RESEARCH REPORT
SWINE HEALTH

Title: Application of FTA based technology for the collection and transport of clinical samples to detect PRRSV by RT-PCR.- **NPB # 09-220**

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Industry Summary:

The ability for producers to succeed depends in part, on their ability to rapidly respond to emerging and existing disease challenges. Submission of fresh samples to diagnostic laboratories in a timely manner often represents a challenge. The use of FTA cards, a filter paper especially designed for the transport and storage of samples, is one option to safely store and rapidly transport biological samples from the field to diagnostic laboratories at a low cost. FTA cards consist of a cellulose-based matrix paper containing chemicals that lyse the cells in the sample while preserving the nucleic acids. Therefore the infectious agents become inactive while their genetic material is preserved.

The objective of this study was to validate the FTA cards for PRRS virus diagnostics. Specifically this study evaluated the FTA cards as an alternative method to transport and store biologic samples to conduct PRRSv molecular testing. Diagnostic sensitivity and specificity of samples embedded on FTA cards was compared to that of samples tested directly (conventional method). Samples originated from both experimentally infected pigs and field submissions to the Veterinary Diagnostic Laboratory.
In vitro validation indicated that detection of PRRSv in FTA cards was possible and that sensitivity was good although lower than testing the samples directly. Results from the experimentally infected animals showed 100% agreement between PCRs from samples embedded on cards and samples tested directly. Sensitivity and specificity was 100%. The samples included serum, blood and tissues (lung, lymph nodes and tonsils) collected from acutely infected animals shortly after euthanasia. PCR sensitivity for samples stored in FTA cards at room temperature or at 4°C, and stored overnight or for 2 weeks was similar. In addition, sensitivity for field serum samples embedded on FTA cards was 86%. In the case of oral fluids, sensitivity was only 36%.
In summary, diagnostic sensitivity of FTA cards from samples collected from experimentally infected animals was good and similar than testing the samples directly. However, sensitivity was slightly lower when field samples were used. The lower sensitivity for field samples may reflect the variability observed in the field and ultimately may result in false negative results. In addition, further evaluation is required to recommend the use of FTA cards to transport oral fluids.
In conclusion, FTA cards are an alternative method for collecting, transporting and storing sera and tissue samples for PRRSV molecular diagnostics. While the probability of detecting PRRSV in FTA

cards is lower than in fresh samples, FTA cards offer advantages to producers which include: a) ease of sample collection and submission in the field, b) safety of samples embedded in the cards making possible to ship samples in a single envelope without need for biohazard labeling, and c) lower cost of submitting samples.

Keywords: PRRS, FTA, diagnostic, safety, swine

Scientific Abstract:

Submission of field samples for porcine reproductive and respiratory syndrome virus (PRRSv) molecular diagnostics can be a challenge if samples cannot be submitted in a timely manner. This study evaluated the safety and the diagnostic sensitivity and specificity of samples embedded in FTA cards for PRRSv RT-PCR testing. FTA cards consist of a cellulose-based matrix paper containing chemicals that lyse the cells in the sample while preserving the nucleic acids. The cards are a safe and easy-to-use method to store and transport samples to conduct molecular diagnostics. Samples for this study originated from both experimentally infected animals and field samples submitted to the Veterinary Diagnostic Laboratory. The analytical sensitivity of PRRSv detection by RT-PCR from serum and oral fluids embedded in FTA cards was reduced 10^2 to 10^4 times compared to detecting the virus directly from serum and oral fluids respectively. However, the virus could still be detected in FTA cards at a very low concentration (10^1 and 10^3 $TCID_{50}$/ml for serum and oral fluids respectively). The specificity and sensitivity of PRRSv RT-PCR detection from FTA-embedded samples collected from experimentally infected animals was 100%. Sensitivity was the same for samples stored in FTA cards at room temperature or at 4°C, and tested overnight or after 14 days. However sensitivity using field serum samples embedded in FTA cards was only 86.1%. The sensitivity for oral fluids in FTA cards from experimentally infected animals was poor, and estimated at 50% from 2 to 16 dpi. After 16 dpi and until the end of the study at 28 dpi, PRRSv could not be detected when the samples were on FTA cards, despite positive PCR results during all sampling days from 2 to 28 dpi when samples were tested directly. Overall sensitivity for oral fluids in FTA cards was 36%. Further validation is needed to improve sensitivity of detecting PRRSv from oral fluids in FTA cards. In addition, cards inoculated with PRRSv positive samples did not yield replicating virus after cell culture. In conclusion, FTA cards proved to be an alternative, safe, simple, sensitive method to transport serum, blood, lymph nodes, tonsils and lung samples from acutely infected animals for PRRSv RT-PCR diagnostics. However, a decrease in RT-PCR sensitivity should be expected especially from oral fluid samples.

Introduction

Livestock productivity is limited in part because of the effect that infectious diseases have on the health and well being of animals. In pigs one of the most important disease worldwide is porcine reproductive and respiratory syndrome (PRRS), a viral disease that was first diagnosed two decades ago [12, 13] and affects all segments of pig production. The annual economic impact of PRRS on the US swine industry is estimated at over $560 million USD[17]. PRRS virus (PRRSv), the causative agent, is an enveloped single stranded positive sense RNA virus of the Arteriviridae family [2, 20]. Due to the devastating impact of PRRSv on swine production, veterinarians adopt strict biosecurity measures to prevent infection and require rapid, convenient, safe and reliable diagnostic methods to properly identify and monitor the disease and prevent its spread within and between farms.

FTA (Flinders Technology Associates) cards were first developed and reported by Burgoyne[5, 6] and are now commercialized by Whatman Inc . FTA cards consist of a cellulose-based matrix paper containing a mix of chemicals that lyse cells and inactivate bacteria and viruses while preserving the nucleic acids. Nucleic acids become physically entrapped and stabilized and are protected from nuclease, oxidation, UV damage and microbial and fungal degradation [6, 9, 26]. Because samples are rendered noninfectious, samples are protected from microbial growth contamination and the user is protected from potential biohazards present in the sample. Samples embedded in FTA cards are

suitable for molecular diagnostics but not for culture and isolation of infectious agents. Therefore FTA cards can be used to transport biosamples without the requirement of a biohazard-shipping label and can represent significant cost savings since samples can be shipped in standard letter mail envelopes at room temperature. In addition, FTA cards can also facilitate collection of samples in remote locations with the subsequent access to state of the art diagnostic facilities located in countries different from where the samples were collected.

FTA cards have been used for the transport, testing and storage of infectious samples containing a wide range of pathogens affecting humans, plants and animals including bacteria [10, 23], fungi [4, 7], rickettsia [3], protozoa [1, 30], parasites [25] and viruses. Among viruses affecting animals, there are reports of the rabies virus, newcastle disease virus, foot and mouth disease virus, swine influenza virus and porcine reproductive and respiratory syndrome virus [11, 14, 16, 18, 19]. In general, studies reported slight decreased analytical sensitivity of RT-PCR for RNA viruses spiked *in vitro* on FTA cards [11, 16, 18]. On the other hand studies with samples from experimentally infected animals showed no difference in sensitivity of RT-PCR to detect RNA virus in FTA cards compared to RT-PCR performed directly from fresh samples [16, 18] Most of these studies used limited number of samples and validation did not include testing of field samples from naturally infected animals. Only one study performed with 14 field samples by Muthukrishnan *et al* (2008) [16] reported no difference in RT-PCR sensitivity to detect FMDV when using FTA cards compared to RT-PCR directly from fresh samples. Studies using a larger number of samples representing broader scenarios including field settings have not been reported to our knowledge. In addition, there is limited information on whether sensitivity changes overtime or whether conditions of temperature affect card sensitivity.

In the case of PRRSv, a prior study showed that PRRSv could be detected in blood samples embedded in FTA cards [11]. However, no information is available on the sensitivity and specificity of PCR-based methods to detect PRRSv in samples preserved on FTA cards and how these cards may perform under field conditions. Therefore additional validation is needed to assess the suitability of using FTA cards for routine diagnostics of swine pathogens.

Objectives:

The objectives of this study were to: a) evaluate the *in vitro* analytical sensitivity, stability and safety for PRRSv positive fluids embedded on FTA cards, b) evaluate the sensitivity and specificity of PRRSv RT-PCR from tissues and fluids embedded on FTA cards from experimentally infected animals and from samples collected from naturally infected animals under field conditions, and c) evaluate collection and testing conditions in regards to time and storage temperature. The overall objective was to assess whether FTA cards can be used as a reliable method for the submission and testing of swine samples for routine molecular PRRSv diagnostics.

Materials & Methods:

Animals and experimental infection. Twelve 3-week-old pigs purchased from a PRRSv negative farm were confirmed PRRSv negative by serology (HerdChek® PRRS 2XR Antibody ELISA, IDEXX Laboratories, Westbrook, Maine USA) and RT-PCR (Taqman RT-PCR kit, Applied Biosystems, Foster City, California USA). Upon arrival pigs were individually identified and randomly housed in two separate rooms at the University of Minnesota animal research units. Pig housing, care and management procedures had been previously approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC).

Pigs were divided into infected (n=8) and control (n=4) groups. Pigs in the infected group were inoculated intramuscularly with 2 ml/pig of PRRSv isolate MN-184 at 10^5 $TCID_{50}$/ml. Pigs in the control group received an injection of saline buffer. Pigs were monitored daily for clinical signs of disease and sampled on a weekly basis as described below.

Sample collection and processing. All pigs were sampled prior to inoculation and at 7, 14, 21 and 28 days post infection (DPI). Confirmation of PRRSv infection was done at 4 days post inoculation by serum RT-PCR. Samples from known positive (infected) and known negative (control) pigs were used to assess the collection, handling, shipping and laboratory processing of samples using FTA cards. At each time point, one control pig and two inoculated pigs were necropsied and sampled. Additionally, oral fluids were collected from both rooms (infected and control) three times per week according to procedures described by Prickett *et al.*, 2008 [21]. At necropsy, blood samples as well as tissues (lung, tonsil and inguinal lymph nodes) were taken. One hundred µl of the fluid samples were transferred to the FTA cards shortly after collection. A fragment of each tissue sample was transferred to the FTA card in the form of directly imprinting 1 cm^2 of the tissue in the card, and by means of swabbing the interior of the tissue and then rubbing the swab onto the card. Each collected sample was transferred to FTA cards in quadruplicates and submitted for RT-PCR as "fresh" form. FTA cards were stored and processed after collection as follows: 4°C / 24 hours, 4°C / 14 days, 25°C / 24 hours and 25°C / 14 days.

Field samples. Forty-five pig serum samples were selected from field submissions arriving at the University of Minnesota Veterinary Diagnostic Laboratory for PRRSv RT-PCR testing. Each serum sample was side-by-side tested directly by RT-PCR and after inoculation onto FTA cards. FTA card samples were stored overnight at room temperature for drying and tested within 24 to 48 h.

Virus. MN-184 strain of PRRSv at concentration of 10^5 $TCID_{50}$/ml was used for animal experimental infection and *in vitro* assays.

FTA cards and punching methods. Classic indicating FTA cards (Whatman Ltd. Catalog number WB 120206) were used for this study. Two punching protocols were used to obtain the FTA card disks for the RNA elution. The first protocol used three "small" punches of 2mm of diameter each obtained with the 2 mm Harris punch (Whatman Ltd. Catalog number WB 100040). The second protocol used one "large" 6 mm punch and obtained with a 6mm diameter office paper punch (McGill Inc., catalog number 803 CR ¼). The Harris puncher was disinfected using 99% ethanol solution, dried using disposable wipes and punching 2 blank cards before punching each sample. The office punchers were disinfected using 1:100 Synergize disinfectant (Preserve International) in a 10 min bath and autoclaved for 10 min at 200°C. The office punchers were also compared to a 6mm Harris punch. To validate the disinfection protocol and to rule out the possibility of cross contamination between samples, one negative control (card embedded with 100µl of saline solution) was punched after every 5^{th} positive control samples for a total of 10 negative controls. Once the disinfection protocol was validated, one negative control was punched after every 10^{th} test sample.

RNA elution. To elute the RNA from the FTA card, the RNA Rapid Extraction Solution (Ambion-Applied Biosystems) was used as recommended by the manufacturer. One 6 mm disk from the FTA card was punched and 125 µl of RNA Rapid Extraction Solution were added to the mix.

RNA extraction and RT-PCR. RNA extraction and RT-PCR were performed at the University of Minnesota Veterinary Diagnostic Laboratory using routine protocols. RNA was eluted from the FTA card with RNA rapid extraction solution (Ambion-Applied Biosystems). RNA was further purified and concentrated using high throughput magnetic bead-based technology (Ambion-Applied biosystems). The RT-PCR protocol used was a real time, TaqMan-based multiplex protocol that detects both North American and European Strains of PRRSv developed and standardized at the University of Minnesota Veterinary Diagnostic Laboratory (MN-VDL)[8].

Determination of the analytical sensitivity (limit of detection). Eight 10-fold dilutions of a pure culture of PRRSv containing 10^5 $TCID_{50}$/ml were spiked into PRRSv-negative serum and oral fluid samples. One hundred twenty-five µl of each dilution from each of the three samples (pure

culture, serum and oral fluids) was inoculated on FTA cards, while another aliquot was saved and stored at 4º C for direct PCR. FTA cards were stored overnight at room temperature. RNA extraction and RT-PCR was performed for all FTA eluted samples and liquid samples as described above.

Determination of the stability of the RNA in FTA cards. Duplicates of the FTA cards inoculated with dilutions of PRRSv-positive culture media, serum and oral fluids were left at room temperature for 14 days. These samples were then processed and tested by RT-PCR.

Determination of the safety of samples embedded in FTA cards. FTA cards inoculated with 100 µl of 10^5 $TCID_{50}$/ml of PRRSv RFLP 1-8-4 solution or with the same amount of serum or oral fluids collected from pigs 7 days post experimental infection were stored at room temperature for overnight testing. The cards were then resuspended in viral culture media and tested for viral growth on PAM and MARC-145 cells.

Results:

Analytical sensitivity, stability and safety of inoculated FTA cards

The *in vitro* analytical sensitivity of PRRSv detection by RT-PCR from fluids embedded in FTA cards, as well as sample stability testing in cards over 14 days of storage is shown in table 1. The analytical sensitivity from serum and culture media embedded on FTA cards was reduced 100 times compared to detecting the virus directly from serum or culture media. However, viral RNA in FTA cards could still be detected at concentrations of 10^1 $TCID_{50}$. There was no loss of sensitivity when comparing detection on FTA cards at 24h or 14 days of storage.

The analytical sensitivity using saliva spiked samples was lower for samples embedded in FTA cards at 24 hours compared to samples embedded in cards for 14 days and similarly to serum, sensitivity was reduced about 100 times compared to testing the spiked samples directly. Nevertheless, virus in saliva could be detected at low dilutions of 10^3 and 10^1 $TCID_{50}$/ml at 24h and 14 days respectively.

No virus growth was observed in MARC-145 or PAM cell culture from FTA inoculated cards after 24 hours of culture.

Diagnostic sensitivity and specificity of inoculated cards with samples from experimentally infected animals

All pigs originated from a PRRSv negative farm and were confirmed PRRSv negative by RT-PCR prior to the experimental infection.

The specificity analyses of samples are shown in tables 2, 3 and 4. All serum, blood, oral fluids and tissues collected from the control group (non-infected animals) were PRRSv RT-PCR negative at all time points when tested as fresh or after the four FTA card treatments: tested after overnight storage at 25ºC, tested after 14 days of storage at 25ºC, tested after overnight storage at 4ºC or tested after 14 days of storage at 4ºC.

The sensitivity analyses of samples collected from experimentally infected animals are shown in tables 5, 6 and 7. All serum, blood and tissue samples collected from the PRRSv infected group where PRRSv RT-PCR positive at all time points with a sensitivity of 100%. Sensitivity for cards tested with storage at 4º C or 25º C, or at 24h or 14 days post inoculation was the same also estimated at 100%. Sensitivity of PRRSv RT-PCR was lower in oral fluid samples embedded in FTA cards compared to fresh samples. The overall sensitivity for FTA cards with oral fluids was 36%. Differences in sensitivity

increased as the infection progressed in time. Between 1 dpi and 16 dpi sensitivity was 50%, but it decreased to zero from 19 dpi until the termination of the study at 26 dpi.

Diagnostic sensitivity and specificity of inoculated FTA cards using field samples

Results from field samples are shown in table 6. Sensitivity of detecting PRRSv from FTA cards compared to detecting PRRSv directly from the fresh samples was 86.11% and the specificity was 97.8%. The diagnostic agreement between testing the field samples directly or from the FTA cards was 84.4%.

Comparison of punching protocols and puncher disinfection

Sensitivity was slightly better when using the single 6mm punch compared to the three 2 mm punch (results not shown). However, there was no difference in sensitivity comparing the 6mm Harris punch to the 6mm office punch. However, the protocol with Harris punch yielded more false-positives than the protocol with the office puncher (results not shown).

Discussion:

Submission of fresh samples to diagnostic laboratories often represents a challenge for PRRSv diagnostics. The use of FTA cards as a solid medium is one option to safely store and rapidly transport biological samples from the field to reference laboratories, ensuring sample collection and submission at the first opportunity without compromising the quality of the sample.

This study compared sensitivity and specificity of PRRSv RT-PCR performed directly in fresh samples and samples embedded in FTA cards. Storing conditions (time and temperature) were also compared between different FTA treatments.

Analytical sensitivity for RT-PCR from FTA cards decreased about 100 times compared to the analytical sensitivity from the samples directly. A decrease in the analytical sensitivity of this magnitude for FTA cards has been reported before in a prior study with PRRSv [11], as well as in studies with foot and mouth disease virus[16], and with New Castle virus [18]. The decrease in the analytical sensitivity can be explained by the differences in the amount of sample used in the PCR reaction for testing the sample directly or the FTA card. While 100 µl of sample is used when conducting the PCR directly from the sample, only a portion of the sample (a punch) equivalent to 5.7% is used in the PCR reaction for the FTA card. A 6mm diameter disk is punched from the card and used for RNA extraction and subsequent PRRSv RT-PCR testing. This estimate assumes that the sample is equally distributed in the FTA card area. For oral fluids, 175 µl are used for the direct RT-PCR test, but only the equivalent of 3.3% is used for the punch from the FTA card.

Different puncher sizes are available ranging from 1 to 8 mm of diameter [11, 16, 18, 19, 24, 26]. In this study, we evaluated two puncher sizes of 2 and 6mm of diameter (results not shown). The sensitivity of PRRSv RT-PCR detection from FTA cards was slightly better using the 6mm punch (data not shown) and we used the 6mm throughout the rest of the study. Other studies have also reported increase in diagnostic sensitivity when punch size was increased [16, 26].

In addition, when the manufacturer's instructions were used to disinfect the Harris punch between samples (punching two blank FTA cards between cards with samples), false-positive results were observed indicating cross-contamination between samples. To overcome the cross-contaminations, an additional step of disinfecting the punch using 99% ethanol solution followed by drying using disposable wipes before punching 2 blank cards prior to punching each sample was added. After this additional step, the cross-contamination issue between samples was resolved although this protocol remained complex to implement in the laboratory.

Overall the 6mm office puncher was preferred to the 6mm Harris puncher. The office puncher offered advantages compared to the Harris puncher protocol including: a) easier disk removal, b) lower cost (U$ 1.50 vs U$ 20.00) and c) more effective disinfection since the office puncher was single use and more easily disinfected and autoclaved.

In regards to storage conditions, there was no difference in PRRSv RT-PCR sensitivity between 24 hours or 14 days of storage. Similarly, data from other studies have shown that chicken genomic DNA is detectable by PCR in FTA cards embedded with chicken blood 44 months after storage [26]. Likewise, a rabies study showed that rabies virus is detectable in FTA cards embedded with infected dog brain tissue after 7, 14, 21 and 28 days of storage. In that same study rabies virus could be detected in FTA card samples after 222 days of storage when a nested PCR was used [28].

A decrease in sensitivity in samples embedded in FTA cards was not observed in serum, blood and tissue samples collected from experimentally infected animals. However, the estimated sensitivity in serum samples embedded in FTA cards collected from field cases was only 86.1%. Differences in sensitivity can be explained by the disease status at the time of sample collection. Experimentally infected pigs were acutely infected at the time of sample collection with significant viral loads distributed throughout the tissues. On the other hand, disease stage was unknown for the field samples selected for the study. In the case of PRRSv, it is known that viral load decreases over time throughout the course of infection [29].

Under the conditions of this study, the PRRSv RT-PCR analytical sensitivity from oral fluids samples was better in FTA cards stored for 14 days compared to 24h storage. Oral fluids are composed of saliva and transudates originating from the circulatory system, including an array of substances such as mucin, amylase, lysozyme, lipase and proline-rich glycoproteins [22]. Some of these oral fluids components can potentially interfere with RT-PCR sensitivity and we speculate that the chemical properties of FTA matrix have a neutralizing effect on these inhibitors, what enhanced diagnostic sensitivity of PRRSv detection on samples embedded in FTA cards for 14 days compared to 24 hours. Alternatively, differences may be attributed to the uneven antigen distribution in saliva or when placed in the card. Nevertheless, we concluded that sensitivity from oral fluids in FTA cards is lower and that storage conditions did not have a significant effect since we could not see an increase in sensitivity for oral fluids from experimentally infected animals tested at 14 days vs. 24 hours.

Attempts to sequence an ORF-5 portion of the PRRSv genome directly from the FTA cards did not yield results. The sequence length of the target fragment was of 605 bp, which is considered fairly large compared to the gene portions of other nucleic acids that have been sequenced directly from the cards. A 386 bp sequence of white spot syndrome virus could be sequenced from haemolymph from infected shrimp embedded in cards [27]. Similarly, RFLP (restriction fragment length polymorphism) was sucessful when DNA fragments from *Mycoplasma gallisepticum* and *Mycoplasma synoviae* ranging from 237 and 395 bp were used [15]. In addition, genome from RNA viruses is less stable than DNA viruses, being more prone to degradation which could also explain the lack of success in sequencing the PCR product. Nevertheless further attempts have to be made to sequence additional fragments.

In summary, under the conditions of this study FTA cards proved to be an alternative, safe, simple, sensitive method to transport serum, blood, lymph nodes, tonsils and lung samples from acutely infected animals for PRRSv molecular diagnostics. However, a slight decrease in sensitivity was observed in serum field samples. In addition, use of FTA cards for oral fluids warrants further investigation and is not recommended at this time.

Tables and figures:

Table 1 – Limit of detection (analytical sensitivity) of PRRSv RT-PCR from FTA cards tested at 24h or 14 days post inoculation. FTA cards were embedded with culture media, serum and oral fluids, and spiked with eight 10-fold PRRSv dilutions

	Direct	FTA* 24h	FTA 14 days
Culture Media	10^{-1}	10^{1}	10^{1}
Serum	10^{-1}	10^{1}	10^{1}
Oral fluids	10^{-1}	10^{3}	10^{1}

Table 2 – PCR positive results from control (non-infected) pigs throughout the study.

	7 dpi						14 dpi						21 dpi						28 dpi					
Treatment	S*	B	LI	LS	LN	T	S	B	LI	LS	BL	T	S	B	LI	LS	BL	T	S	B	LI	LS	BL	T
Fresh	0/4[a]	0/4	0/1	0/1	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/2	0/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
FTA																								
25ºC/24 hrs	0/4	0/4	0/1	0/1	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/2	0/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
25ºC/14 days	0/4	0/4	0/1	0/1	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/2	0/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
4ºC/24 hrs	0/4	0/4	0/1	0/1	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/2	0/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
4ºC/14 days	0/4	0/4	0/1	0/1	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/2	0/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

*S: serum, B: blood, LI: lung imprint, LS: lung swab, LN: bronchial lymph node, T: tonsil

[a] Number of positive PCR / total number of pigs

Table 3 – PRRSv RT-PCR results from oral fluids collected from control (non-infected) pigs throughout the experimental study.

	Days post infection										
Treatment	2	5	7	9	12	14	16	19	21	23	26
Fresh	-*	-	-	-	-	-	-	-	-	-	-
FTA 25º C, 24 hrs	-	-	-	-	-	-	-	-	-	-	-
FTA 25º C, 14 days	-	-	-	-	-	-	-	-	-	-	-
FTA 4º C, 24 hrs	-	-	-	-	-	-	-	-	-	-	-
FTA 4º C, 14 days	-	-	-	-	-	-	-	-	-	-	-

* One sample collected on each collection day; (-): negative

Table 4 - PCR positive results from infected pigs throughout the study. Number of positive PCR/number of infected pigs per treatment and per sample, at 7, 14, 21 and 28 days post infection

	7 dpi						14 dpi						21 dpi						28 dpi					
Treatment	S*	B	LI	LS	LN	T	S	B	LI	LS	BL	T	S	B	LI	LS	BL	T	S	B	LI	LS	BL	T
Fresh	8/8[a]	8/8	2/2	2/2	2/2	2/2	6/6	6/6	2/2	2/2	2/2	2/2	4/4	4/4	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
FTA																								
25ºC/24 hrs	8/8	8/8	2/2	2/2	2/2	2/2	6/6	6/6	2/2	2/2	2/2	2/2	4/4	4/4	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
25ºC/14 days	8/8	8/8	2/2	2/2	2/2	2/2	6/6	6/6	2/2	2/2	2/2	2/2	4/4	4/4	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
4ºC/24 hrs	8/8	8/8	2/2	2/2	2/2	2/2	6/6	6/6	2/2	2/2	2/2	2/2	4/4	4/4	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
4ºC/14 days	8/8	8/8	2/2	2/2	2/2	2/2	6/6	6/6	2/2	2/2	2/2	2/2	4/4	4/4	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2

[a]Number of positive PCR/total number of pigs.

*S: serum, B: blood, LI: lung imprint, LS: lung swab, LN: bronchial lymph node, T: tonsil

Table 5 - PCR results from oral fluids collected from experimentally infected pigs:

	Days post infection										
Treatment	2	5	7	9	12	14	16	19	21	23	26
Fresh	+*	+	+	+	+	+	+	+	+	+	+
FTA 25º C, 24 hrs	+	sus	+	-	+	sus	+	-	-	-	-
FTA 25º C, 14 days	+	+	-	sus	sus	-	+	-	-	-	-
FTA 4º C, 24 hrs	-	sus	+	+	+	+	+	-	-	-	-
FTA 4º C, 14 days	+	+	+	-	+	-	+	-	-	-	-

* One sample collected on each collection day; (+): positive; (-): negative; sus: suspect

Table 6 –PRRSv RT-PCR results from field serum samples tested directly or onto FTA cards.

		FTA-embedded samples		
		Positive	Negative	Total
Fresh	Positive	**30**	**6**	36
	Negative	**1**	**8**	9
	Total	31	14	**45**

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