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Towards the veterinary diagnostics of the future

Main topic : Surveillance and control of emerging diseases

Controlling for normal variation in PRRSV RT-qPCR testing (ECqs)

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Introduction. Normalization, the process of controlling for normal variation in testing, can be achieved in diagnostic PCRs by converting quantification cycles (Cqs) to “efficiency standardized Cqs” (ECqs). In Equation 1, E = amplification efficiency and ?Cq = (Sample Cq - Reference Standard Cq): Equation 1. $ECq = E^{-?Cq}$

Normalization is routine in basic research[1]. The objective of this study was to adapt this approach to a commercial PRRSV RT-qPCR used for routine diagnostics.

Methods. Serum (n = 132) and individual oral fluid (n = 130) samples from 12 pigs vaccinated with a PRRSV MLV from -7 to 42 days post vaccination (DPV) were tested using commercial reagents (IDEXX Laboratories, Inc.) and the MIC PCR™ Cycler (Bio Molecular Systems).

Reference standards (RS) were created from PRRSV modified-live vaccine (Ingelvac® PRRS MLV). A commercial vaccine was used as the reference standard because the requirements of vaccine manufacture assured its uniformity and availability. The vaccine was rehydrated and diluted with serum (1×10^{-4}) or oral fluid (1×10^{-5}) to match the sample matrix to be tested. Four reference standards were run on each plate and subsequently used to calculate plate-specific estimates of E and RS Cqs (Equation 1).

Results. Following testing, sample Cqs converted to ECqs (Equation 1). Mean plate amplification efficiencies were 1.75 to 2.6 for serum and 1.7 to 2.3 for oral fluid. Mean plate reference standard Cqs were 29.1 to 31.3 for serum and 29.2 to 31.5 for oral fluids.

Receiver operating characteristic analyses (R.4.2.1., package ‘pROC’) were conducted for each specimen type to estimate the area under the curve (AUC) and estimate the diagnostic specificities and sensitivities for a range of ECq cutoffs. Receiver operating characteristic analysis calculated the area under the curve for serum and oral fluid sample ECqs as 0.999 (95% CI: 0.997, 1.000) and 0.947 (0.890, 1.000), respectively. For serum, the diagnostic sensitivity and specificity of the commercial PRRSV RT-qPCR was estimated as 97.9% and 100% at a cutoff of ECq ? 0.20 and, for oral fluid, 82.6% and 100%, respectively, at a cutoff of ECq ? 0.45.

Conclusions. Simply put, ECqs represent a practical approach for accounting for run-to-run variation and variability in amplification efficiency. ECqs are interpreted as the fold change of the target in a sample relative to a reference standard (RS). Accounting for amplification efficiency (E) improves test accuracy because sample Cqs are directly related to E and assuming 100% E will lead to the over estimation of target concentration. In addition, all results have an ECq numeric value, including truncated or “indeterminate” Cqs. Therefore, it is possible to calculate cutoffs and evaluate diagnostic performance using receiver operating characteristic analysis.