



Development of a novel real-time qPCR assay for the dual detection of canine and phocine distemper virus

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Poster Title	Development of a novel real-time qPCR assay for the dual detection of canine and phocine distemper virus.
Abstract (300 words approx.)	<p>In a commercial diagnostic setting streamlining and optimization is an important factor when the goal is to provide high quality diagnostic results while remaining competitive. In the PCR diagnostics unit at DTU National Veterinary Institute part of this optimization programme is to replace conventional PCR assays with real-time PCR assays to obtain a uniform assay palette. The present work describes the development of a novel real-time RT-qPCR assay for the dual detection of canine and phocine distemper virus. The assay is relevant for the future detection of outbreaks of canine distemper virus in e.g. in farmed mink and wildlife and phocine distemper in seals.</p> <p>A set of primers and dual labelled probe was designed based on an alignment of distemper sequences in GenBank from various species and in-house sequences from recent outbreaks in Danish farmed mink. The assay amplifies a segment of 151 bp in the Phosphoprotein (P) gene of the distemper virus genome. The dynamic range and PCR efficiency (E) was experimentally determined using 10-fold dilutions of a specially designed distemper DNA-oligo in addition to extracted RNA from clinical samples.</p> <p>E of the real-time assay was shown to range between 0.95 and 1.05 when using both clinical samples and the distemper DNA-oligo. Comparing the real-time RT-PCR assay to a currently used conventional assay showed that the real-time assay detects a 10² –fold further dilution of a clinical sample. Tests of distemper positive clinical samples from a wide range of different species performed in parallel with the conventional PCR assay revealed that whereas both assays detect canine and phocine distemper virus, only the conventional assay could detect the dolphin distemper variant. Furthermore it was shown that the Taqman assay does not amplify influenza, a common differential diagnosis in mink and seals.</p> <p>Further testing is needed to determine the effects of PCR inhibitors in clinical samples. In addition, an elaborate panel of potential differential diagnostic agents must be tested in order to determine the assay specificity.</p>

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