

PY5: Development of Prototype Multiplexed Lateral Flow Assay (LFA) for Rapid Detection of Invasive Fungal Infections

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ABSTRACT

Introduction: Invasive fungal infections (IFIs), especially caused by the fungal genera *Candida*, *Aspergillus* and *Fusarium*, can be fatal to patients with weakened immune systems. **Method:** This study was conducted to develop a prototype multiplexed lateral flow assay (LFA) based on the sandwich format to detect the presence of invasive *Candida*, *Aspergillus* and *Fusarium* at the genus-specific level. DNA was extracted from human blood spiked with pure cultures of *Candida albicans*, *Aspergillus fumigatus* and *Fusarium solani* and underwent multiplexed PCR using fungi-universal and genus-specific primers targeting the internal transcribed spacer (ITS) region. These primers were labelled with different dyes at the 5' position to serve as antigens. The resultant double stranded PCR products were tested on the prototype LFA strips, each pre-lined with 5 antibodies (3 raised against the respective reverse primer dye labels on the genus-specific primers, 1 against the fungi-universal reverse primer and 1 anti-streptavidin line). Colour production was via red coloured gold nanoparticle (GNP)-streptavidin. Unbound GNP-streptavidin binds to the anti-streptavidin line to serve as internal control. **Results:** All positive samples produced 3 red lines on the LFA strip (1 line at their respective complementary genus-specific antibody positions, 1 line at the fungi-universal antibody position and finally 1 line at the internal control position). Negative control (phosphate buffer) and negative PCR product only produced 1 line at the internal control. **Conclusion:** The prototype LFA successfully detected the presence of the 3 selected fungi genera in spiked blood sample.

KEY WORDS:

LFA; fungal; IFI; Candida; Aspergillus; Fusarium