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ePoster Viewing
Diagnostic bacteriology – non-culture based, including molecular and MALDI-TOF

A rapid 3base™ real-time PCR for the detection of carbapenemase-resistant organisms using multi-coloured fluorogenic probes

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Background: Beta-lactam antibiotics are the most commonly used antibiotics worldwide in the treatment of bacterial infections. The recent emergence of carbapenemase resistant bacteria is a significant global concern in healthcare settings, as standard treatments may be rendered ineffective. Thus accurate and timely detection of carbapenemase resistant organisms will have a significantly impact on patient management. We have developed a rapid real-time PCR (RT-PCR) assay to detect the six most significant and commonly encountered beta-lactam resistance genes, bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48}$ and the variant $bla_{OXA\text{-}181}$. In order to produce a single tube assay using a standard 4-channel RT-PCR instrument we utilised multi-coloured fluorescent probes[1] (Figure 1). This strategy increases the multiplex capability of a standard 4-channel instrument from four to ten and up to fifteen or more on a 5-channel instrument.

Material/methods: The nucleic acids were converted to a 3base™ form during the DNA isolation in order to yield better multiplexed PCR performance (www.geneticsignatures.com). The assay sensitivity was determined using synthetic DNA constructs and assay performance assessed by using reference material from ATCC (Manassas, USA), Zeptometrix (Buffalo, USA) and QCMD (Glasgow, Scotland). The clinical performance of the assay was assessed by using over 100 clinical isolates obtained from St. Vincent's Hospital (Sydney, Australia). DNA extraction and PCR set up was performed on a GS1 automated extraction platform (Genetic Signatures, Sydney, Australia) resulting in a significant reduction in hands on time. PCR was performed on a CFX real-time PCR instrument (Bio-Rad, Caliofornia, USA) with integrated software calling.

Results: The sensitivity of each component in the final multiplexed assay was found to be less than 5 copies of target when introduced into the RT-PCR reaction. Mixed infections were easily detected using the multi-coloured probe approach, as they appear in different combination of colours and Ct values. The specificity of the assay was assessed on a cross reactivity panel which showed no cross reactivity. Results from the validation panels yielded 100% concordance with the expected resistance patterns. Results from testing the clinical samples will be presented in detail including sensitivity data obtained from quantitative real-time PCR analysis.

Conclusions: The use of multi-coloured fluorogenic probes enables the expansion of the multiplexing capabilities of standard PCR instrumentation. In addition, novel variant or new resistant markers can readily be incorporated into existing assays easily without the need to rely on using multiple wells, thereby improving the throughput of such assays. The optimised assay provides a sensitive and specific alternative for the detection of carbapenemase resistant organisms and can be carried out in less than 4 hours with minimal hands on time for laboratory technicians.

Figure 1. Increasing the multiplex capabilities of standard 4 channel PCR instruments