Developing a method to specifically and simultaneously measure the concentration of infective enterovirus serotypes in environmental samples

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INTRODUCTION

Detection and quantification of viruses in the environment is usually done by qPCR, which does not give indication on the infectivity of the viruses. Integrated cell culture qPCR (ICC-qPCR) combines the propagation of the viruses on cells with detection by qPCR, allowing to selectively measure the infective concentration of viruses. The objective of this study is to develop an ICC-qPCR method capable of selectively quantifying the infective concentration of different enterovirus (EV) serotypes, specifically different coxsackievirus (CV) and echovirus (E) serotypes, in a single sample.

1. PRIMER DESIGN

2. PRIMER EFFICIENCY

An ideal primer causes the concentration of the amplicon to double during each PCR cycle. The efficiency of a primer set corresponds to the ratio of the theoretical vs. actual cycle number needed to achieve a doubling in amplicon number. An efficiency of 1 is optimal. Primer sets with an efficiency of 0.8 or above: 9/15. Primer sets with an efficiency of 0.7 or above: 12/15.

4. WORKFLOW OF ICC-qPCR

6 well-plates are seeded with BGMK cells and RD cells in duplicate.
- Once confluency is reached, the virus inoculum is spread onto the cells.
- One of the plates is placed in the incubator at 37°C for 24 hours, while RNA extraction is immediately performed on the replicate plate.
- After 24 hours, RNA from the first plate is also extracted.
- The increase in viral genome copies concentration in these 24 hours is proportional to the amount of infective virus in the inoculum.
- Standard curves created with each serotype in the laboratory define this relationship (see part 5.)

5. STANDARD CURVES

Ten-fold dilutions of a stock solution containing known infective concentrations of all serotypes were inoculated onto cells. The inocula were collected after 0 and 24 hours and were analyzed by qPCR using serotype-specific primers. The number of genome copies of each serotype was determined by comparison with a standard. The difference in genome copies after and before amplification was plotted against the known infective concentration in the inocula.

To date, standard curves were successfully obtained for three serotypes: CVB2, CVB4 and CVB5.

SUMMARY

We have designed serotype-specific primers which allow to selectively and efficiently amplify the different targeted EV serotypes.

We showed a direct relationship between the increase in genome copies during amplification of the serotype on cells and the infective concentration of the serotype in a mixed sample.

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- Using standard samples containing multiple EV serotypes, combine cell culture and serotype-specific primers to establish ICC-qPCR standard curves.