

Introduction

NG-Test CTX-M MULTI is a qualitative rapid immunoassay for the detection of CTX-M groups 1, 2, 8, 9 and 25 in a bacterial colony obtained from culture. It is an *in vitro* diagnostic assay, for professional use only, that aids to detect extended-spectrum β -lactamases (ESBLs) produced by *Enterobacteriaceae*.

Summary

β -lactams are first-line antibiotics for the treatment of infections caused by *Enterobacteriaceae*. Nevertheless, since the beginning of their massive use in the 1940s, their efficacy has been challenged by the production of enzymes that inactivate them: β -lactamases. Among them, the extended-spectrum β -lactamases (ESBLs) hydrolyse most β -lactams sparing only cephamycins (as ceftioxin) and carbapenems.

The epidemiology of ESBLs among *Enterobacteriaceae* recently changed with widespread dissemination of CTX-M-type enzymes. In the 1990s, the main ESBLs were derived from TEM- and SHV-type enzymes, and mainly diffused within hospital clones of *Klebsiella pneumoniae* and *Enterobacteriaceae spp.* Diffusion of CTX-Ms within the *Escherichia coli* species changed this situation. Currently, more than 170 CTX-M variants have been identified and described in 5 main groups (CTX-M groups 1, 2, 8, 9 and 25). Dominant variants are geographically different. However, CTX-M-15 (group 1) and CTX-M-14 (group 9) are the most detected around the world, followed by CTX-M-2 (group 2).

Wherever their location in the world, CTX-Ms now represent the majority of ESBLs, to the point that their diffusion is qualified as pandemic. They are equally isolated in community and hospital settings and seem to be endemic in long-term care institutions. The increasing prevalence of ESBLs in community settings creates an unprecedented problem: the influx of multiresistant bacteria from the community setting to the hospital setting.

Principle

NG-Test CTX-M MULTI is a ready for use rapid immunoassay for the detection of groups 1, 2, 8, 9 and 25 CTX-M β -lactamases in a bacterial colony sampled on a solid agar medium after culture (an overnight) and processed in an extraction buffer.

The assay is carried out by dispensing the sample in the cassette well. The sample migrates towards the conjugate pad and, if present, the CTX-M enzymes react with labelled anti-CTX-M mouse monoclonal antibodies. The complex migrates through nitrocellulose membrane by capillarity and interacts with the anti-CTX-M mouse monoclonal antibodies immobilized on the membrane, on the test region "T".

The control line C, is formed by labelled streptavidin and monoclonal antibodies reacting with biotin-BSA and goat anti-mouse polyclonal antibodies immobilized on the membrane.

If the sample is positive for CTX-M from groups 1, 2, 8, 9 or 25, a red line will appear on the test region "T" and on the control region "C" of the membrane. If not, only one red line will appear on the control region "C".

Reagents and materials supplied

Each kit contains:

- 20 Test cassettes in aluminium pouches with desiccant
- 20 Eppendorf tubes
- 20 Disposable pipettes of 100 μ L
- 1 Extraction buffer solution in a plastic bottle (4,5 mL)
- 1 Leaflet

Materials required but not supplied

- Timer
- Single use gloves
- Loop
- Vortex

Precautions

- *In vitro* diagnostic test. For professional use only.
- All the operations must be carried out according to good laboratory practices.
- Do not use after the expiry date.
- The devices must remain in the sealed pouches until they are used.
- Handle the samples as if they were potentially infectious.
- After use, discard the device in an infectious waste container.
- Do not reuse the device.

Storage and stability

Store the devices in their sealed pouches between 4 and 30°C. Do not freeze. Kits are stable until the expiry date indicated on every kit.

Culture and sampling

The samples to be tested shall be obtained and handled according to the standardised microbiology procedures. A colony will be collected in a solid agar-based culture, then will be suspended in the extraction buffer provided into the kit. It is highly recommended to use fresh bacterial colonies for the assay performance to be optimal.

Validated culture media

Mueller Hinton (MH) agar, URIsellect 4 (URI-4), Columbia agar + 5% horse blood, ChromID ESBL agar, Drigalski (DRIG) agar.

Operating procedure

1. Wear protective gloves.
2. Bring the kit components at room temperature for at least 10 minutes.

Preparing the sample

1. Dispense 5 drops (150 μ L) of extraction buffer in one of the microtubes provided into the kit.
2. From a solid agar-based culture, collect a colony with a loop, and then suspend it in the microtube containing 150 μ L of extraction buffer.
3. Close the microtube.
4. Vortex to homogenise the mixture before use.

NOTE: Mucous colonies can lead to migration problems, due to their high viscosity. Vortex for 3 minutes a colony in extraction buffer and incubate for 10 minutes at room temperature before performing the test.

Carrying out the test

1. Open the pouch, and take out the device. Once opened, use the test immediately.
2. Using the provided pipette, add 100 μ L of the prepared mixture (sample must reach the black line indicated on the pipette to accurately aspirate 100 μ L) in the sample well labelled "S".
3. Read the results at 15 minutes and interpret them as indicated below.

NOTE: Do not interpret the test results after 20 minutes, as they may vary possibly causing false positive results.

Result interpretation

Negative result



If only one red line appears on the control region (C): the sample does not contain any CTX-M enzyme or non-detectable level of this one and must be interpreted as a negative result.

Negative

Positive result

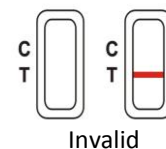


If two red lines appear, one on the control region (C) and one on the test region (T): the sample contains CTX-M enzyme and must be interpreted as a positive result.

Positive

NOTE: The intensity of the red test line (T) may vary depending on the CTX-M enzyme level in the sample. A weak line should be considered as a positive result.

Invalid result



If the control line (C) does not appear, the test result is invalid. Insufficient sample volume or an incorrect procedure are the most likely reasons for control line failure. Deterioration of the test kit may have occurred. Repeat the procedure using a new test. If the problem persists, do not reuse the kit and contact your distributor.

Quality control

An internal control is included in the test. When the control line develops, it confirms the sample volume was sufficient and the procedure was correct.

Limitations

This test is a qualitative assay, so it cannot yield any quantitative result. This first-line test allows a rapid stratification of the patients. The obtained results must be confirmed with alternative or complementary diagnostic procedures. A positive or a negative test does not rule out the presence of other mechanisms of antibiotic resistance.

Performances and characteristics

Detection limit

The detection limit was determined using purified recombinant enzymes.

Group 1 / CTX-M-15	250 pg/mL
Group 2 / CTX-M-2	600 pg/mL
Group 9 / CTX-M-14	100 pg/mL

NG-Test CTX-M MULTI detects the following variants:

- Group 1: CTX-M-1, -3, -10, -15, -32, -37, -55, -57, -71, -82, -101, -182
- Group 2: CTX-M-2
- Group 8: CTX-M-8
- Group 9: CTX-M-9, -13, -14, -17, -18, -19, -24, -27, -65, -93
- Group 25: CTX-M-94, -100

Evaluation study

NG-Test CTX-M MULTI was evaluated on 165 isolates (PCR characterized ESBL content) at the NRC (AMR French National Reference Centre) of CHU Kremlin Bicêtre Paris-France.

Table 1

NG-Test Status	Positive NG-Test CTX-M MULTI	Negative NG-Test CTX-M MULTI	Total
Positive result	149	0	149
Negative result	0	16	16
Total	149	16	165










Sensitivity: 100% **95% confidence interval [97,5%; 100%]**
Specificity: 100% **95% confidence interval [80,6%; 100%]**

Bibliography

- Hervé Boutal and Al. 2018. A multiplex lateral flow immunoassay for the rapid identification of CTX-Ms producing *enterobacteriaceae*. In : Poster ECCMID 2018.
- Hervé Boutal and Al. 2017. CTX-M-15 detection in clinical samples: evaluation of an immune chromatographic test. In : Poster ANSM 2017.
- Hasan Afzali and Al. 2016. Characterization of CTX-M-Type Extend-Spectrum β -Lactamase Producing *Klebsiella* spp. in Kashan, Iran. In : Jundishapur J Microbiol. 2015 October; 8(10): e27967.
- R. Bonnet, 2004. Growing Group of Extended-Spectrum -Lactamases: the CTX-M Enzymes. In: Antimicrobial agents and chemotherapy, Jan. 2004, p. 1–14
- Etienne Ruppé, and Al. 2009. CTX-M β -Lactamases in *Escherichia coli* from Community-acquired Urinary Tract Infections, Cambodia. In: Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 15, No. 5, May 2009
- Wei-Hua Zhao and Zhi-Qing Hu, 2013. Epidemiology and genetics of CTX-M extended-spectrum β -lactamases in Gram-negative bacteria. In: Critical Reviews in Microbiology, 2013; 39(1): 79–101 © 2013 Informa Healthcare USA, Inc
- Rafael Cantón, José María González-Alba and Juan Carlos Galán, 2012. CTX-M enzymes: origin and diffusion. In : Frintier in microbiology, volume 3, articles 110.
- Hiran Dhanjia, Michel Doumitha, Olivier Clermont, Erick Denamur, Russell Hope, David M. Livermore, Neil Woodford. Real-time PCR for detection of the O25b-ST131 clone of *Escherichia coli* and its CTX-M-15-like extended-spectrum-lactamases; International Journal of Antimicrobial Agents 36 (2010) 355–358.

- David L. Paterson¹ and Robert A. Bonomo. Extended-Spectrum-Lactamases: a Clinical Update. Clinical Microbiology reviews, Oct. 2005, p. 657–686 Vol. 18, No. 4.
- Martha Valiadi & Sumit Kalsi & Isaac G. F. Jones & Carrie Turner & J. Mark Sutton & Hywel Morgan. Simple and rapid sample preparation system for the molecular detection of antibiotic resistant pathogens in human urine. Biomed Microdevices (2016) 18: 18.
- Sridhar Rao P.N- Assistant Professor Dept.of Microbiology JJMMC,Davangere.CTX-M β -lactamases. www.microrao.com 03rd July, 2012.
- Sun Hee Park. Third-generation cephalosporin resistance in gram-negative bacteria in the community: a growing public health concern - Korean J Intern Med 2014;29:27-30
- Wei-Hua Zhao and Zhi-Qing Hu. Epidemiology and genetics of CTX-M extended-spectrum β -lactamases in Gram-negative bacteria. Critical Reviews in Microbiology, 2013; 39(1): 79–101.
- Wageih S. El-Naghy, Amal A. Wafy, Nashwa N. Elfar, Atef Taha, Abeer Shahba, Nashwa M. Noor-eldeen and Nahla A. Nosair. Multiplex PCR for Detection of bla CTX-M Genes among the Extended Spectrum Beta Lactamase (ESBL) Producing Gram-negative Isolates. Egyptian Journal of Medical Microbiology, July 2014 Vol. 23, No. 3.
- L. Dortet, L.Poirel, P. Nordmann. Rapid Detection of Extended-Spectrum-Lactamase-Producing Enterobacteriaceae from Urine Samples by Use of the ESBL NDP Test. Journal of Clinical Microbiology- October 2014 Volume 52 Number 10.

Symbols

	Content for 20 assays		Expiry date
	<i>in vitro</i> diagnostic medical device		Do not re-use
	Batch number		Catalogue reference
	Consult instructions for use		Temperature limit
	Manufacturer		



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This test was developed in collaboration with the CEA*.
*The French Alternative Energies and Atomic Energy Commission is a key player in research, development and innovation.

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