Should we be concerned about the effect of sample handling on PRRSV detection by RT-qPCR?

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Background

Sample quality is a key issue in PRRSV RT-qPCR and recommendations for sample handling to optimize nucleic acid detection include prompt chilling or freezing of the specimen after collection on the farm and maintenance of the cold chain through testing. However, it is not always logistically possible to meet these recommendations and samples may have been exposed to inappropriate temperatures or multiple freeze-thaw cycles. One approach to dealing with the question of sample quality is by the use of reference genes inherent to the specimen, i.e., internal sample controls (ISC), to verify that nucleic acid integrity was sufficient for testing. That is, detection of the reference gene indicates that the specimen was of sufficient quality for testing.

Looking at the question more closely, it can be seen that there is actually very limited information on the effect of sample handling on PCR-detectable PRRSV RNA or companion reference genes. To address this information gap, we characterized the effect of temperature × time (*Study 1*), freeze-thaw cycles (*Study 2*), and high PRRSV RNA concentrations (*Study 3*) on the detection of PRRSV and a porcine-specific ISC in serum, oral fluid, and fecal samples using a commercial PRRSV RT-qPCR assay.

Methods

Study 1 (temperature × time): Individual aliquots (n = 28 per specimen) of PRRSV-positive serum, oral fluid and feces were assigned to 1 of 28 combinations of storage temperature (4°, 10°, 20°, or 30°C) × time (24, 48, 72, 96, 120, 144, or 168 h). *Study 2* (freeze-thaw): Individual aliquots (n = 4 per specimen) of serum, oral fluid, and fecal samples assigned to 1 of 4 freeze-thaw treatments (2, 5, 10, or 15 cycles). *Study 3* (high PRRSV RNA): A PRRSV MLV (Ingelvac PRRS MLV) was resuspended with 5 mL of PRRSV-negative serum, oral fluid, and feces (20% w/v fecal suspension). Thereafter, six 10-fold serial dilutions (10^{-1} to 10^{-6}) were created to obtain different PRRSV RNA concentrations.

In each of the 3 studies, samples were randomized within specimen and tested with a commercial PRRSV RT-qPCR for PRRSV and ISC RNAs after the treatments were completed.

Results

In *Study 1*, the effect of temperature × time on PRRSV and ISC detection was shown to be specimen dependent. In serum stored at 4, 10, or 20°C, PRRSV detection was consistent for up to 168 h, but storage at 30°C reduced detectable PRRSV RNA. The ISC RNA was stable in serum held at 4 and 10°C, but not at 20 and 30°C. In contrast, PRRSV and ISC RNAs in oral fluid and fecal samples continuously decreased in all temperature × time treatments. In *Study 2*, freeze-thaw cycles had little impact on PRRSV and ISC detection, but more so in oral fluids than serum or fecal samples. In *Study 3*, the ISC was not affected by high concentrations of PRRSV RNA in serum, oral fluid, or fecal samples.

Conclusions

Based on these data, serum samples should be stored at ≤20°C to optimize PRRSV RNA detection. In contrast, oral fluid and fecal samples should be frozen in a non–self-defrosting freezer until tested. Moreover, freeze-thaw cycles in oral fluids should be minimized before RT-qPCR testing to prevent loss of detectable PRRSV RNA. The detection of the ISC was highly consistent among specimens. Thus, failure to detect the ISC indicates a significant problem at some point between sampling and testing.

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