Improving the transport of clinical samples for the molecular diagnosis of PRRSV

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Introduction & Objectives

The Porcine reproductive and respiratory syndrome (PRRS) caused by the PRRS virus (PRRSV) is the most economically significant disease of swine worldwide. Testing for PRRSV by RT-qPCR on clinical samples is challenging, due to the denature of the virus RNA during transit which is a problem when samples have to travel long distances. For international transport, the need to refrigerate the samples and customs constraints make their transport even more difficult. Alternative methods of transporting clinical samples for PCR diagnosis of PRRS have been tested. However, none of them has allowed the stabilization of the virus RNA. This study aimed at testing an alternative liquid transport media (LTM) for shipping and subsequent detection of the PRRSV on clinical samples by RT-qPCR.

Methods

Study design: In order to validate the LTM in our laboratory, three different studies were performed comparing unmixed and mixed samples with LTM. The first study evaluated the analytical sensitivity by calculating the detection limit of the RT-qPCR with both sample types. Afterwards, in the second study different time points were analyzed to check RNA stability along time. Finally, in the third study clinical samples were tested in order to check the feasibility of this alternative methodology in the field.

Samples: A ten-fold serial dilution experiment using the type 1 PRRSV reference strain VP-046 was carried out. The obtained suspensions were mixed and unmixed with LTM to assess the variation in the analytical sensitivity of the RT-qPCR according to the type of sample. To asses RNA stability over time a non-diluted suspension of the VP-046 PRRSV strain was mixed with LTM and tested by qPCR on weekly bases. Finally, the feasibility of preserving the RNA by the LTM was tested with 86 clinical samples (48 oral fluids and 38 blood samples of unknown PRRSV status) from commercial swine farms in Spain, Italy and Belgium. They were mixed and unmixed with LTM (Figure 1) and tested by RT-qPCR for PRRSV.

RNA extraction: Total RNA was extracted and purified using RNeasy Mini Kit (Qiagen, Germany) in the automated QIACUBE platform (Qiagen, Germany).

RT-qPCR: The extracted products were then amplified with a previously described ORF7 RT-qPCR2. Results were compared in terms of negative/positive agreement and Ct values.

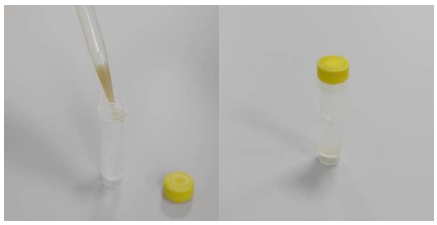

Figure 1. Inoculation of an oral fluid clinical sample in liquid transport media (LTM).

Results

Analytical sensitivity

Type 1 PRRSV reference strain was detected up to the same dilution in both mixed and unmixed viral suspensions (Table 1).

Table 1. Results of the RT-qPCR on ten-fold serial dilutions of the VP-046 PRRSV strain mixed and un mixed with liquid transport media

	RT-qPCR results (Ct* values)	
Dilution	Unmixed	Mixed
10e-1	18	20.8
10e-2	21.7	24.7
10e-3	25.5	28
10e-4	31.2	32
10e-5	33.6	34.9
10e-6	Negative	Negative

*Ct: Threshold cycle. Positive results Ct>35.

RNA stability

Type 1 PRRSV reference strain was stable at least up to 1 month at room temperature after mixing with LTM (Table 2).

Table 2. Results of the RT-qPCR on a PRRSV suspension at different time points after being mixed with LTM.

	RT-qPCR results (Ct* values)			
	Day 0	Day 7	Day 14	Day 28
VP-046 PRRSV strain	17.3	19.18	17.6	21.2

*Ct: Threshold cycle. Positive results Ct>35.

Clinical samples

Up to 37/86 (43%) and 39/86 (45%) samples were RT-qPCR- positive after being unmixed and mixed with LTM, respectively (Figure 2). A total of 78 samples (90.7%) showed the same qualitative results (positive/negative) both mixed and unmixed. However, 3/86 (3.5%) of the samples were positive only when were not mixed with LTM, and 5/86 (5.8%) the other way around. Even so, the variation in the results of the samples that did not match was very low, with an average difference in the Ct = value of 0.16.

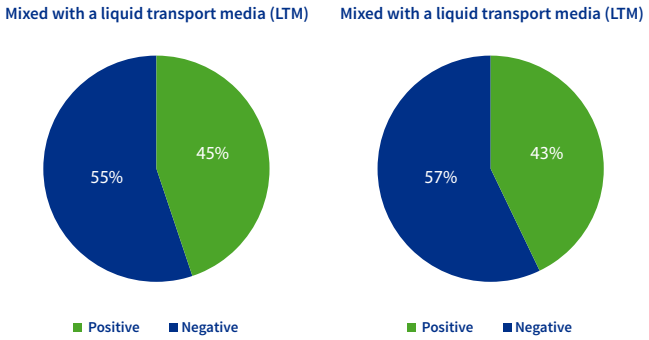

Figure 2. Percentage of PRRSV RT-qPCR-positive and -negative clinical samples mixed and unmixed with the liquid transport media (LTM).

Conclusions

These results demonstrate a comparable sensitivity for the ORF-7 RT-qPCR for PRRSV, whether the sample is mixed with the LTM tested or not. Moreover, PRRSV RNA showed a prolonged stability at room temperature when mixed with the alternative LTM. All-in-all, these results indicate that the alternative LTM tested is valid for the transport of clinical samples with suspicion of carrying PRRSV, as shown by the RT-qPCR results.

Acknowledgments

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References

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