AN UPDATE ON THE USE OF REAL-TIME PCR FOR THE DIGNOSIS AND CONTROL OF PORCINE EPIDEMIC DIARRHEA VIRUS AND PORCINE DELTACORONAVIRUS

Christa Goodell[1], Martina Kahila[2], Lori Plourde[1], Wendy Witbeck[1*], Kathy Velek[1], Lisa Gow[1], Valerie Leathers[1], and Michael Angelichio[1]

[1]IDEXX Laboratories, Inc., Westbrook, Maine, U.S.A. [2]IDEXX Switzerland AG, Liebefeld-Bern, Switzerland

wendy-witbeck@idexx.com

Introduction

Porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV) represent new threats to the swine industry. To aid in early detection of virus, monitor shedding, or differentiate viral species, PCR has been a useful diagnostic tool. To this end, a multiplex real-time PCR test was developed to detect and differentiate the presence of viral RNA from PEDV and PDCoV. Additionally, tests for PEDV, PDCoV and TGEV have been developed. All tests use an internal control approach based on detection of endogenous swine RNA, referred to as the Internal Sample Control (ISC) reaction.

Materials and Methods

Reaction mixes contained equal parts of RealPCR™ RNA Master Mix and target-specific detection mix for a total volume of 20 µL x number of samples tested. Samples (5ul per reaction) consisted of either synthetic oligonucleotides or nucleic acid purified from clinical samples. Clinical samples (fecal swabs and oral fluids) were purified using a commercial total nucleic acid extraction kit. The cycling program consisted of one cycle at 50°C for 15 minutes and 95°C for 1 minute, followed by 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds.

Results

The efficiencies and correlation coefficients for each test design were determined using serial dilutions of synthetic DNA. All test designs maintained efficiencies of 95%–105% with R^2 values of ≥0.994 and detected at least 10 copies per reaction. To ensure no interference and/or competition between target and ISC reactions, multiplexed sensitivity testing was performed for all test designs. Copies of the target sequence (PEDV or PDCoV) were amplified in the presence or absence of artificially high concentrations of ISC. High levels of ISC had no impact on the detection of 10 copies of either PEDV or PDCoV. To confirm the ISC design detects swine RNA and not genomic DNA, the reverse transcriptase (RT) contained in the RealPCR RNA Master Mix was inactivated before addition of sample. Inactivation of RT resulted in complete loss of ISC signal. Test sensitivity and specificity for PEDV and PDCoV were evaluated using purified total nucleic acid from samples of known status. The PEDV/PDCoV multiplex test had PEDV sensitivity of 99.5% (n=191) and PDCoV sensitivity of 100% (n=44). Both designs had 100% specificity.

Conclusions

These results demonstrate the high sensitivity and specificity of the RealPCR swine coronavirus tests. The tests are configured as either single target, PEDV, PDCoV, and TGEV tests, or as a PEDV/PDCoV multiplex test. All configurations include an ISC for the detection of swine RNA as an internal control. Early detection of PEDV and PDCoV is crucial in the control of these viruses and ongoing monitoring is essential to avoid new introductions of the diseases. For this purpose real-time PCR is the most sensitive and efficient tool available.

References

1. Lowe et al., Emerging Infectious Diseases, 20:872-874.