Use of an internal control is not necessary for detecting Influenza in combined nose and throat swabs!

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- **Introduction and purpose**
- The purpose was to assess the need for an internal control (IC) for nucleic acid tests on nose and throat swabs, as previous data from our laboratory, in 2004, had not shown any inhibition in 1500 serum samples processed for Dengue real time RT-PCR.
- Our interest in avoiding an IC is that it consumes one channel, which is precious when most PCR instruments have 5 or fewer channels.
- Our concern is that some authorities demand the use of an IC. The IC verifies lack of inhibition and satisfactory assay performance in each tube. If the IC target is a human gene, the IC also serves to verify that the sampling method did successfully collect human material, which is especially pertinent for preparations from tiny samples such as shavings of formalin fixed paraffin-embedded tissues.
- **Methods**
- 1 We performed a gel based PCR for a human housekeeping gene on combined nose/throat swabs submitted from 40 subjects over a three month period to assess the quality of sampling as well as inhibition.
- 2 We retrospectively collated extraction control data from 1,456 routine PCR runs over two years, conducted by multiple different routine staff of varying degrees of experience.
- All extractions were with an EasyMag (Biomerieux).
- **Results**
- All 40 samples showed an IC band at the expected weight; 39 were strong and one was weak. We did not have a single failure of sample set up or extraction.
- **Conclusion**
- 1 Inhibition is not a major factor in these samples.
- 2 The quality of sampling is good despite the samples being collected by a wide variety of staff over three months.
- The extraction process is reliable. While this data cannot verify each individual tube in the future, or that each swab will have been collected properly, the standard of routine processes and commercial reagents is so high that it is reasonable to argue that an IC is not mandatory in these combined nose and throat swabs.
- **Discussion**
- We prefer to use the extra channel for detecting more targets. We use a Stratagene Mx3005P PCR instrument, with 5 channels, to detect Influenza A, Influenza B, H3, H1 and, when epidemiologically indicated, either H5, H7 or MERS-CoV; all in one multiplex.
- Consider that internal controls are not used in culture methods in microbiology. Urine sample are often culture 'false negative' due to prior administration of antibiotics but few laboratories report the presence of antimicrobials in culture negative samples.
- A retrospective analysis reported (1) from another laboratory of 386,706 specimens representing a variety of matrix types determined the overall inhibition rate to be 0.87%. For FFPE tissue it was 1.72%, the only matrix with an inhibition rate above 1%.
- References

1 J. Clin. Microbiol. 2014, 52(6):2139. DOI:10.1128/JCM.03389-13.

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