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RE: modification to the in house method for the detection of mumps virus by RT-PCR

Assay modification

This letter is to notify you that we have made a modification to the existing test method for detection of mumps virus by RT-PCR. Our existing method, as communicated on the NML Guide to Services website (specifically at https://cnphi.canada.ca/gts/reference-diagnostic-test/5038?labId=1016, accessed 2023-12-06), consists of a real-time RT-PCR method which targets the fusion (F) gene of the mumps virus. This method is duplexed with an internal quality control target, human RNase P. We have modified this method with the addition of another primer / probe set, developed by the US CDC (protocol available at https://www.cdc.gov/mumps/downloads/simple-protocol-MuV-rRT-PCR.pdf, accessed 2023-12-06), which targets the mumps nucleoprotein (N) gene. All three primer / probe sets are run in a multiplex RT-PCR. Our verification of this modified method demonstrated comparable performance to the existing method with respect to clinical specificity and sensitivity, using a panel of 167 specimens. With respect to analytical sensitivity, the modified method has a detection limit of 9 copies of genomic RNA per reaction (1.8 copies / µl). A full report detailing the verification process has been prepared (document code VESTD-PR-TS-013A-3); a copy of which can be provided upon request. It is our intention as well to publish the method and its verification.

Current status and next steps

We have implemented the modified (triplex) mumps RT-PCR assay in parallel with the existing (duplex) mumps RT-PCR since October. Test results have been and continue to be reported on the basis of the existing method. We will continue this practice and intend to switch over to solely using the triplex method on **March 11, 2024**.