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Main topic : Other Topics

Effect of storage temperature x time on PRRSV detection by RT-qPCR

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PCR targets in diagnostic samples are subjected to a variety of potentially adverse conditions in the course of collection, storage, and transport to the laboratory, but the effect of these conditions on PCR testing results is largely unexplored. Herein, the effect of storage temperature x time on the detection of PRRSV RNA and a pig-specific RNA internal sample control (ISC) inherent to all pig-derived specimens, was evaluated in serum, oral fluids, and fecal samples by RT-qPCR.
Serum samples (n = 5) used in the study were from pigs experimentally inoculated with contemporary wild-type PRRSV-2 isolates. Oral fluids (n = 5), and fecal samples (n = 5) were from individually housed pigs vaccinated with a PRRSV MLV (Ingelvac® PRRS MLV).
Each sample was divided into 28 aliquots (500 uL) and each aliquot was subjected to one combination of (temperature x time), i.e., 4, 10, 20, or 30°C by 24, 48, 72, 96, 120, 144, or 168 hours. After completing all treatments, samples were tested using a commercial RT-qPCR that detected both PRRSV and the ISC simultaneously (IDEXX Laboratories, Inc.). RT-qPCR results (Cqs) were re-expressed as “efficiency standardized Cqs (ECqs)”, ECqs were cube root transformed for analysis, and the effect of storage temperature x time was analyzed by a mixed-effects regression (MRM) model using R 4.1.0 (R core team, 2020).
In serum, PRRSV was stable at 4, 10, and 20°C ($p > 0.05$) but affected by storage at 30°C ($p < 0.05$), with an estimated decrease of 0.11 ECqs every 24 h. For the ISC, an ECq loss of 0.07 and 0.10 every 24 h was observed at 20 and 30°C, respectively. In oral fluids, a decrease in PRRSV and ISC was observed at all temperatures ($p < 0.05$). Specifically, the estimated ECq decrease every 24 h at 4, 10, 20, and 30°C was 0.03, 0.05, 0.07, and 0.08 for PRRSV, and 0.02, 0.04, 0.05, and 0.05 for ISC, respectively. In fecal samples a significant ($p < 0.05$) daily decline in PRRSV ECqs of 0.05 and 0.06 was observed at 4°C and 10°C, and 0.05 for 20 and 30°C. For ISC, the ECqs declined by 0.06 for 4°C and 10°C and 0.10, 0.08 for 20 and 30°C, respectively.
Overall, the results showed that storage of serum at 4, 10, and 20°C for up to 168 h had little impact on PRRSV RNA, but detection was affected by storage at 30°C. The ISC in serum was stable at 4 and 10°C, but a discernible effect was observed at 20, and 30°C. In oral fluids and feces, a significant decrease in PRRSV and ISC RNAs was observed when stored at either 4, 10, 20, or 30°C and at all time points. Based on these data, serum samples intended for PRRSV RT-qPCR testing should be maintained at < 20°C and oral fluid and fecal samples should be kept frozen.