Evaluation of a new lateral flow assay for the detection of VIM-producing bacteria

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Introduction & Objectives

- Rapid detection of carbapenemase in Gram-negatives (GN) is essential for early appropriate therapeutic management and infection control purposes.¹
- Currently available tests such as Carba NP² are well suited as rapid screening test for the detection of carbapenemase producing organisms (CPO) but they do not allow identification of the carbapenemase type (only achievable by molecular tests which are expensive and not routinely available in most laboratories).
- We recently developed lateral flow immunochromatographic assays (ICAs) OXA-48 and KPC, NDM K-SeT Coris BioConcept, for direct confirmation of OXA-48-like, KPC and NDM carbapenemases based on monoclonal antibodies generated by immunization in mice.³
- Here we have evaluated several new VIM detecting ICA prototypes aimed at identification of VIM-type-producing bacteria.

Methods

- Antibodies: Monoclonal antibodies were raised by immunizing mice with highly purified recombinant VIM-2 protein. Eighty-four hybridoma supernatants were screened to select antibodies recognizing VIM-2 and VIM-4. From the 34 initially selected antibodies, a subset of the 6 best-binding antibodies were used to develop 6 prototypes sandwich ICAs which all did recognize VIM-2 and VIM-4 purified protein.
- Bacterial strains: 19 well-characterized Enterobacteriaceae and Pseudomonas spp. collection strains (producing different VIM variants (Table 1) were used to select the best prototype. This prototype was hence challenged on a collection of 143 carbapenem non-susceptible Pseudomonas aeruginosa (PA) isolates originating from 49 acute care hospitals in the setting of a large Belgian national survey. All tested isolates were characterized phenotypically (identification and antimicrobial susceptibility testing) and genotypically (PCR-sequencing)⁴ for their mechanisms of resistance to beta-lactam agents according to the procedures at the Belgian National reference center (Table 2).
- Reading and data recording: Immunochromatographic assays (ICA) performed on fresh culture isolates (18-24 h growth on TSA blood agar plates). Time to signal of the visualized band was recorded by one technologist and the final results were confirmed at 15 minutes by another independent technologist. A negative result was recorded when the control band appeared without the VIM band observed.
- Performance analysis: Sensitivity and specificity of the VIM ICA was determined by comparing to the results of multiplex PCR targeting carbapenemase on all tested strains.

Results

1. Evaluation of 6 different VIM Immunochromatographic assay (ICA) prototypes with 19 VIM-producing collection isolates

- Prototype 2 gave the best detection results: all VIM variants but one VIM-5 are detected (VIM-5 production remains to be confirmed as NDM was also present in the strain)
- Time to signal ranged between 30 sec to 14 min
- No cross reactivity has been observed (not shown)

Table 1. Species and VIM variants evaluated with the 6 VIM ICA prototypes

Table 2. Metallo-beta-lactamase (MBL) and ESBL identified in clinical PA isolates

2. Evaluation of VIM ICA Prototype 2 on clinical P. aeruginosa (PA)

- The isolates were collected in 2016 from 49 Belgian hospitals
- 142 P. aeruginosa were multidrug resistant and tested
- 127 carbapenemase resistant (including 47 VM-2 and 14 VM-4) and 15 Carba S (including 4 VM-2)

The VIM K-SeT presented:
- 100% specificity (IC95: 94-100%);
- 100% sensitivity (IC95: 93-100%)
- Time to signal varies between 20 sec to 1 min 30 sec (median time 50 sec)

Conclusions

- The VIM-K-SeT² ICA prototype offers a reliable and convenient tool for rapid direct detection of VIM-producing P. aeruginosa. This assay may be of particular interest owing to the lack of specificity of phenotypical synergy tests using MBL inhibitors (DPA, EDTA,...)
- Current development includes the combination of VIM and NDM enzymes in one single assay and KPC and OXA-48 in a second single assay.

References


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