Diagnostic Performance of a RT-PCR Test for the Detection of PRRS Virus in Serum

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Diagnostic Performance of a RT-PCR Test for the Detection of PRRSV Virus in Serum

Abstract
Polymerase chain reaction (PCR)-based testing is being widely implemented for diagnostic purposes. The PCR technique is generally considered to be both analytically sensitive (i.e., detects small quantities of a substance) and analytically specific (i.e., few cross reactions). There are few studies, however, of diagnostic sensitivity and specificity (i.e., the likelihood of correctly identifying infection status). The purpose of the following study was to estimate the diagnostic sensitivity and specificity, as well as the analytical sensitivity, of a RT-PCR currently being used in a midwestern diagnostic laboratory for the detection of PRRSV RNA.

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Diagnostic Performance of a RT-PCR Test for the Detection of PRRS Virus in Serum

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Introduction

Polymerase chain reaction (PCR)-based testing is being widely implemented for diagnostic purposes. The PCR technique is generally considered to be both analytically sensitive (i.e., detects small quantities of a substance) and analytically specific (i.e., few cross reactions). There are few studies, however, of diagnostic sensitivity and specificity (i.e., the likelihood of correctly identifying infection status). The purpose of the following study was to estimate the diagnostic sensitivity and specificity, as well as the analytical sensitivity, of a RT-PCR currently being used in a midwestern diagnostic laboratory for the detection of PRRSV RNA.

Materials and Methods

To estimate RT-PCR diagnostic specificity, sera from 195 PRRSV-negative swine were collected and used as known negative samples. All animals were from one of 2 PRRSV-negative herds that had been clinically, virologically, and serologically monitored for PRRS virus infection over a period of several years. Animals were confirmed negative by testing sera for the presence of PRRS antibodies (IDEXX HerdCheck® PRRS ELISA), virus, or both. Virus isolation (VI) was carried out on pulmonary alveolar macrophages (PAMs) and/or MARC-145 cells.

To estimate RT-PCR diagnostic sensitivity, 103 of the 195 negative pigs were intranasally inoculated under experimental conditions with the same lot and strain of PRRSV (isolate ISU-P). All animals were shown to have become infected by VI, ELISA, or both. Day 7 post-inoculation serum samples were used as known positive samples for the RT-PCR.

To examine analytical sensitivity, sera were collected from 7, 7-week-old PRRSV naïve pigs, pooled, and divided into 40, 1 ml aliquots. Ten aliquots received no virus, and the remainder were spiked with PRRSV isolate ISU-P to yield a virus titer of 3.2 (n=10), 0.9 × 10^1 (n=10), or 1.2 × 10^2 (n=10) fluorescence foci unit (FFU) per milliliter. The 338 samples were completely randomized, re-numbered, and submitted for PCR analysis.

Results and Discussion

In the initial testing, diagnostic sensitivity and specificity of the RT-PCR test was 25.2 and 96.4%, respectively. After Trial 1 results were known, a subset of the samples was resubmitted and re-run at the laboratory’s request. Trial 2 was composed of 174 known negative and 93 known positive animals. In Trial 2, the diagnostic sensitivity of the test was 68.8% and the diagnostic specificity of the test was 99.4%. Regarding analytic sensitivity and specificity, the assay detected PRRSV RNA in 10 of 10, 6 of 10, 0 of 10, and 0 of 10 samples containing approximately 1.3 × 10^2, 0.9 × 10^1, 3.2, and zero FFU/ml, respectively.

Few studies have been done to assess the diagnostic performance of the PCR-based assays currently in use. The assumption has been that because analytical sensitivity is often excellent that these tests possess nearly perfect diagnostic performance. Based on the results of this study, it is apparent that the diagnostic performance of PCR assays should be evaluated more carefully using samples from animals of known infection status. In addition, longitudinal studies need to be carried out to determine which clinical samples are best for accurate detection over time. Evaluation of PCR performance, optimization of sample selection, and comparison with other diagnostic techniques should be done before PCR is implemented as the test of choice. In the mean time, decisions made on the basis of PCR diagnostic tests may need to be evaluated based on this data.