Saturday - 428

CTX-M-15 Detection in Clinical Samples: Evaluation of an ImmunoChromatographic Test

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Revised abstract

Background The dissemination of broad-spectrum ß-lactamases (ESBLs) such as CTX-Ms among Enterobacteriaceae is a matter of great concern given the major role of these pathogens as causes of nosocomial infections and of community-acquired infections. Detection of those 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 evaluate a Lateral Flow ImmunoAssay (LFIA) to detect CTX-Ms producers from agar plates, urine or blood cultures. Results are interpretable within 15 minutes.

Methods A manufactured LFIA for detection of group 1 (G1) CTX-Ms (CTX-Ms G1 LFIA) has been validated with 175 enterobacterial isolates with PCR characterized ß-lactamase content. A colony from agar culture was suspended in extraction buffer (lysis step) and added to the device. Prospectively, 100 clinical isolates with decreased susceptibility to broad spectrum cephalosporinases were also tested. In order to evaluate the CTX-Ms G1 LFIA direct use in urine and blood cultures, both types of samples were spiked with CTX-Ms producing or non-producing strains. Before testing, extraction buffer was added to those samples. In each case, the result was eye read 15 minutes after the sample deposition. A homemade CTX-Ms G1-2-9 was also evaluated in the same conditions.

Results CTX-Ms G1 LFIA is able to detect the variants CTX-M-1-3-15-55-82-101 and-182. During validation, no false positive or false negative were observed considering the CTX-Ms G1 detection. Concerning the spiking in urine and blood culture, a positive result was observed only when a CTX-M G1 was expressed. The homemade CTX-Ms G1-2-9 LFIA showed similar performances with an extended specificity to G2 and G9 CTX-Ms In this evaluation no matrix effects were observed.

Conclusions Our CTX-Ms G1 LFIA showed 100% sensibility and specificity on agar media grown strains with a limit of detection closed to 10^6 cfu/ml. Its performances are similar in urine and blood culture. The homemade CTX-Ms G1-2-9 LFIA targets enzymes from 4 CTX-M groups and could provide the detection of 99% CTX-M expressing strains worldwide. The promising results will allow us to start an evaluation study with clinical samples from hospitalized patients.

CTX-Ms G1 LFIA validation: manufactured tests from NG Biotech

One colony from agar plate was suspended in 150µl of extraction buffer (lysis step). After vortexing 100µl were loaded on the cassette. Migration 15' before naked eye reading

Retrospective evaluation

175 strains with PCR characterized ß-lactamase content (from the french National Reference Center (NRC) for antibiotic resistance)

- ✓ **70 CTX-Ms group 1 producers gave positive results**
- ✓ **105 non CTX-Ms G1 producers gave negative results**

Routine use

100 isolates (from urine, blood culture, bile…)

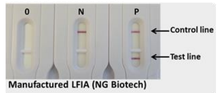
- ✓ **82% CTX-Ms Group 1 gave positive results**
- ✓ **16% CTX-Ms Group 2 and 9 gave negative results**
- ✓ **2% Other ESBLs gave negative results**

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*

100% sensititvity and specificity for CTX-Ms G1 LFIA

Variants detected: CTX-M 1/3/15/32/37/55/57/71/101/182

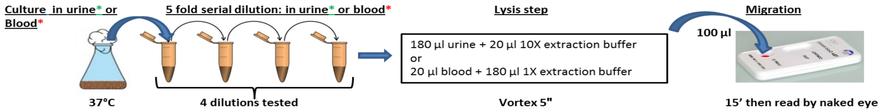
Results illustration



O: cassette before use, N: negative result, P: positive result

CTX-Ms G1 LFIA evaluation in urine and blood culture

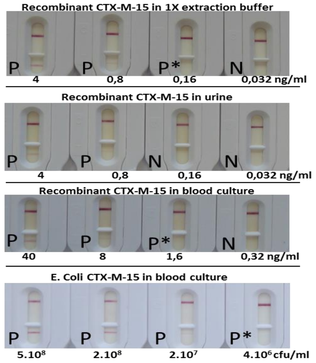
Protocole



Urine*: recovered from first author. Blood *: negative blood culture (Bact/Alert®FA Plus/Biomérieux) from clinical laboratory

Limit of detection (LOD) in urine and blood culture

Recombinant CTX-M-15 was serially diluted either in 1X extraction buffer, negative urine or blood culture and each dilution treated as detailed above (lysis step and migration)



P positive result; N negative result; * does not appear on the picture due to camera sensitivity

Limit of detection in urine and blood culture

With CTX-Ms G1 expressing strains

- *E. coli* expressing CTX-M-1
- *E. coli* expressing CTX-M-15
- *K. pneum.* expressing CTX-M-15

CTX-Ms G1 non-expressing *E. coli*, *K. pneum. and P. mirabilis* were also tested

LOD in urine (3H culture)

- *E. coli* CTX-M-1 ≈ 8.10^5 cfu/ml
- *E. coli* CTX-M-15 ≈ 1.10^6 cfu/ml
- *K. pneum.* CTX-M-15 ≈ 2.10^6 cfu/ml

LOD in « blood » (1H culture)

- *E. coli* CTX-M-15 ≈ 4.10^6 cfu/ml
- *K. pneum.* CTX-M-15 ≈ 2.10^6 cfu/ml

Urine and blood cultures did not influence the background on the membrane

Non-expressing strains gave negative results in both sample types

The CTX-Ms G1 LFIA could also detect CTX-M-32-37-57 (*E. coli*) and CTX-M-71 (*P. mirabilis*) expressing strains in urine and blood culture (data not shown)

Homemade CTX-Ms G1-2-9 LFIA

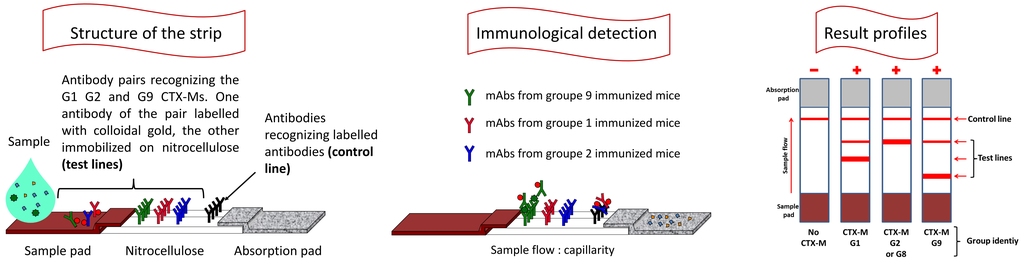
Monoclonal antibodies (mAbs), production and selection

Mice were immunized with purified recombinant CTX-M-15 (group 1), CTX-M-2 (group 2) or CTX-M-14 (group 9)

20 mAbs were selected and purified per enzyme

One pair selected per enzyme

Immunochromatographic assay principle

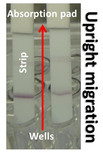


Evaluation of the CTX-Ms G1-2-9 LFIA in urine and blood culture

Protocole

Culture, dilution, lysis step were performed as previously described

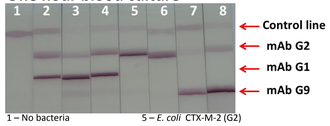
For the migration, 100µl of each dilution were added to 10µl of gold-labelled mAbs (mix of the 3 labeled mAbs) in a microtitration plate well. Then the strips were dropped into the well and results eye read after 30'



Results after culture in urine and blood culture

CTX-Ms expressing strains were grown as previously described

One hour blood culture



1 – No bacteria
2 – *E. coli* CTX-M-1 (G1)
3 – *E. coli* CTX-M-15 (G1)
4 – *K. pneu.* CTX-M-15 (G1)
5 – *E. coli* CTX-M-2 (G2)
6 – *E. coli* CTX-M-8 (G8)
7 – *K. oxytoca* CTX-M-14 (G9)
8 – *E. coli* CTX-M-27 (G9)

- ✓ All strains detected with result profiles correponding to the CTX-M group
- ✓ Results after culture in urine (3H) were very similar (not shown)
- ✓ CTX-M non-expressing strains (*E. coli, K. pneum., P. mirabilis, C. freundii)* gave negative results (not shown)
- ✓ **This homemade CTX-Ms G1-2-9 LFIA also recognized CTX-M-8 (G8)**
- ✓ **LODs for CTX-Ms G1 were similar whatever the test format (manufactured or homemade)**
- ✓ **LODs for CTX-Ms G2-8-9 were between 1.10^6 and 1.10^7 cfu/ml**

Conclusions and perspectives

- ✓ The CTX-Ms G1 LFIA will soon be commercially available
- ✓ Its validation for direct detection in urine and blood culture is still in progress
- ✓ The CTX-Ms G1-2-8-9 LFIA will soon be validated with the NRC collection and will follow the commercialization process
- ✓ Those two LFIAs could directly be used with samples such as urine and blood culture using the described protocol
- ✓ **Four CTX-M groups out of five could be detected including:**
  - **CTX-M-1-15-32-37-55-57-71-101-182 (group 1), CTX-M-2 (group 2), CTX-M-8 (group 8) and CTX-M-14-18-19-27 (group 9)**
- ✓ Results have to be confirmed with significant number of clinical samples:
  - blood cultures just after they flagged positive in several commercial media
  - urine from patients and not only spiked urine from healthy donor
  - swabs should also be tested