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Development of a Multiplex Immunochromatographic Assay for Rapid Detection of the Five Main Carbapenemases in Enterobacteriaceae

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Revised abstract

Background The emergence and spread of carbapenemase producing Enterobacteriaceae (CPE) is a matter of great Public Health concern. A rapid detection and identification of CPEs is essential to prevent further spread and provide appropriate antimicrobial therapy. Simultaneous detection and identification of carbapenemases is mainly based on molecular tests that are rather expensive and require trained staff. Here, we validated a multiplex Lateral Flow ImmunoAssay (LFIA) to detect NDM-, OXA-48-, KPC-, VIM- and IMP-like producers within 15 minutes.

Methods LFIA (strip + cassette) were manufactured using our monoclonal antibodies previously produced and selected. Retrospectively, 179 reference enterobacterial isolates with characterized ß-lactamase genes (grown on UriSelect™4 medium, Biorad), and prospectively 116 clinical isolates showing a decreased susceptibility to at least one carbapenem and referred to the French National Reference Centre (NRC) for antibiotic resistance during a one month period were studied. One colony was suspended in extraction buffer (lysis step) and after vortexing dispensed on the LFIA. Migration was allowed for 15 minutes and results were subsequently monitored with a strip reader (prototype) from NG Biotech (Guipry, France).

Results Considering the carbapenemases 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 multiplex "5carba LFIA" gave no false positive and no false negative results. The media didn't have any influence on the results even when colonies showed strong coloration.

Conclusions Our multiplex 5carba LFIA is able to detect the targets with 100% sensitivity and 100% specificity, even when they are expressed with other ß-lactamases. It is compatible with samples handled in clinical laboratories. It could be relevant in area with high prevalence of NDM-, OXA-48-, KPC-, VIM- or IMP-like producers to discriminate them from each other, especially for initiating proper treatment. This test is sensitive, specific, easy to use, cost effective and could thus be implemented in any microbiology laboratory around the world.

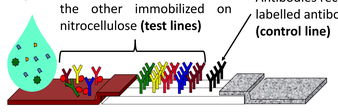
Immunochromatographic assay principle

Structure of the strip

Antibody pairs recognizing the 5 main carbapenemases. One antibody of the pair labelled with colloidal gold, the other immobilized on nitrocellulose **(test lines)**

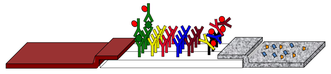
Sample

Antibodies recognizing labelled antibodies **(control line)**



Sample pad — Nitrocellulose — Absorption pad

Immunological detection



Sample flow : capillarity

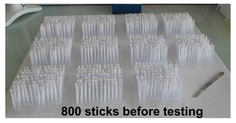
Result

- ✓ The control line appears:: the test is correct
- ✓ One or several test lines appear: positive test for the corresponding carbapenemase(s)
- ✓ No test line appears: negative test for the 5 carbapenemases

Monoclonal antibodies: production and selection

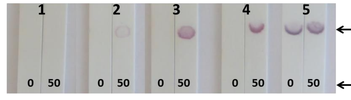
Mice were immunized with purified recombinant carbapenemases (rec. carbapenemases)
20 monoclonal antibodies (mAbs) were selected and purified per carbapenemase

For each targeted carbapenamase:
Pair-selection: each mAb used as either immobilized or gold-labelled antibody
20 mAbs ⇨ 400 pairs ⇨ 800 strips (handmade, 0 or 50 ng/ml of rec. target)



800 sticks before testing

Pairs selection - signals obtained



1-no specific signal
2-low specific signal
3-medium specific signal
4-high specific signal (U-shape)
5-high specific and non-specific signal

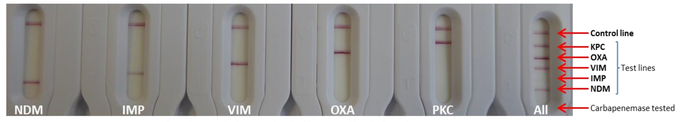
Pairs with high specific signal are tested with serial dilution of rec. carbapenemase and carbapenemase expressing bacteria.

The pairs with the best limit of detection (LOD) with bacteria is selected for the 5 carba LFIA.

Manufactured 5carba LFIA (NG Biotech)

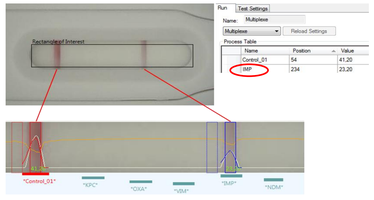
Performances: LODs with rec. carbapenemases
- KPC: 600 pg/ml
- OXA-48: 300 pg/ml
- VIM: 300 pg/ml
- IMP: 200 pg/ml
- NDM: 150 pg/ml

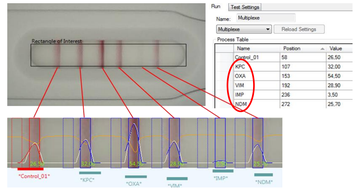
Results illustration with carbapenemase expressing strains



NDM, IMP, VIM, OXA, KPC: one colony in 150µl of extraction buffer, 100µl loaded on the cassette
All: 25µl of each previous extract pooled, 100µl loaded on the cassette

Results analysis (NG Biotech reader)





Reader only gives results for positive test lines with the corresponding target name

5carba LFIA validation

Retrospective evaluation (NRC collection)

179 isolates with PCR characterized ß-lactamase content, including:
- ✓ 54 non-carbapenemases producers:
  - ESBL and/or acquired AmpC cephalosporinases ± impermeability, and extended spectrum oxacillinases OXA-163 and OXA-405
- ✓ 125 carbapenemases producers:
  - 30 Ambler class A: 22 KPC, 3 IMI, 2 SME, 1 NMC-A, 1 FRI, 1 GES
  - 52 metallo-ß-lactamases, Ambler class B: 23 NDM, 17 VIM, 11 IMP, 1 GIM
  - 37 OXA-48-like, 1 OXA-372, Ambler class D
  - 5 both NDM and OXA-48-like

Species variability: *E. coli, K. pneumoniae, K. ozoenae, E. asburiae, P. mirabilis, E. aerogenes, M. morganii, C. koseri, P. stuartii, P. rettgeri, S. enterica, E. cloacae, C. freundii, S. marscesens, Shewanella bicestrii*

LFIA results
Non-carbapenemase producers:
- ✓ 51 gave negative results
- ✓ 2 OXA-163 and 1 OXA-405 gave positive results

Carbapenemase producers:
- ✓ 3 IMI, 2 SME, 1 NMC-A, 1 FRI, 1 GES, 1 OXA-372* gave negative results (* not related to OXA-48 like)
- ✓ 116 others gave positive results at the corresponding test line(s)

Prospective evaluation

116 isolates with decreased suceptibility to at least one carbapenem referred to the F-NRC (April 2017)

LFIA results
- ✓ 46 isolates gave negative results
- ✓ 70 isolates gave positive results:
  - 1 Ambler class A: KPC
  - 11 Ambler class B: 9 NDM, 2 VIM
  - 57 OXA-48-like, Ambler class D
  - 1 NDM and OXA-48-like

PCR results
- ✓ 45 non-carbapenemase producers
- ✓ 71 carbapenemase producers
  - 2 Ambler class A: 1 KPC, 1 IMI
  - 11 Ambler class B: 9 NDM, 2 VIM
  - 57 OXA-48-like, Ambler class D
  - 1 NDM and OXA-48-like

The major carbapenemases were detected in less than 15' migration and subsequent reading. Only one IMI-1 producer was missed , but is not supposed to be detected by 5Carba LFIA

Conclusion

The 5carba LFIA showed 100% sensibility and 100% specificity for the targeted ß-lactamases
The 5carba LFIA is rapid (15'), user friendly and affordable
The 5carba LFIA detects at least the following variants:
- **NDM-1 -4 -5 -6 -7 and -9**
- **KPC-2 and -3**
- **IMP-1 -8 and -11**
- **VIM-1 -2 -4 and -19**
- **OXA-48 -181 204 -232 -244 -517* -519* and -535***
  (* see posters P0232, P0233, P0234 from Laura Dabos, ECCMID 2017)

But also OXA-163 and OXA-405 (OXA-48-like extended spectrum oxacillinases)