Rapid tests for antibiotics resistance detection

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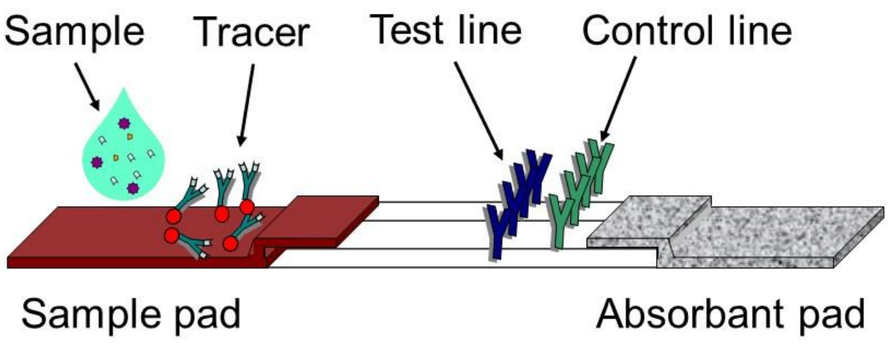
Background : The dissemination of broad-spectrum ß-lactamases (ESBLs and carbapenemases) among Enterobacteriaceae is a matter of great clinical concern given the major role of these pathogens as causes of nosocomial infections (and, for E. coli, also of community-acquired infections), and the major role of expanded-spectrum cephalosporins and carbapenems in the treatment of those infections. Detection of these multidrug resistant clones 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 are developing rapid immunodiagnostic tests (lateral flow formats) for the six most clinically-relevant ß-lactamases: CTX-M-15, NDM-1, OXA-48, KPC-1, IMP-1 and VIM1/2

Material and methods: Monoclonal antibodies were obtained by immunizing mice with recombinant beta-lactamases and after spleen lymphocytes fusion with myeloma cells, the hybridomas were screened by detecting specific antibodies in the culture supernatant. Antibody pairs were selected by combinatorial testing using an immunochromatographic format. Detection limits were determined using the best pairs with an immunochromatographic assay using recombinant proteins and various strains expressing beta-lactamases. The specificity of the tests was determined using different ß-lactamases including mutants.

Principle:



Lateral flow immunoassays of ß-lactamases need two complementary mAbs with high affinity and specificity

Results:

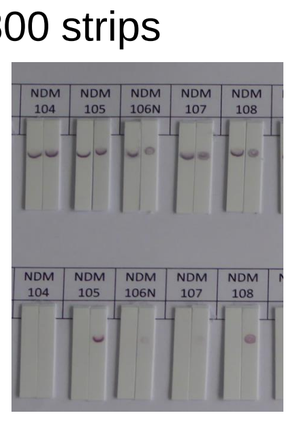
Positive hybridomas obtained:

NDM-1: 220
OXA-48: 390
CTX-M-15: >15
KPC-1: 130

} 20 monoclonal antibodies for each ß-lactamase

Combinatorial testing:

20 monoclonal antibodies, 400 pairs, 800 strips

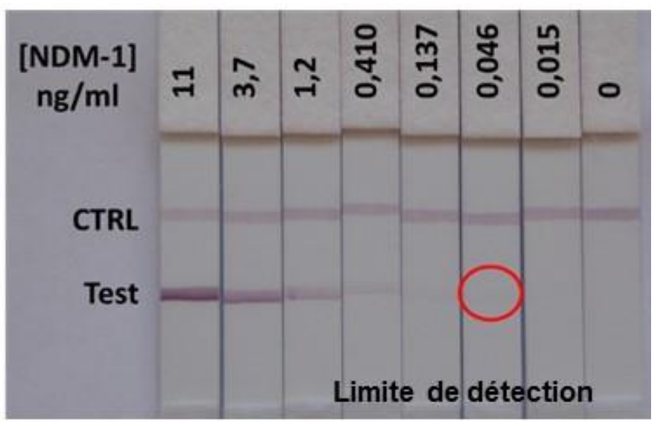


Tested pairs for accurate selection

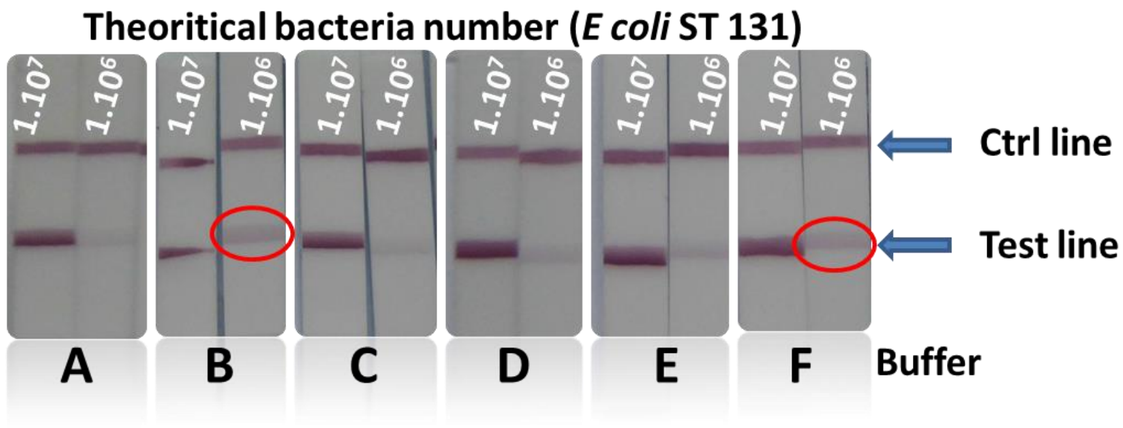
CTX-M-15: **7**
OXA-48: **49**
KPC-1: **5**
NDM-1: **24**

Limit of detection of the best pairs

NDM-1: ~**46 pg/ml**
OXA-48: < **100pg/ml**
CTX-M-15: < **100pg/ml**

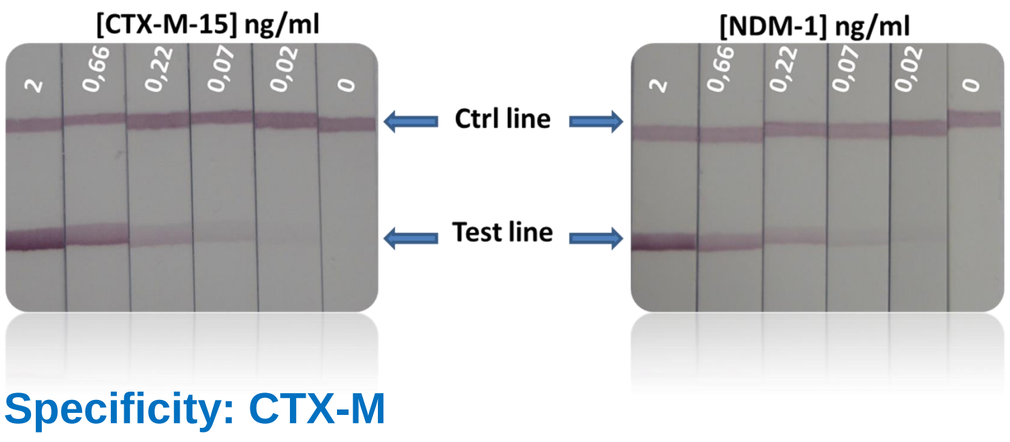


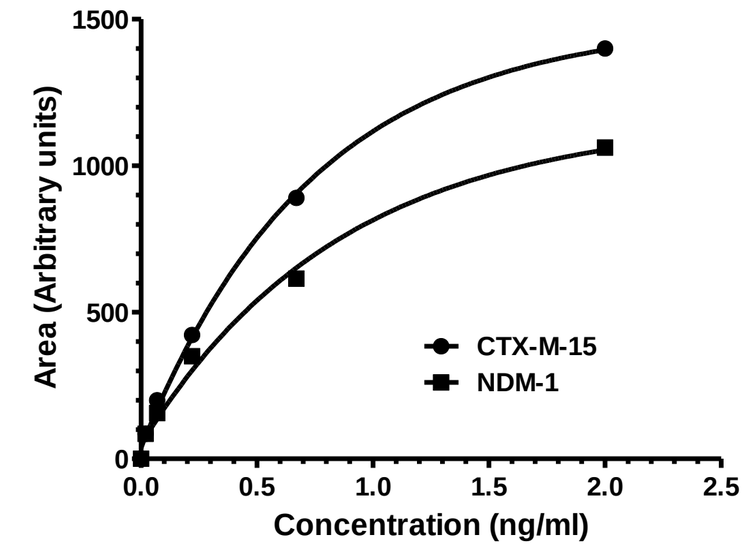
Extraction buffers selection with CTX-M-15 test



Limit of detection with buffer B

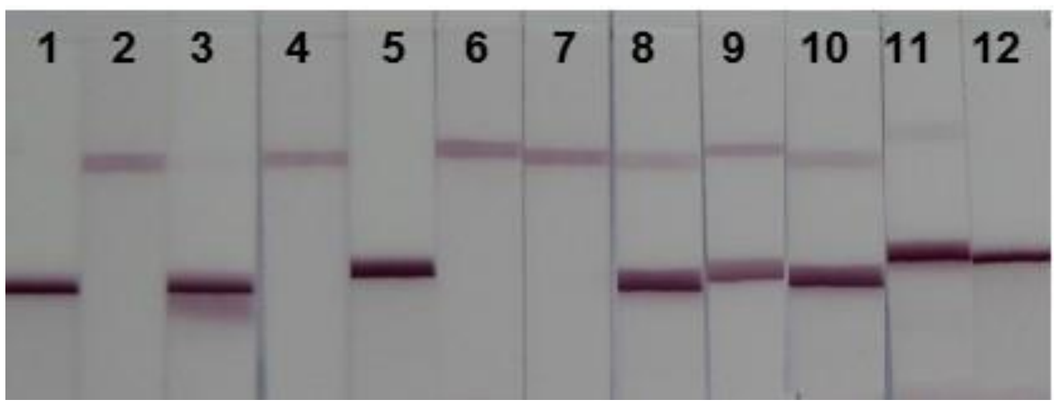
Recombinant ß-lactamases diluted in buffer B





Limit of detection for both tested ß-lactamases is about 20 pg/ml

Specificity: CTX-M



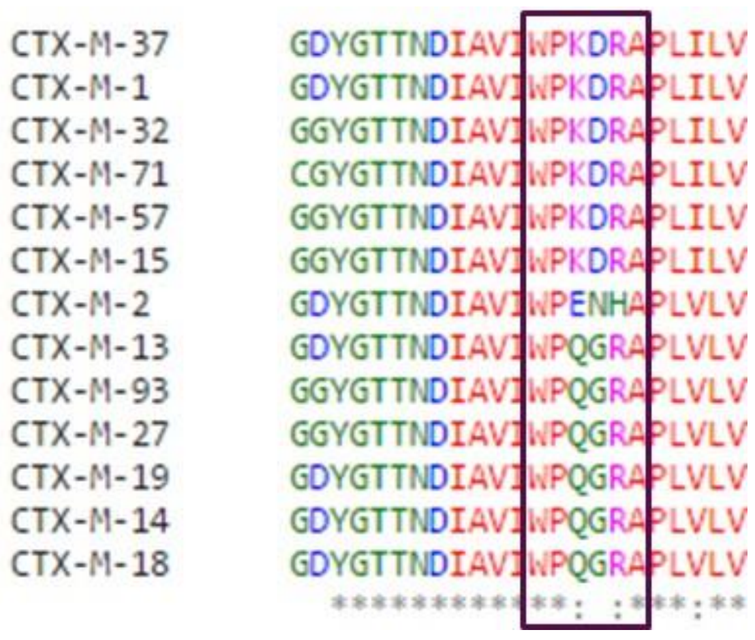
Positive controls

1 : CTX-M-1, *C. freundii*
2 : CTX-M-2, *E. coli*
3 : CTX-M-13, *C. freundii*
4 : CTX-M-14, *K. oxytoca*
5 : CTX-M-15, *E. coli (ST 131)*
6 : CTX-M-18, *K. pneumoniae*
7 : CTX-M-19, *K. pneumoniae*
8 : CTX-M-27, *E. coli*
9 : CTX-M-32, *E. coli*
10 : CTX-M-37, *E. coli*
11 : CTX-M-57, *E. coli*
12 : CTX-M-71, *P. mirabilis*

Negative controls

13 : *C. freundii*
14 : *E. cloacae*
15 : *E. coli*
16 : *K. pneumoniae*
17 : *P. mirabilis*

Epitope
mAb ligne test



⟹ Use of mAbs with other specificity

Perspectives:

Optimization of a ß-lactamases extraction method
Validation of selected pairs with clinical strains and clinical samples (CNR Kremlin Bicêtre)
Production of monoclonal antibodies against IMP-1 and VIM1/2
Development of an inexpensive rapid multiplex immunochromatographic assay
Industrial transfert