

New microfluidic platforms for the simultaneous detection of pathogens using quantitative PCR

Astrid Ferlinz¹, Lyn Healy², Kelly Warrington¹, Jon Sherlock¹, Jacqueline Fryer⁴, Anna Gottlieb⁴, Lesley Young²

¹ Life Technologies, Carlsbad, USA

² UK Stem Cell Bank, National Institute for Biological Standards and Control, Hertfordshire, UK

³ Department of Virology, National Institute for Biological Standards and Control, Hertfordshire, UK.

Over the past decade, nucleic acid amplification tests have been developed for a broad range of pathogens and increasingly replaced traditional methods. The key drivers for this shift of technology are the high sensitivity of TaqMan® probe based Real-time PCR Assays and also the ability to multiplex several assays in one reaction vessel. Here we introduce microfluidic tools (branded TaqMan® Array Cards) that allow the simultaneous detection of up to 384 pathogens in one sample.

The validation of one application of this technology, the “TaqMan Array Human Viral Screening Card”, will be documented in this study. We first tested a panel of ‘real-time’ quantitative PCR (qPCR) reagents for their specificity and sensitivity using 96well plates. Single tube TaqMan® Assays were designed against conserved regions of BKV, JCV, HIV-1, HIV-2, HTLV-I, HTLV-II, HCV, HBV, HPV-16, SV40, HHV7, CMV, Adenovirus C, HSV-1, HSV-2 and HHV4 genomes.

All assays successfully and specifically detected their respective synthetic target sequence oligonucleotide (“artificial template”) down to the single copy level. Sensitivity and specificity were also tested in dilution series of nucleic acid extracted from cell cultures infected with those viruses. In summary, all tested assays showed excellent linearity and efficiency with a very high degree of specificity for their targets. No cross-reactivity was detected between assays of viruses belonging to the same family.

TaqMan Assays for 15 pathogen targets plus one internal positive control are pre-loaded on the TaqMan Array Human Viral Screening Cards allowing the simultaneous assessment of these targets in triplicate amplifications from 8 samples in parallel. We tested sensitivity and specificity using both artificial templates and nucleic acid extracted from cell cultures infected with those viruses on these cards. Again, all TaqMan Assays showed excellent linearity and efficiency and a very high degree of specificity for their targets, even in such a small reaction volume (2µl). In conclusion, the TaqMan Array Human Viral Screening Cards will be used as invaluable tools in ensuring that for example human ES cell cultures, particularly from novel sources, are pathogen-free.

Moreover, additional TaqMan Array Cards for the screening & detection of viruses, bacteria and parasites are currently under development and will be made available in the coming months.