

Recommendations for sampling and shipment of materials for PCR-tests for surveillance of PRRS-virus in Danish herds

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Background & Objectives

A program to reduce the prevalence of porcine reproduction and respiratory syndrome (PRRS) in Denmark was initiated in 2022. The efforts to eliminate PRRS-virus demands more RT-qPCR-tests for PRRSV using a variety of sample materials including non-invasive samples like Processing Fluid (PF), Tongue Tip Fluid (TTF), and oral fluid (OF). The aim of the project was to develop validated recommendations for optimal collection, storage and transport of the different sampling material for PRRSV detection to avoid false negative test results.

Materials and Methods

The influence of storage temperature and time on the decay of PRRSV RNA in OF and PF was tested by spike of negative sampling material with a PRRSV-1 cell culture isolate. The spiked materials were stored at a combination of different temperatures and times and finally extracted viral RNA was tested by RT-qPCR to monitor the impact of the different processes.

Results

The results showed that storage of serum, OF and PF at 4C; -20C and -80C for up to 30 days had limited impact on the Cq value of the RT-qPCR. One round of freeze-thaw had limited impact of the Cq value whereas 2-3 rounds had a more pronounced negative effect especially on OF samples. In contrast, storage at room temperature (RT) for just one day resulted in a significant increase in Cq values for all sampling materials, showing a severe impairment of detection of PRRS-virus.

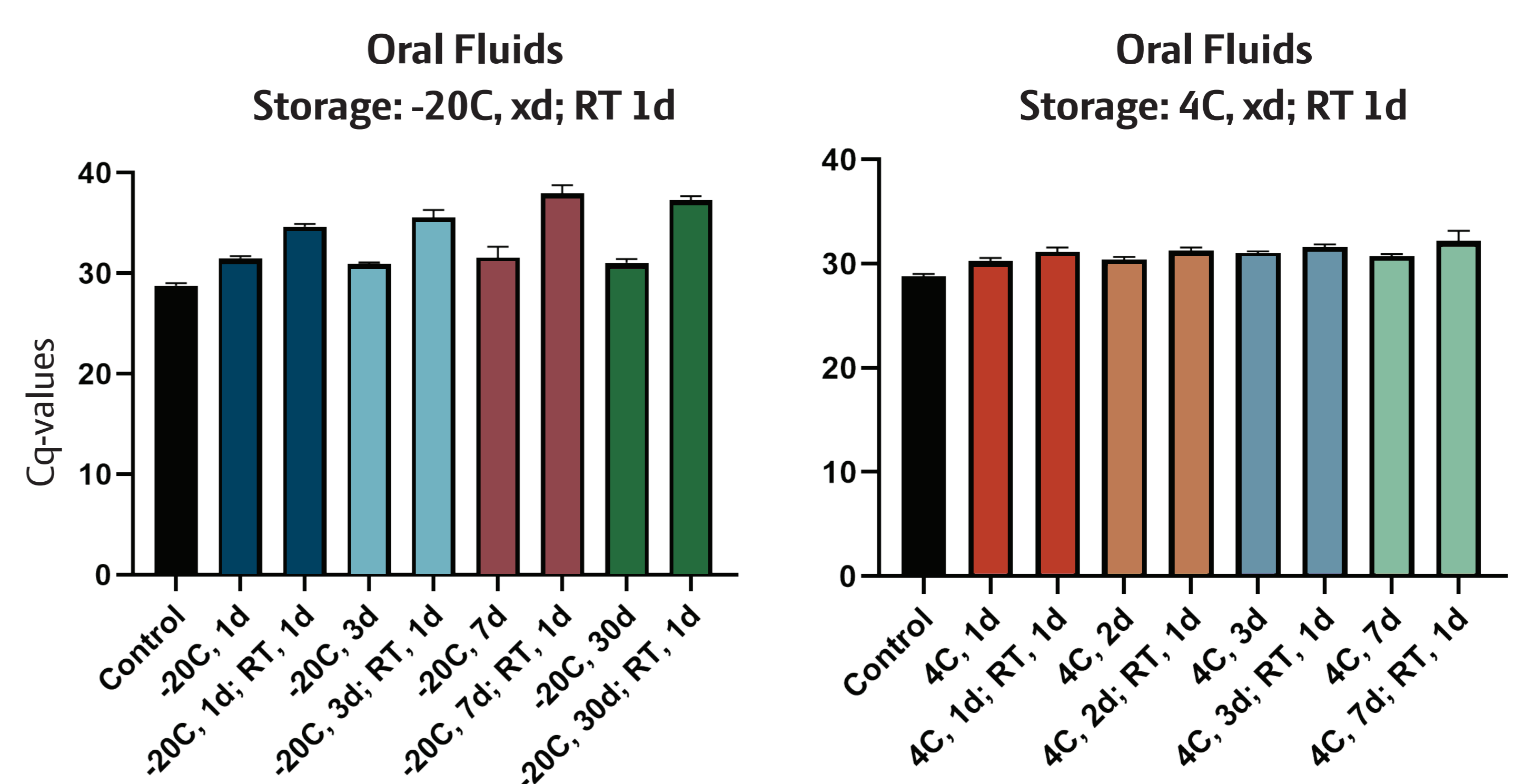
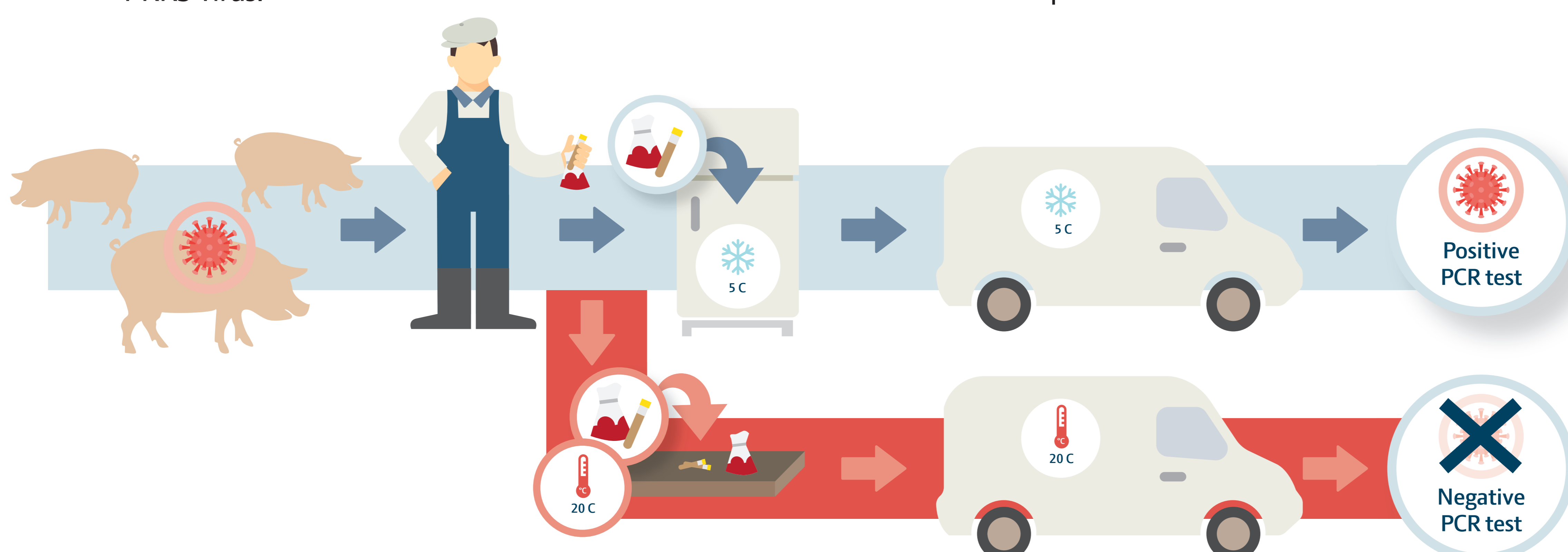


FIGURE. Oral Fluids PRRSV RT-qPCR Cq-values at storage temperatures -20C,+4C, Room temperature RT in 1 day, 3 days and/or 30 days.

Discussion & Conclusion

Correct handling of samples for PCR analyses are essential to avoid false-negative test results. Based on the results of this study we recommend that PF and TTF samples should be frozen at -20C immediately after sampling and then be thawed at a temperature not exceeding 4-8C before the fluid is collected. Samples should also be kept cooled during transportation to the laboratory and therefore styrofoam cool box and ice packs are advised for all types of material.



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