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Effect of heating or diluting swine oral fluid samples on qPCR detection

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Introduction. Reports in the literature state that detection of nucleic acids in human oral fluids is improved by heating and/or diluting the sample. For example, "outstanding" direct RT-qPCR performance for the detection of SARS-CoV-2 in human oral fluid samples was reportedly achieved by diluting the sample with tris borate EDTA (TBE) and then heating at 95°C for 30 minutes[1]. The objective of this study was to test the effect of heating (95°C × 30 m) or diluting (TBE) swine oral fluid samples on the detection of PRRSV, IAV, PEDV, or *Mycoplasma hyopneumoniae* (MHP).
Methods. Experiment 1: oral fluid samples containing PRRSV (n = 8), IAV (n = 8), PEDV (n = 8) or MHP (n = 8) were 2-fold serially diluted (neat, 1:2, 1:4, 1:8) using oral fluid free of PRRSV, IAV, PEDV, and MHP as diluent (n = 32 aliquots per pathogen). Each aliquot was split into 4 and randomized to one of 4 procedures: (P1) heat (95°C × 30 m) and direct qPCR; (P2) heat, cool (25°C × 20 m) and direct qPCR; (P3) heat, cool, nucleic acid extraction, and direct qPCR; (P4, i.e., control) extraction and qPCR.
Experiment 2: oral fluid samples containing PRRSV (n = 9), IAV (n = 10), PEDV (n = 10), or MHP (n = 10) were split into three aliquots: (D1) undiluted; (D2) diluted 1:2 with oral fluid free of PRRSV, IAV, PEDV, and MHP; (D3) diluted 1:2 with TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA). Samples were randomly ordered and then tested.
Nucleic acid extraction and qPCRs were done using commercial products (IDEXX Laboratories, Inc.).
Results. In Experiment 1, standard procedures (P4, control) produced 32/32 aliquots positive for PRRSV, 32/32 for IAV, 32/32 for PEDV, and 32/32 for MHP. In contrast, the aggregated results for P1, P2, and P3, produced 7/96 aliquots positive for PRRSV, 14/96 for IAV, 13/96 for PEDV, and 65/96 for MHP, i.e., multiple false negatives. For positive results in all pathogens, standard procedures (P4) produced lower Cqs than procedures P1, P2, or P3.
The results (means) from Experiment 2 showed no gain with D2 or D3:
PRRSV - undiluted Cq = 32.3; D2 Cq = 33.5; D3 Cq = 34.8.
IAV - undiluted Cq = 28.9; D2 Cq = 30.0; D3 Cq = 29.8.
PEDV - undiluted Cq = 25.1; D2 Cq = 25.6; D3 Cq = 25.0.
MHP - undiluted Cq = 32.9; D2 Cq = 33.5; D3 Cq = 33.9.
Conclusions. In conclusion, the heat and dilution treatments described in the literature were detrimental to the detection of PRRSV, IAV, PEDV, and MHP nucleic acids in oral fluid samples by qPCR. Interestingly, examination of the literature showed that these reports often did not include comparisons with standard methods. Therefore, we recommend that research along these lines include quantitative measures of the gain or loss in performance achieved by alternative methods. In this study, the inclusion of comparisons showed that optimum results were obtained using standard extraction and amplification methods.