

SWINE HEALTH

Title: Detection and differentiation of field strains and commonly used vaccine strains of Type 2 PRRS virus in the US, **NPB #16-196**

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Industry Summary:

PRRS remains the most economically devastating swine disease with an estimated annual loss of \$664 million in the US. The PRRS genome is constantly changing, making accurate diagnosis difficult. Many pairs of primers have been used in some commercial PRRS PCR kits to cover the genetic diversity of the PRRS genomes. Hypothetically, using more primers can bring potential issues such as non-specific amplifications. On the vaccine side, four PRRS vaccines have been used in the US, namely PrimePac; Ingelvac MLV, Ingelvac ATP and Fosterera. Due to their close relationships to some field strains, differentiation between vaccine strains and field strains are challenging using common real-time PCR methods. Currently the differentiation is mostly done by PRRS ORF5 sequencing, which is expensive and time consuming. Luminex technology allows us to detect more molecular targets in a single reaction with the cost similar to that of one real-time PCR reaction. Beads of different colors are coupled with target-specific, i.e., pathogen-specific, capture oligos in the Luminex platform. A short piece of pathogen genome is amplified by target-specific primers. A region in the PCR product is complementary to the capture oligo on the beads, thus is hybridized to the beads for specific target identifications. Designing a Luminex PCR assay without a probe that is required in most real-time PCR assays, can simplify the designing process, and can potentially resolve the multiplexing problem encountered in real-time PCR assays. By analyzing all available full- and near full-genome sequences of PRRS, we were able to design two pairs of primers that are predicted to detect 98% of filed PRRS strains. We were also able to design a pair of primers for each vaccine strains, which can differentiate PRRS strains that are 98% or less identical to the vaccine strains. Analytical sensitivity of this Luminex PRRS assay is one half log to one log lower than that of a typical real-time PCR assay. Testing on the vaccine strains and 489 PRRS field strains indicated that the Luminex PRRS assay we have developed can detect 95.2% of the field strains, and can differentiate majority of the vaccine-like strains especially for MLV-like strains. Although Luminex assays do not provide quantification data as these Ct values generated by real-time PCR assays, it provides a cost-effective way of comprehensive detection of rather divergent PRRS strains; at the same time, differentiates the PRRS vaccine strains without using the more expensive sequencing procedure.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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