1).
Sequencing strategy applied depends on PRRS viral load and quality of the sampling process: sample collection, storage, shipment (Figure 2).
For 82 samples containing a high/medium PRRS viral load with a high quality sampling, RNA-Seq or Long Range protocols on PGM instrument were applied in order to obtain whole PRRS genome sequences.
For 20 samples containing a weak viral load or with a poor quality, capillary electrophoresis protocol on Genetic Analyzer was performed in order to obtain a specific target sequence of PRRS genome.

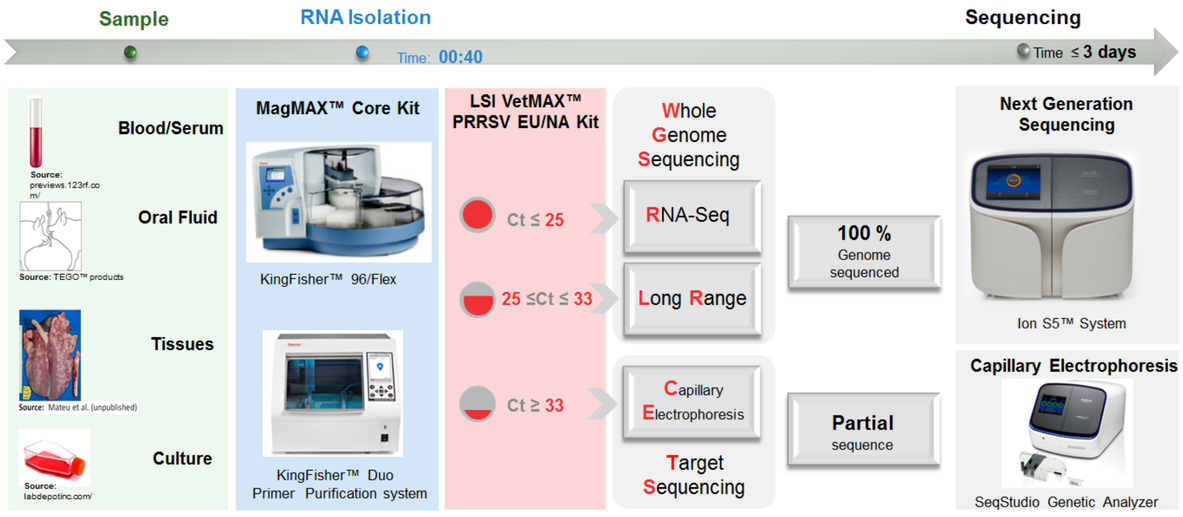
Figure 1. Sample origin coming from more than 10 countries



More than 100 PRRSV positive samples were sequenced :
- Serum/Blood samples
- Cultures
- Oral fluids
- Tissues
- RNA from various sample type

Different viral load were obtained for all samples:
a majority of sample containing high/medium PRRS viral load and some samples containing a weak PRRS viral.

Figure 2. Sequencing strategy– Global workflow



The analytical strategy is divided into different steps: Viral RNAs are isolated using the MagMAX™ Core Nucleic Acid Purification Kit on KingFisher machines. Isolated RNA is amplified using LSI VetMAX™ PRRSV EU/NA Kit on QuantStudio 5 real-time PCR system.
Depending on the PRRS viral load estimation into samples, two sequencing strategies were applied:

- Samples containing a high/medium PRRS viral load, RNASeq or Long Range protocols were applied in order to obtain complete PRRS genome sequences.
  - Using the RNASeq protocol, no additional step is needed between isolated RNA and Sequencing step.
  - Using the Long Range protocol, 2 additional steps are required before the sequencing: Step 1, full-length cDNA synthesis. Step 2, cDNA amplification (4 fragments of 4Kb). Each fragment is used as a template for the sequencing.
- Samples containing a weak viral load, capillary electrophoresis protocol was performed in order to obtain a specific target sequence of PRRS genome (ORF7 sequence).

RESULTS

Figure 3. Bioinformatics Analysis – Identification of most conservative region

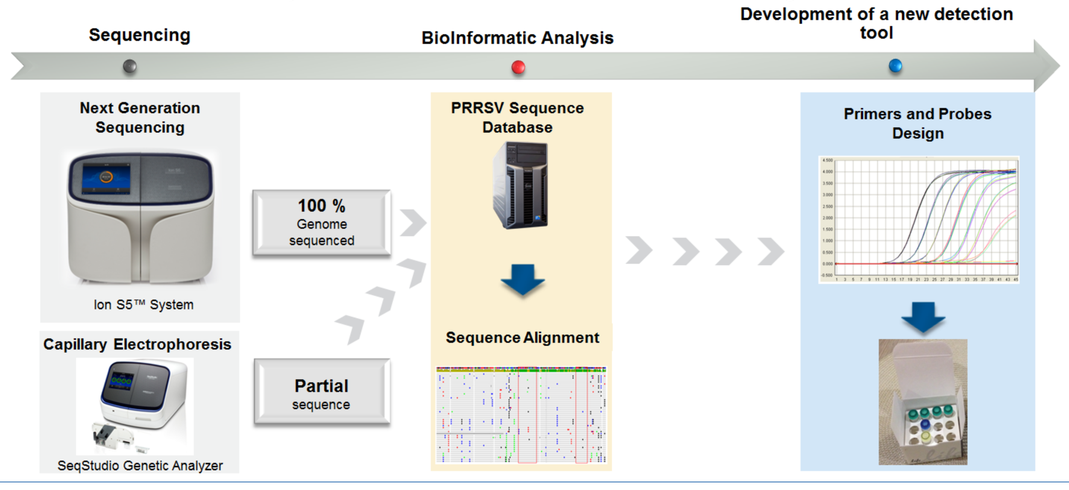


Table 1: Inclusivity results

Genotype	Number	VetMAX PRRSV EU & NA assay
EU Subtype 1	≈ 185 Samples	Detected
EU Subtype 2	9 Strains	Detected
EU Subtype 3	5 Strains	Detected
EU Subtype 4	1 Strain	Detected
EU Atypical	1 Strain	Detected
NA	18 samples	Detected
NA High Pathogenic	1 strain	Detected

- The VetMAX PRRSV EU & NA assay in development allows the detection of the 4 subtypes of the PRRSV European genotype, including Eastern Europe strains and atypical European strains.
- The VetMAX PRRSV EU & NA assay in development allows the detection of the North American PRRSV genotype, including High pathogenic Chinese strain.

Table 2: Characteristics of VetMAX PRRSV EU & NA assay in development

Characteristics	Standard mode EU	Standard mode NA	Fast mode EU	Fast mode NA	Remarks
Specificity (Inclusivity/Exclusivity)	Specific detection of PRRSV				200 PRRSV strains 22 Porcine pathogens
Efficiency	94,3%	101,9%	97,4%	99,1%	Evaluated on transcript RNA
Limit of Detection (MonoInfection) *	9 copies / RT-PCR		9 copies / RT-PCR		95% of confidence
Limit of Detection (Coinfection) *	9 copies / RT-PCR		9 copies / RT-PCR		
Repeatability	CV<1,26%	CV<1,10%	CV<1,56%	CV<1,86%	Evaluated on 3 concentration levels (High/Medium/Low)
Reproducibility	CV<1,69%	CV<2,10%	CV<1,60%	CV<1,87%	
Robustness	Non affected by different parameters (Variation of RNA or MMx volumes, T°C and Hybridization time)				Evaluated at 2X LD_{RT-PCR}
Stability	In progress over a 15 months period				Evaluated at the LD_{RT-PCR}

Table 3: Diagnostic sensitivity on a panel of 200 positive field samples

		Prototype		
	EU	Pos +	Neg -	
Sequencing	Pos +	198	2	200
	Neg -	0	0	0
		198	2	200

➢ **Diagnostic sensitivity : 99%**

The diagnostic sensitivity was evaluated on a panel of 200 field samples, determined positive in European PRRSV by sequencing. These samples were collected from different countries (see Figure 1).
The diagnostic sensitivity of the VetMAX PRRSV EU & NA assay in development was estimated to 99%.

CONCLUSIONS

PRRSV is a highly mutating, so we consistently monitor PRRSV strains to be sure to offer the most up-to-date PCR solution to enable our customers to work with confidence and detect all strains of concern.

The monitoring of circulating European PRRSV strains, using sequencing technologies enables the sequencing of RNA directly isolated from field samples.

Sequencing approaches offer the possibility to identify new PRRSV strains, increasing the performance of a diagnostic tool for PRRSV detection.

The VetMAX PRRSV EU & NA assay in development is designed to reinforce the efficacy of PRRSV surveillance program in the field, with the detection of the 4 subtypes of the PRRSV European genotype, and a diagnostic sensitivity of 99%.

Thermo Fisher Scientific offers a range of adapted workflows from the sampling, extraction methods to the sequencing solutions

ACKNOWLEDGEMENTS

- **Sarah McGowan – Jean Pierre Frossard**, Virology, Animal and Plant Health Agency (APHA), UK
- **Katarzyna Podgorska**, Swine Diseases Department, National Veterinary Research Institute (NVRI), Poland
- **Tomasz Stadejek**, Warsaw University of Life Sciences, Faculty of Veterinary Medicine, Poland
- **Ivan Toplak**, Nacionalni veterinarski inštitut (NVI), Slovenia
- **Beatrice Boniotti**, Istituto Zooprofilattico Sperimentale della Lombardia e Dell' Emilia Romania (IZS), Italy
- **Brigitte Cay**, Coda Cerva, Belgium
- **Jordi Serra Marinez**, BIOFAR Laboratoris, S.L, Spain
- **Enric Mateu**, CRESA, Spain
- **Adolf Steinrigl – Sandra Revilla-Fernandez**, Agentur für Gesundheit und Ernährugssicherheit GmbH (AGES), Austria

TRADEMARKS/LICENSING

•Applied Biosystems™ MagMAX™ Core Nucleic Acid Purification kit**
•Applied Biosystems™ LSI VetMAX™ PRRSV EU/NA kit*
•Applied Biosystems™ QuantStudio™ 5
•Thermo Scientific™ KingFisher™
•Ion Torrent™**
•Ion S5™ system**

*For veterinary use only. Regulatory requirements vary by country; products may not be available in your geographic area.
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