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Paper Poster Session III

Recent advances in molecular diagnosis and epidemiology of viral respiratory infections

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

T. Barkham[1], J.W.Y. Leong[1]

[1]*Tan Tock Seng Hospital, Singapore, Singapore*

Objective

Laboratories often implement home-brew nucleic acid tests for viral targets such as Influenza without an internal control (IC). In contrast, some authorities demand the use of an IC, which 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 pertinent for swabs. Our interest in avoiding an IC is that it consumes one channel, which is precious when most PCR instruments have 5 or fewer channels.

The aim was to assess the need for an IC for nucleic acid tests on nose and throat swabs.

Methods

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. We retrospectively collated data from extraction controls, extracted with an EasyMag (Biomerieux), tested in routine runs over two years.

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 after 1,456 PCR runs.

Conclusion

Inhibition is not a major factor in these samples. 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. We do continue to run a positive control in each run so we can verify the automated extraction and sample set up and that reagent and instrument performance are satisfactory.

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. Most patients with possible H5, H7 or MERS-CoV are more likely to have seasonal influenza, so it is productive to include both seasonal influenza types and subtypes when entertaining requests for less likely pathogens. This is efficient and cost effective for patients and public health authorities. Our processes also include a back up; when clinically indicated we repeat the test with a fresh sample as viral load may vary over time.