Development and validation of a novel Taqman-based real-time RT-PCR assay suitable for demonstrating freedom from viral haemorrhagic septicaemia virus

Viral haemorrhagic septicaemia (VHS) is a serious disease in several fish species. VHS is caused by the rhabdovirus viral haemorrhagic septicaemia virus (VHSV). To prevent spreading of the pathogen, it is important to use a fast, robust, sensitive and specific diagnostic tool to identify the infected fish. Traditional diagnosis based on isolation in cell culture followed by identification using, for example, ELISA is sensitive and specific but slow. By switching to RT-PCR for surveillance and diagnosis of VHS the time needed before a correct diagnosis can be given will be considerably shortened and the need for maintaining expensive cell culture facilities reduced. Here we present the validation, according to OIE guidelines, of a sensitive and specific Taqman-based real-time RT-PCR. The assay detects all isolates in a panel of 79 VHSV isolates covering all known genotypes and subtypes, with amplification efficiencies of approximately 100%. The analytical and diagnostic specificity of the real-time RT-PCR is close to 1, and the analytical and diagnostic sensitivity is comparable with traditional cell-based methods. In conclusion, the presented real-time RT-PCR assay has the necessary qualities to be used as a VHSV surveillance tool on par with cell culture assays.
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