Porcine Reproductive and respiratory syndrome virus (PRRSV) is a single-stranded RNA virus and a worldwide cause of significant respiratory disease and reproductive failure in swine. Two different types of PRRSV, the European (EU) and North American (US) type exist. Boar semen can harbor PRRSV (1) and the virus can be transmitted by this route, creating a need for diagnostic tests to ensure a PRRSV-free semen supply. PCR is an obvious method for such testing, and especially nested and TwoStep RT-PCR methods have been extensively used for this purpose. However, OneStep RT-PCR offers a more convenient and safe diagnostic procedure, since cDNA synthesis and PCR is performed sequentially without inbetween opening of the PCR-tubes, thus eliminating a substantial contamination risk. The aim of the present study was to validate a real-time OneStep RT-PCR assay for the simultaneous detection and discrimination of PRRSV EU and US types in semen.