Proposal of a Validation Method for Automated Nucleic Acid Extraction and RT-qPCR Analysis: an Example with Bluetongue Virus

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The control of bluetongue virus (BTV) in Central-Western Europe is greatly complicated by the coexistence of several BTV serotypes. Rapid, sensitive and specific assays are therefore needed to identify correctly the currently circulating BTV serotypes in field samples. In the present study, four serotype-specific real-time reverse-transcription quantitative polymerase chain reaction assays (RT-qPCR) are described for the detection of BTV-1, 6, 8 and 11. The analytical sensitivity of BTV-1/S2, BTV-6/S2, BTV-8/S2 and BTV-11/S2 serotype-specific RT-qPCR assays is comparable to the earlier described serogroup-specific pan-BTV/S5 RT-qPCR assay. *In silico* and *in vitro* analyses indicated that none of the assays cross-reacted with viruses which were symptomatically or genetically related to BTV and only detected the intended BTV serotypes. All assays exhibited a linear range of at least 0.05–3.80 $\log_{10}$ $TCID_{50}$/mL and a PCR-efficiency approaching the ideal amplification factor of two per PCR cycle. Both intra- and inter-run variations were low with a total coefficient of variation of 1–2% for clear positive samples, and below 10% for very weak positive samples. Finally, the performance of the described assays was compared with commercially available kits for the detection of BTV-1, 6 and 8. Three in-house assays gave the same diagnostic results (positive/negative) as the commercial assays and can thus be used interchangeably. Together with the earlier-described serogroup-specific pan-BTV/S5, the serotype-specific RT-qPCR assays form a flexible and properly validated set of tools to detect and differentiate the BTV serotypes currently circulating in Central-Western Europe.

Keywords: Bluetongue – Serotype – PCR.

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