RESEARCH REPORT
SWINE HEALTH

Title: Development of a simple on-site diagnostic test to detect PRRSV acute infection in boar studs – **NPB #06-154**

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II. Industry Summary

Because PRRSV can be transmitted through artificial insemination with semen from infected animals, boar studs are constantly being monitored for the presence of PRRSV. Current monitoring protocols for PRRSV in most boar studs in North America consist of routine sampling of boars and testing of these samples by RT-PCR in diagnostic laboratories. Considering the large number of samples being run in a large boar stud, and the importance of a quick turnaround time of results, it would be advantageous to run the diagnostic test on site. Unfortunately, RT-PCR requires sophisticated equipment, specialized labor and cannot be implemented successfully outside a specialized laboratory. The objective of this study was to investigate the feasibility of using a new diagnostic test (RT-LAMP) for the detection of PRRSV. RT-LAMP is a recently described diagnostic test reported to be simple, inexpensive, fast and accurate that can be performed in a simple heat block.

The RT-LAMP was designed to detect both the North American and European strains of PRRSV. The RT-LAMP was able to detect seven different PRRSV isolates. However, it failed to detect small amounts of virus (the limit of detection ranged between 10^2 and 10^4 $TCID_{50}$/ml). Further evaluation included validation of the RT-LAMP using samples from animals of known infection status. The ability of RT-LAMP to detect PRRSV in serum from acutely infected animals (sensitivity) was evaluated with 114 serum samples from 18 experimentally inoculated boars. Forty-nine of these samples tested positive by RT-LAMP, while 94 were positive by RT-PCR. Furthermore, the ability of the test to correctly identify negative samples (specificity), evaluated with 100 known negative sera, was estimated as 99%.

The feasibility of RT-LAMP to detect PRRSV was demonstrated in this study. The RT-LAMP reaction could be performed in just 1 hour with a simple and inexpensive heat block with good specificity. However, the sensitivity was lower than that of RT-PCR. Nevertheless, there is potential for this technique to be applied in situations where RT-PCR is too expensive or too sophisticated to be implemented.

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III. Scientific Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important pathogen of swine. The objective of this study was to investigate the feasibility of using reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) for the detection of PRRSV. RT-LAMP is a recently described DNA amplification technique reported to be simple, inexpensive, fast and accurate. The RT-LAMP reaction was setup using two sets of primers that were designed to detect North American and European strains of PRRSV and performed successfully in a simple heat block. The specificity of the amplified product was demonstrated by restriction analysis. The RT-LAMP was able to detect seven different PRRSV isolates. However, the limit of detection ranged between 10^2 and 10^4 $TCID_{50}$/ml. Further evaluation included validation of the RT-LAMP using samples from animals of known infection status. The ability of RT-LAMP to detect PRRSV in serum from acutely infected animals was evaluated with 114 serum samples from 18 experimentally inoculated boars. Forty-nine of these samples tested positive by RT-LAMP, while 94 were positive by RT-PCR. The diagnostic specificity, evaluated with 100 known negative serum samples, was estimated as 99%. The feasibility of RT-LAMP to detect PRRSV was demonstrated in this study. The RT-LAMP reaction could be performed in just 1 hour with a simple and inexpensive heat block. However, the sensitivity of this technique was significantly lower than that of RT-PCR.

IV. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an important disease of swine caused by *porcine reproductive and respiratory syndrome virus* (PRRSV), a RNA virus from the genus *Arterivirus*, family *Arteriviridae*. This virus, which causes abortions in pregnant sows and respiratory disease in growing pigs, has a tremendous economic impact in swine production worldwide.[20] Because PRRSV can be transmitted through artificial insemination with semen from infected animals, boar studs are constantly being monitored for the presence of PRRSV. Current monitoring protocols for PRRSV in most boar studs in North America consist of routine sampling of large numbers of boars and testing of these samples by RT-PCR in diagnostic laboratories.[16] RT-PCR, a highly sensitive and specific test, is considered the best option for PRRSV detection during the acute phase of infection. However, because of the need of a specialized laboratory there is a lag of 12 to 48 hours between sampling the animal and obtaining the results of the test. Considering the large number of samples being run in a large boar stud, and the importance of a quick turnaround time of results, it would be advantageous to run the diagnostic test on site. The ideal scenario for a monitoring protocol would be to be able to test all the boars whose semen is going to be shipped with a highly sensitive, simple, inexpensive and rapid test that could be run at the boar stud. Unfortunately, RT-PCR requires sophisticated equipment, specialized labor and cannot be implemented successfully outside a specialized laboratory.

A novel diagnostic technique called loop-mediated isothermal amplification (LAMP) has been recently described.[14] Like PCR, this technique uses DNA primers and a polymerase enzyme to amplify a specific fragment of DNA of the target pathogen. However, LAMP has some advantages compared to PCR. A polymerase with strain-displacement capabilities is used and therefore there is no need for changing the temperature to facilitate DNA denaturation. For that reason, the entire reaction can be performed in a simple water bath or heat block, at a constant temperature of 63 C. This characteristic has two important implications: there is no need for sophisticated and expensive equipment such as a thermal cycle, and, because there are no changes in the temperature, the DNA amplification is not interrupted and the processing time can be substantially reduced to one hour. Furthermore, LAMP has been reported to amplify DNA very efficiently, needing only a few copies of target DNA.[14] The sensitivity of LAMP has been reported to be comparable to that of PCR[8,14,18] and, since this technique uses 4 different DNA primers that recognize 6 specific sequences of the target DNA, it is considered highly specific. Coupled with reverse transcription (RT-LAMP), the LAMP technology has been used to detect RNA viruses such as *severe acute respiratory syndrome virus*,[8] *human influenza virus*,[15] *food and mouth disease virus*[6] and *measles virus*.[7]

V. Objective

To develop a rapid, inexpensive, and effective on-site diagnostic test for PRRSV that could be used in negative boar studs to detect acute infection using RT-LAMP technology.

VI. Materials and Methods

Primer Design:

Four primers that hybridize with 6 specific sequences of the target DNA are used in LAMP. Each of the two outer primers (F3 and B3) hybridizes with a specific sequence of the target DNA of about 20 nucleotides. In contrast, each of the two inner primers (forward inner primer (FIP) and backward inner primer (BIP)) hybridizes with 2 different DNA sequences in the target DNA. Primer FIP consists of sequences F1c and F2, while primer BIP consists of sequences B1c and B2.[14] Additionally, two more primers can be used to accelerate the reaction.[13] These primers hybridize with a region of the LAMP product that forms a loop and are therefore called loop primers (LF and LB).

For the design of PRRSV RT-LAMP primers, the whole genome RNA sequences of the original North American (VR-2332, Genbank accession code U87392) and European (Lelystad Virus, Genbank accession code A26843) PRRSV isolates were obtained from Genbank, aligned[a] and compared[b] in order to identify a conserved region.[4] A conserved stretch of at least 200 base pairs (bp) is needed to design LAMP primers. Due to the large divergence between these two PRRSV strains, a conserved region of that size was not found. Therefore, a duplex RT-LAMP test including primers for both the North American and the European strains was attempted. The web-based primer design software Primer Explorer V4[c] was used to design the primers targeting the conserved regions as recommended by the software developer.[2]

Each of the sequences F3, B3, F1c, F2, B1c, B2, LF and LB of the designed primers were compared to available nucleotide sequences in the Genbank with the basic local alignment search tool (BLAST),[d] to account for possible binding to DNA with an origin other than PRRSV. The BLAST analysis was done using the default conditions recommended for finding primer binding sites.[1]

RNA extraction:

RNA was extracted from virus culture and serum samples using the QIAmp Viral RNA Mini Kit,[e] following manufacturer's instructions.

RT-LAMP reaction:

The RT-LAMP reaction was performed in a 25 µl reaction mixture containing: 1.6 µM of each inner primer, 0.2 µM of each outer primer, 0.8 µM of each loop primer, 1.4 mM of each dNTP,[f] 0.8 M of betaine,[g] 2.5

μl of 10x ThermoBuffer,[h] 8 mM $MgSO_4$,[i] 8 Units of Bst DNA Polymerase,[j] 2 Units of enhanced avian myeloblastosis virus reverse transcriptas,[k] and 2 μl of the extracted target RNA.[8,14,18] The reaction was performed in a heat block[l] at 63 C for 1 hour.[2] The detection of the RT-LAMP reaction product was performed by electrophoresis in a 2% agarose gel stained with ethidium bromide. The presence of a smear or a pattern of multiple bands of different molecular weights was considered a positive result.[14] A molecular marker[m] was used to estimate product size.

Analysis of the product of the RT-LAMP reaction:

The RT-LAMP reaction product is a combination of fragments of DNA of different sizes. These fragments are made of repetitions of six smaller units or building blocks previously described[14] as F+, F-, B+, B-, + and – . These 6 units are arranged in the final products in a predictable manner and the exact nucleotide sequence of each of them can be predicted from the sequences of the target and the primers. Therefore, by digesting the sample with a restriction enzyme that cuts the DNA in specific recognition sites of these six units it is possible to predict the size of the spliced products after the digestion. This method serves as a control of the specificity of the product of the LAMP reaction because the unspecific amplification of DNA would yield a different pattern of bands after digestion.[6,8,14]

Restriction enzymes were selected with the NEBcutter V2.0 online software.[n] For the restriction analysis of the PRRSV North American strain product, the PRRSV isolate MN30-100 and the enzyme EaeI[o] were used. For the restriction analysis of the product from the European PRRSV strain, the Lelystad virus isolate and the restriction enzyme NlaIV[p] were used. The digestion reactions were performed by incubating overnight 2 μl of RT-LAMP product obtained from a sample containing 10^5 $TCID_{50}$/ml of PRRSV, in the presence of 12 units of restriction enzyme, in a total volume of 25 μl.

Evaluation of alternative detection methods:

Three alternative methods to detect the product of the RT-LAMP reaction were evaluated:

1. Turbidity: the accumulation of magnesium pyrophosphate, a by-product of the DNA amplification reaction, results in the increase of the turbidity of the sample.[12] The turbidity was evaluated by visual inspection of the samples, comparing them to a negative control.

2. Color change: one microliter of 10,000x SYBR Green I nucleic acid stain[q] was added to the tube after the reaction. Samples turning yellow-green were considered positive while samples turning orange were considered negative.[18]

3. Fluorescence from calcein: calcein is a chelating molecule that binds to manganese. If the manganese is released, the calcein molecule becomes fluorescent. In a positive RT-LAMP reaction, the pyrophosphate binds to the manganese ion, releasing free calcein which emits fluorescence.[3] To evaluate this method of detection, 1

μl of fluorescent reagent[r] containing calcein was added to the LAMP reaction mixture before the reaction. Samples showing fluorescence under a UV hand lamp[s] at 365 mn wave length were considered positive. Samples were compared to a negative control to account for background fluorescence.

To compare the methods of turbidity, color change, fluorescence from calcein and agarose gel electrophoresis, a RT-LAMP reaction was performed on replicates of a sample containing 10^4 $TCID_{50}$/ml of PRRSV North American strain MN30-100. One set of replicates was used to evaluate the methods of turbidity, change in color and agarose gel electrophoresis. Another set of replicates, set up with detection reagent in the reaction mix, was used to evaluate the method of detection by fluorescence from calcein. To generate samples with different amounts of RT-LAMP product, the replicates were allowed different reaction times of 20, 30, 40, 50 and 60 minutes. Each replicate was matched with a negative control sample and all replicates were run simultaneously.

Evaluation of the analytic sensitivity and specificity:

To evaluate the analytic sensitivity, seven well characterized PRRSV isolates were grown in MARC 145 cells. Six of the isolates (VR2332, MN30-100, MN-184, ATP, JA-142 and MLV) were North American type isolates and one (Lelystad Virus) was a European type isolate. Each isolate was titrated in microtitration plates (8 replicates per isolate) using cytopathic effect and fluorescent antibody staining to assess infectivity. The infectious dose was calculated by the Spearman-Karber formula. Based on the obtained titer, each isolate was diluted in culture media to obtain ten fold dilutions containing 10^5 $TCID_{50}$/ml through 10^{-1} $TCID_{50}$/ml. RNA extraction and RT-LAMP was performed on each dilution as previously described to determine the limit of detection for each isolate.

To evaluate its analytical specificity, the RT-LAMP test was performed on a panel of viral isolates commonly isolated from pigs. The panel included *influenza virus* H1N1 and H3N2, *porcine respiratory coronavirus, transmissible gastroenteritis virus, porcine pseudorabies virus, encephalomyocarditis virus, porcine enterovirus, bovine viral diarrhea virus 1* and *2, hepatitis E virus, porcine parvovirus, porcine cytomegalovirus* and *porcine circovirus 1* and *2.*

Validation with serum samples:

To evaluate the ability of RT-LAMP to detect PRRSV in serum samples from acutely infected animals, the test was performed on 114 serum samples from 18 experimentally inoculated boars that were part of a previous study.[17] These samples were obtained at 1, 3, 5, 8, 10, 12 and 15 days post-inoculation with PRRSV and 94 of them tested positive for PRRSV by RT-PCR[17] (Table 2). The aliquots used for the present study had been kept at -80 C for a year and had never been thawed.

Diagnostic specificity (proportion of truly negative samples that tests negative by RT-LAMP) was estimated using serum samples from 100 pigs from known PRRSV-negative farms. Ascertainment of farm infection status was based on continuous routine monitoring for PRRSV (monthly or more frequent testing of at least 30 pigs).

VII. Results

Primer Design:

Thirty-two North American PRRSV whole-genome sequences available in the Genbank were aligned (Genbank accession numbers EF112446, EF075945, EF112447, EF112445, AY150321, AY032626, AY262352, AY545985, AY424271, DQ988080, AF325691, AF494042, NC_001961, AF046869, AF331831, AF159149, EF153486, DQ459471, AF066183, DQ056373, AY612613, AY585241, AF176348, DQ176021, U87392, AY150564, AY457635, DQ779791, AF184212, DQ473474, DQ176020, DQ176019). A relatively conserved region of 330 bp was found between nucleotides 14,591 and 14,920 of the reference VR-2332 strain (Genbank accession number U87392). The percentage of nucleotide identity between the reference strain and other isolates in this region ranged from 94 to 99%. This region lies within the ORF 6 of the virus. Primers targeting this region were designed with the Primer Explorer V4 software as recommended by the developer[2] and are shown in Table 1. Similarly, 7 whole genome RNA sequences of the European PRRSV strain available at the Genbank were aligned (AY588319, M96262, A26843, DQ864705, AY366525, DQ489311, EU076704). A relatively conserved region was identified between nucleotides 14,769 and 15,134 of the reference Lelystad Virus strain (Genbank accession number A26843) and primers targeting this region were designed (Table 1). The percentage of nucleotide identity between the reference strain and other isolates in this region ranged from 95 to 100%. A BLAST analysis for each target sequence in the designed primers showed 100% identity only with previously published sequences of PRRSV.

Restriction analysis of the RT-LAMP reaction product:

The RNA was successfully reverse transcribed and the DNA amplified from both North American and European strains of PRRSV by RT-LAMP (Fig. 1). Taking into account the target sequence, the restriction sites and the different possibilities for the theoretical arrangement of the amplified products,[14] the digestion of the RT-LAMP products from the North American PRRSV strain should result in 3 main fragments of 242, 187, and 132 bp and 3 residues of 21, 76 and 162 bp. Figure 1 shows the RT-LAMP product before and after digestion with EaeI. Before digestion, the product looks like a smear due to the presence of DNA fragments of multiple sizes ranging from more than 10,000 to less than 200 bp. After digestion, 3 bands are distinguishable, 1 of size

between 300 and 200 bp and 2 of sizes between 200 and 100 base pairs. The 3 bands are consistent with the predicted sizes of 242, 187, and 132 bp. Similarly, the digestion of the RT-LAMP products from the European PRRSV strain should result in 2 main fragments of 101 and 113 bp and 2 residues of 96 and 102 bp. Figure 1 shows that after digestion there was a single very intense band of around 100 bp, consistent with the predicted fragment sizes.

Alternative detection methods:

The amplified product can be detected by electrophoresis in an agarose gel stained with ethidium bromide, as described above. However, although this method is very sensitive, it is time consuming and requires specialized equipment and therefore is not practical for the routine use of the test. Therefore, three alternative methods were evaluated. The products of the RT-LAMP reaction were detected by agarose gel electrophoresis after 40, 50 and 60 minutes of reaction but not after only 20 or 30 minutes (Fig. 2A). In addition, the intensity of the smear was lower after 40 minutes than after 50 or 60 minutes, suggesting a smaller amount of RT-LAMP products. With the methods of turbidity and change in color, only the samples run for 50 and 60 minutes were positive. Positive and negative samples were easily distinguishable with these two methods. In the samples that were added calcein, background fluorescence was observed under the UV light in the negative control samples. Samples run for 20 and 30 minutes showed a weak fluorescent signal comparable to that of the negative control. The samples run for 50 and 60 minutes showed a strong fluorescent signal, more intense than the background fluorescence. More difficult was the interpretation of the sample run for 40 min, which showed an intermediate fluorescent signal.

Based on these results, the evaluation of the analytic sensitivity and specificity of RT-LAMP and further validation with serum samples were performed using the turbidity and change in color methods only. The calcein detection method was discarded because of the potential subjectivity of the interpretation of the results.

Analytic sensitivity and specificity:

The limit of detection was 10^2 $TCID_{50}$/ml for isolates JA-142 and Lelystad Virus, 10^3 $TCID_{50}$/ml for isolates MN-30-100, MN-184 and MLV, and 10^4 $TCID_{50}$/ml for isolates ATP and VR2332. The RT-LAMP test was negative for all the samples in the panel of viruses frequently isolated from swine.

Validation with serum samples:

Forty-nine of the 114 samples from experimentally infected boars tested positive for PRRSV by RT-LAMP. Therefore, RT-LAMP detected PRRSV in 43% of all the serum samples and in 52% of the PCR-positive samples (Table 2). One of the 100 known negative serum samples tested positive by RT- LAMP. When

the test was repeated, this sample tested negative. This represents a diagnostic specificity of 99%. All the samples were evaluated by turbidity and by color change with identical results.

VIII. Discussion

The feasibility of RT-LAMP to detect PRRSV was demonstrated in this study. With this new diagnostic technique it is possible to detect PRRSV by specifically amplifying a DNA fragment complementary to the RNA of the virus, and detecting the amplified product. The RT-LAMP reaction can be performed in just 1 hour with a simple and inexpensive heat block.

Four different detection methods were evaluated. The electrophoresis in agarose gel is a very sensitive method to detect DNA and was used as a reference method. This method was slightly more sensitive than the other three. However, this method is not intended for routine use because it is time consuming and requires specialized equipment. Under the conditions of this study, the detection method based on fluorescence from calcein could be subject to misinterpretation due to the presence of fluorescent background on negative samples. The methods of turbidity and change in color were selected for their simplicity and ease of interpretation. These two methods were further compared with the serum samples used for validation and yielded identical results in each of the 214 serum samples tested. However, the method of change in color requires opening the tube at the end of the reaction. This represents an important limitation for an on-site test due to the high chances of contamination with the end product of the reaction. For that reason and because of its simplicity, the detection based on turbidity is preferred. The limitation of this method is that the interpretation can be somewhat subjective. To address this limitation, other studies used a special heat block that has a turbidimeter incorporated.[8] This device can measure the turbidity of the sample with high accuracy in real time. Nevertheless, in the present study positive and negative samples were clearly distinguishable and the results with this method perfectly matched those obtained with the change in color method.

Since 6 different sequences on the target DNA are recognized by the LAMP primers, this is theoretically a very specific technique.[14] In the present study, the restriction analyses confirmed the specificity of the amplified DNA fragments. The evaluation of the specificity was completed by the lack of amplification of DNA from other swine viruses and by testing 100 known negative serum samples. One of these 100 samples tested positive. Since this sample tested negative after retest, the false positive result is likely due to contamination of the tube while setting up the reaction.

The main challenge in the development of molecular tests for PRRSV is the large genetic heterogeneity of this virus.[9,11] In this study, seven different PRRSV isolates were detected by RT-LAMP. After the development of the test, 10 additional field isolates (9 of the North American strain and one of the European

strain) have been tested and 9 of them have been successfully detected by RT-LAMP (data not shown). The isolate that could not be detected was a North American variant that recently emerged in the Northwest of the US and is known as 1-24-2. This emphasizes the need for a constant update of the primers of molecular diagnostic tests for PRRSV. Most current RT-PCR tests include two or more sets of primers to capture most of the variability observed in the PRRSV genome.[11] Multiplex tests have been described for the LAMP technique as well.[5,10] In this study, two sets of primers were included and it is likely that a third set of primers could be added without a detrimental effect on the performance of the test.

The main limitation of the diagnostic test developed was the sensitivity. The limit of detection ranged from 10^2 to 10^4 $TCID_{50}$/ml, far from the limits reported for RT-PCR tests.[11,19] The results obtained from serum samples also showed a reduced sensitivity compared to RT-PCR. Specifically, the RT-LAMP failed to detect the virus in samples with low copy numbers of virus (data not shown), mainly obtained during the first 1 to 5 days post-inoculation.[17] On the other hand, the RT-LAMP detected the virus in at least one sample from each of the 18 infected boars.

This is the first study that has explored the use of RT-LAMP technology in a diagnostic test for PRRSV. The test was simple, specific, rapid and was able to detect PRRSV from serum samples from acutely infected animals. However, the sensitivity was lower than that of RT-PCR. Nevertheless, there is potential for this technique to be applied in situations where RT-PCR is too expensive or too sophisticated to be implemented, such as boar studs or diagnostic laboratories in developing countries.

Sources and Manufacturers

a) ClustalW2 software, Online Service at the European Bioinformatics Institute, (www.ebi.ac.uk/Tools/clustalw2).

b) Jalview version 2.3, Barton Group, University of Dundee, Dundee, Scotland.

c) Primer Explorer V4 (http://primerexplorer.jp/e), Eiken Chemical Co, Ltd, Tokyo, Japan.

d) Basic Local Alignment Search Tool (BLAST)(http://blast.ncbi.nlm.nih.gov), National Center for Biotechnology Information (NCBI), Bethesda, MD.

e) QIAamp Viral RNA Mini-Kit®, Qiagen, Inc., Valencia, CA.

f) Master Mix dNTP, Denville Scientific Inc., Metuchen, NJ.

g) Betaine 5M, Sigma, St Louis, MO.

h) 10x ThermoBuffer, New England Biolabs Inc., Ipswich, MA.

i) $MgSO_4$, New England Biolabs Inc., Ipswich, MA.

j) Bst DNA Polymerase, New England Biolabs Inc., Ipswich, MA.

k) Enhanced avian myeloblastosis virus reverse transcriptase, Sigma, St Louis, MO.

l) Accu Block D1200, Labnet International Inc., Woodbridge, NJ.

m) Hi-Lo DNA Marker, Minnesota Molecular Inc, Minneapolis, MN.

n) NEBcutter V2.0 (http://tools.neb.com/NEBcutter2), New England Biolabs Inc., Ipswich, MA.

o) EaeI restriction enzyme, New England Biolabs Inc., Ipswich, MA.

p) NlaIV restriction enzyme, New England Biolabs Inc., Ipswich, MA.

q) 10,000x SYBR Green I, Molecular Probes Inc., Eugene, OR.

r) Loopamp Fluorescent Reagent, Eiken Chemical Co., Tokyo, Japan.

s) Spectroline Enf-24, Spectronics Corp., Westbury, NY.

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Table 1: DNA sequences of the primers used in the RT-LAMP.

Primer	Sequence
NA F3	5'-GGGAGCAGTAGTTGCACTC-3'
NA B3	5'-GGACGACAAATGCGTGGTTA-3'
NA FIP	5'-GCACAAACGGCATCTGGAGGTTTGGGGGGTGTACTCAGC-3'
NA BIP	5'-TAGGCCGCAAGTACATTCTGGCCATTTGCCGCAATCGGATG-3'
NA LF	5'-GATGAATTTCCAGGTTTCT-3'
NA LB	5'-CCTGCCCACCACGTTGA-3'
EU F3	5'-TGCTGCAGGTCTCCATTCA-3'
EU B3	5'-CCCCATCGGAGCTGTACT-3'
EU FIP	5'-AGTCCTGGTACTAGAGTGCCGTTGGTAACCGAGCATACGCT-3'
EU BIP	5'-GGCGGCAAACGAGCTGTTAAACTGGCTCTGGTTTTTACCGG-3'
EU LF	5'-TCACTGATGTTAGTCCGGGC-3'
EU LB	5'-AGGAGTGGTTAACCTCGTCAA-3'

Table 2: RT-LAMP and RT-PCR results for the 114 serum samples obtained from boars experimentally inoculated with PRRSV.

		RT-PCR	
		+	-
RT-LAMP	+	49	0
	-	45	20

Figure 1: Restriction analysis of the PRRSV RT-LAMP products. The first and forth lanes show the molecular marker (M); the second and third lanes show the products of the RT-LAMP reaction for the PRRSV North American strain before (BD) and after digestion (AD) with enzyme EaeI; the fifth and sixth lanes show the products of the RT-LAMP reaction for the PRRSV North American strain (BD) and after digestion (AD) with enzyme NlaIV. For a better visualization of the bands, 5 times less product was loaded in AD compared to BD. The sizes of the main bands of the molecular marker are indicated in base pairs.

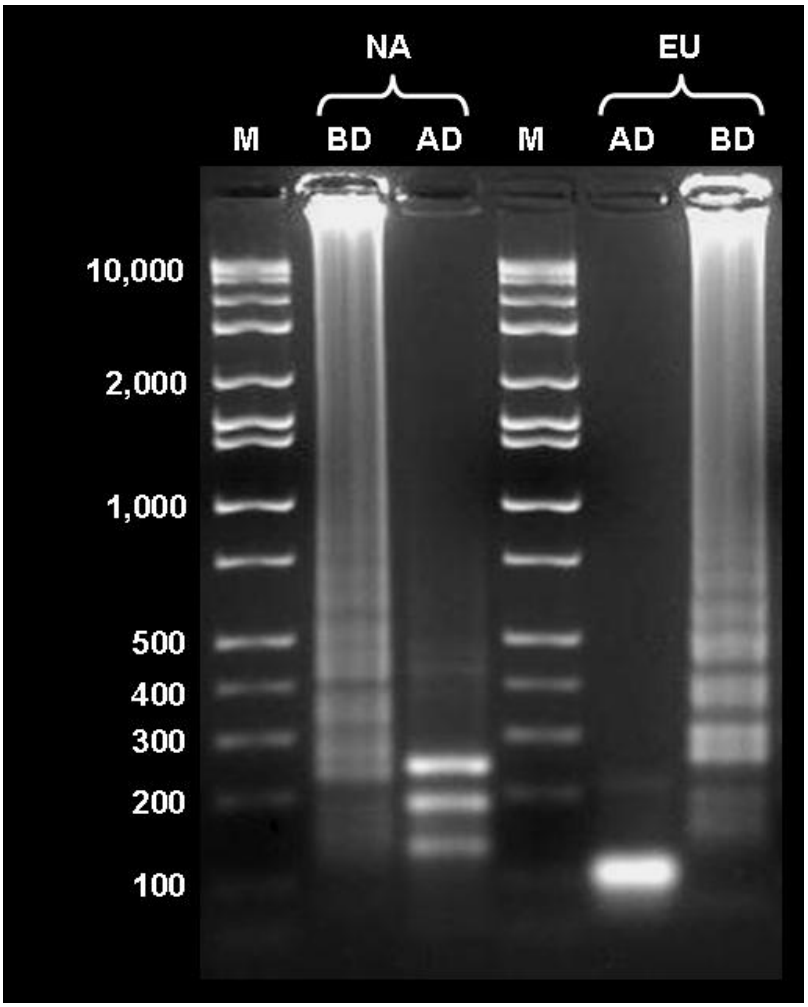
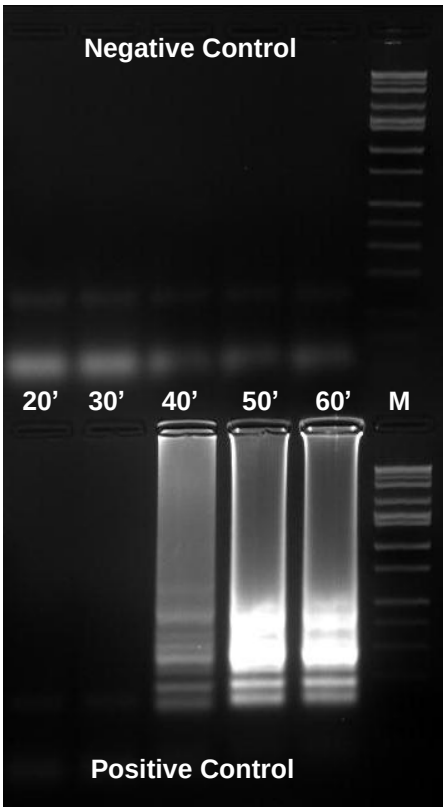
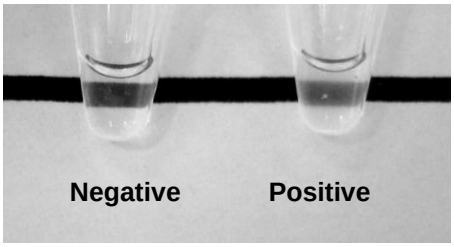


Figure 2: Evaluation of two different detection methods for RT-LAMP product detection. (**A**) Agarose gel electrophoresis. Positive and negative control samples were run for 20, 30, 40, 50 or 60 minutes.; (**B**) Turbidity. Positive and a negative control samples are shown.

A


Negative Control
20’
30’
40’
50’
60’
M
Positive Control

B


Negative
Positive