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Towards the veterinary diagnostics of the future

Main topic : Other Topics

Effect of freeze-thaw on PRRSV RNA detection by RT-qPCR

MUNGUIA-RAMIREZ B. [1], ARMENTA-LEYVA B. [1], HENAO-DIAZ A. [2], YE F. [1], DOOLITTLE K. [3], ZIMMERMAN S. [3], GIMENEZ-LIROLA L. [1], EGLI C. [3], TROCH J. [3], ZIMMERMAN J. [1]

[1] Iowa State University, Ames, Iowa, United States; [2] PIC Latinoamerica, Queretaro, Queretaro, Mexico; [3] IDEXX Laboratories, Inc, Westbrook, Maine, United States

Re-testing samples is common in diagnostic and research settings. This implies subjecting samples to multiple freeze-thaw cycles, but what is the effect of this process on the detection of PRRSV RNA? Herein, the effect of freeze-thaw on the detection of PRRSV and a porcine internal sample control (ISC) by RT-qPCR was evaluated in serum, oral fluids, and feces. Note: The ISC used in this study is a pig-specific RNA target present in all pig-derived specimens and, therefore, is a measure of sample quality. The RT-qPCR kit used in the study (IDEXX Laboratories, Inc.) detected both PRRSV and ISC RNAs simultaneously.

Serum samples were collected from pigs experimentally inoculated with contemporary wild-type PRRSV-2 (n = 5 pos; n = 5 neg). Oral fluids (6 pos; 4 neg), and feces (5 pos; 5 neg) were from individually housed pigs vaccinated with a PRRSV-2 MLV (Ingelvac® PRRS MLV). Samples were aliquoted into 2 ml tubes to create 4 sets (one set per freeze-thaw "treatment"). Each set was exposed to a specific number of freeze-thaw cycles (2, 5, 10, and 15) by freezing at -80°C and thawing at 4°C overnight. Thereafter, samples (n = 40 per specimen) were tested in technical triplicates for PRRSV and ISC detection using a commercial PRRSV RT-qPCR (IDEXX Laboratories, Inc.). RT-qPCR Cqs were re-expressed as function of the PCR efficiency as “efficiency standardized Cqs (ECqs)” following the equation:

$ECq = E^{-?Cq} = E^{-(Cq\ Sample - Mean\ Cq\ of\ reference\ standards)}$

ECqs were analyzed using a mixed-effects regression model (MRM) in R 4.1.0 (R core team, 2020).

In serum, no freeze-thaw effect was detected in PRRSV and ISC ECqs ($p > 0.05$; MRM). In oral fluids, the slope of the regression line showed a decrease of 0.027 (95% CI: 0.020, 0.033) and 0.052 (95%CI: 0.018, 0.085) ECqs per freeze-thaw cycle for PRRSV and ISC, respectively ($p < 0.05$; MRM). In feces, no freeze-thaw effect was detected for PRRSV or ISC ECqs ($p > 0.05$).

Overall, freeze-thaw cycles had little impact on the detection of PRRSV and the ISC, albeit more so in oral fluids vs serum and feces. Notably, these results apply to PRRSV RNA; further studies are needed to address the effect of freeze-thaw on PCRs for other pathogens and specimen types.