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Validation of lateral flow immunoassays for the rapid detection of CTX-M- group1, NDM and OXA-48 producers

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Background: The dissemination of broad-spectrum ß-lactamases (ESBLs and carbapenemases) among Enterobacteriaceae is a matter of great concern given the major role of these pathogens as causes of nosocomial infections (and, or for *E.coli*, also of community-acquired infections), and the major role of expanded-spectrum cephalosporins and carbapenems in the treatment of these infections. Detection of those multidrug-resistant bacteria is primarily based on indirect detection of antimicrobial resistances. The strategies involve fast identification of the resistance mechanisms, followed by strict hygiene and contact precautions of the patients. Here, we validate Lateral Flow Immuno Assays (LFIA) to detect NDMs, OXA-48-like and CTX-Ms group 1 producers within 15 minutes.

Material/methods: LFIA (strip + cassette) were manufactured using monoclonal antibodies previously produced and selected. 175 enterobacterial isolates with characterized ß-lactamases content, referred to the french National Reference Centre (NRC) for antibiotic resistance between 2008 and 2014, were grown on agar plates. Seven agar media mainly used in clinical laboratories have been assessed for their influence on the detection. One colony was suspended in extraction buffer and then dispensed on the cassette. Migration was allowed for 15 minutes. A prospective evaluation was also done with

clinical isolates showing a decreased susceptibility to at least one carbapenem and referred to the NRC during the validation period.

Results: Considering the ß-lactamases targeted all the results were correlated with the genotype of the strains determined by PCR analysis. Positive results showed a dark pink colored band leading to no ambiguous interpretation. The three LFIAs gave no false positive and no false negative results. The different media didn't have any influence on the results even when colonies showed strong coloration. Validations of LFIAs to detect KPC, IMP and VIM are in progress and preliminary results suggest excellent perfomances, that may be the premise for a multiplex strip capable of detecting the major carbepenamases and ESBLs.

Conclusions: Our LFIA tests are able to detect their specific target with 100% sensitivity and 100% specificity, even when they are expressed with other ß-lactamases. They are compatible with samples handled in clinical laboratories. They could be relevant in area with high prevalence of NDMs, OXA-48 like and CTX-M group 1 producers. These tests are sensitive, easy to use, and cost effective, thus applicable in any laboratory around the world.