

**Title:** Development of Real-time, multiplex PCR/RT-PCR assays for improved PRDC pathogen detection - **NPB #03-114**

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**Abstract:** Porcine respiratory disease complex (PRDC) continues to be a significant economic problem for swine producers. Porcine reproductive and respiratory syndrome virus (PRRSV), Swine influenza virus (SIV), Porcine circovirus-2 (PCV-2), *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, and *Pasturella multocida* are among the most important pathogens contributing to PRDC. Recently, a new generation of PCR/RT-PCR equipment has been developed. These machines use sophisticated, sensitive detection techniques which allow quantification of the amplification reaction as it occurs. This methodology has been termed “real-time” and/or “quantitative” PCR (for DNA pathogen detection) or RT-PCR (for RNA pathogen detection). Although not yet widely used in veterinary diagnostic laboratories, Real-time PCR/RT-PCR offers several advantages over traditional molecular diagnostic techniques. In this project multiplex real-time PCR or RT-PCR assays were developed and validated for the detection of: 1) PRRSV (both NA and European strains) and SIV; 2) PCV-2 and *M. hyopneumoniae*; and 3) *P. multocida* and *A. pleuropneumoniae*. Comparison of the analytical and diagnostic parameters of these assays to traditional culture and PCR/RT-PCR based approaches demonstrated that the real-time assays were consistently equal or superior. Approaches were investigated to improve the efficiency of sample preparation and assay performance. In addition, an analysis of laboratory-associated costs for real-time PCR/RT-PCR was performed.

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