NG-Test MCR-1 For research use only

Rapid test for the detection of Colistin resistance on bacterial colony from culture
For professional in vitro diagnostic use only

Introduction

NG-Test MCR-1 is a qualitative rapid immunoassay for the detection of the MCR-1 enzyme in a bacterial colony obtained from culture. It is an *in vitro* diagnostic assay, for professional use only, that aids in the rapid identification of antibiotic-resistant bacteria.

Summary

The presence of Gram-negative multiresistant bacterial strains represents a global and growing threat. They constitute an increase of nosocomial infections in hospital. Polymyxin E (colistin) is an antibiotic considered as a last line of defense against infections caused by resistant strains.
The appearance of the MCR-1 gene (mobilized colistin resistance) in the genome of bacterial plasmids calls into question the use of colistin.
This gene was first discovered in China in 2011 and is present to date in about 18 countries. Global spread is a challenge in terms of sanitary safety. Indeed, the MCR-1 gene coding for the MCR-1 enzyme confers an antimicrobial resistance to colistin. It allows the addition of a phosphoethanolamine residue to lipid A lipopolysaccharide (LPS) bacteria and thus inhibits the attachment of colistin to LPS, thereby affecting its mechanism of operation. This antibiotic resistance enzyme co-exists with others such as carbapenemases and extended-spectrum β-lactamases, thus causing a problem difficult to anticipate.

Principle

NG-Test MCR-1 is a ready for use rapid immunoassay for the detection of the MCR-1 enzyme 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 MCR-1 enzymes react with labelled anti-MCR-1 mouse monoclonal antibodies. The complex migrates through nitrocellulose membrane by capillarity and interacts with the anti-MCR-1 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 MCR-1, 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 µL
- 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.

Recommended culture media
Luria Broth (LB) and LB agar, Trypticase soy agar (TSA), Mueller Hinton (MH) agar and URI-4, Columbia agar + 5% horse blood, ChromIDTM ESBL agar, ChromIDR CARBA SMART, 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 µL) 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 µL of extraction buffer.
3. Close the microtube.
4. Vortex to homogenise the mixture before use.

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 µL of the prepared mixture (sample must reach the black line indicated on the pipette to accurately aspirate 100 µL) in the sample well labelled "S".
3. Read the results after 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

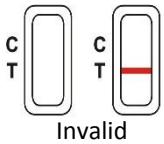
If only one red line appears on the control region (C): the sample does not contain MCR-1 enzyme or non-detectable level of this one and must be interpreted as a negative result.

Positive result

If two red lines appear, one on the control region (C) and one on the test region (T): the sample contains MCR-1 enzyme and must be interpreted as a positive result.

NOTE: The intensity of the red test line(s) may vary depending on the concentration of the MCR-1 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.

NG Biotech, Z.A. Courbouton, Secteur 1, 35480 Guipry, France
Phone : +33(0)2 23 30 17 83 Fax : +33(0) 9 71 70 53 10 email:info@ngbiotech.com

www.ngbiotech.com

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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 test should be used as an aid for the rapid identification of patients bearing a resistance to colistin antibiotics. 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 a purified recombinant MCR-1 enzyme and evaluated at 300 pg/ml.

Validation on a reference strain bank
NG-Test MCR-1 was evaluated on 44 clinical strains at NRC Kremlin Bicêtre Paris (National Reference Center), 123 clinical strains at ANSES (National Health Security Agency) Lyon and 117 clinical strains at NRC Clermont Ferrand.
A total of 284 strains were evaluated.

Cross-reactivity
All results were correlated to the genotype of strains determined by PCR analysis.
NG-Test MCR-1 detects some MCR-2 variants.

Table 1: Results obtained at NRC Kremlin Bicêtre

Status	Positive	Negative	Total
Positive	25	0	25
Negative	3 (mcr-2)	16	19
Total	28 (3 mcr-2 detected)	16	44

Sensitivity : 100% Confidence interval 95% : 86.7% to 100%
Specificity : 81.25% Confidence interval 95% : 62.4% to 94.5%

Table 2: Results obtained at NRC Clermont-Ferrand

Status	Positive	Negative	Total
Positive	17	0	17
Negative	0	100	100
Total	17	100	117

Sensitivity : 100% Confidence interval 95% : 81.6 % to 100%
Specificity : 100% Confidence interval 95% : 96.3 % to 100%

Table 3: Results obtained at ANSES Lyon

Status	Positive	Negative	Total
Positive	59	0	59
Negative	0	64	64
Total	59	64	123

Sensitivity : 100% Confidence interval 95% : 93.9 % to 100%
Specificity : 100% Confidence interval 95% : 94.3 % to 100%

Table 4: Overall results of the three sites for MCR-1

Status	Positive	Negative	Total
Positive	101	0	101
Negative	3 (mcr-2)	180	183
Total	104	180	284

Sensitivity : 100% Confidence interval 95% : 96.3 % to 100%
Specificity : 98.3% Confidence interval 95% : 95.3 % to 99.4%

Bibliography

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Symbols

Σ 20	Content for 20 assays		Expiry date
IVD	*in vitro* diagnostic medical device		Do not re-use
LOT	Batch number	REF	Catalogue reference
	Consult instructions for use	+4°C +30°C	Temperature limit
	Manufacturer	**Contains NaN3**	0.01% sodium azide

NG Biotech
Z.A. Courbouton, Secteur 1
35480 Guipry France
Tel: +33 (0) 2 23 30 17 83 Fax: +33 (0) 9 71 70 53 10
Email: info@ngbiotech.com

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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