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Stability of Porcine Reproductive and Respiratory Syndrome Viral (PRRSV) RNA in Serum and Semen at Different Laboratory Temperatures

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Introduction and Objectives

The detection of PRRSV RNA by TaqMan® reverse-transcriptase polymerase chain reaction (TaqMan) is routinely performed on sera and semen samples from swine in order to assess viremia or shedding of virus in semen. This method of testing is key to many swine health programs as an attempt to evaluate the risk of virus transmission. Since many swine sera and semen samples are collected on farm and delivered to the diagnostic laboratory by courier or mail, the samples are subjected to different temperatures for various periods of time before they can be tested. PRRSV is a somewhat labile pathogen. Virus stability is affected by temperature and pH, with complete inactivation of infectious virus occurring by 48 hours at 37 degrees Celsius (C) [1]. Even though the TaqMan test detects viral RNA and not live virus, questions still remain concerning the stability of PRRSV RNA and whether RNA will degrade over time to a level undetectable by TaqMan.

The goal of this experiment was to assess the stability of PRRSV RNA in porcine serum and semen at different laboratory temperatures.

Materials and Methods

PRRSV negative serum and semen were spiked with PRRSV VR2332 supernatant to a final concentration of approximately 100 $TCID_{50}$/ml. A 0.5 ml aliquot of spiked serum and semen were stored at either approximately 25 C (room temperature) or 4 C (refrigeration temperature) for 24, 48, 72, 96 and 168 hours, then frozen at –70 C until tested. One spiked serum and semen sample each was stored immediately at –70 C.

Viral RNA was extracted on one of two days. RT-PCR tests were performed in duplicate on 5 uL of extracted material with positive and negative controls on the same day. The samples were read in an Applied Biosystems PRISM 7200 reader before and after the RT-PCR, and delta Rn values (measures of fluorescence) were obtained and analyzed. A positive result (e.g., PRRSV RNA is detected) is a sample with a delta Rn reading higher than the cutoff Rn value calculated from the following equation: 4 X (SD of the RN for negative controls) X (t) + (average Rn of the negative control). The value (t) was determined by Applied Biosystems.

Results and Discussion

PRRSV RNA was detectable in serum after storage at room temperature or under refrigeration (Fig. 1). PRRSV RNA was less stable in semen, as viral RNA degraded to below detectable levels after 96 hours at room temperature (Fig. 2). However, there remained detectable RNA in semen even after one week under refrigeration. PRRSV RNA presumably declined in both serum and semen samples after the initial 24 hours at either temperature when compared to samples immediately frozen at –70 C, as is shown by the significant drop in delta Rn values from 0 to 24 hours.

Submitting semen and serum for PRRSV TaqMan testing is a practical approach for monitoring PRRSV shedding. Our results indicate that samples collected, refrigerated, and submitted to a laboratory in a timely manner will retain PRRSV RNA that is detectable by TaqMan. Furthermore, because the RNA is stable in properly stored samples, additional laboratory testing on archived samples is a valid procedure.

Fig 1. Stability of PRRSV RNA in Serum

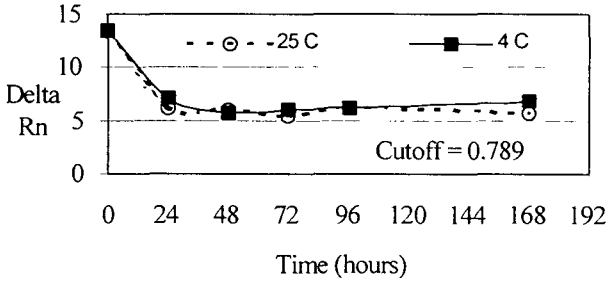

Fig 2. Stability of PRRSV RNA in Semen