Instructions for use

eazyplex® SuperBug mcr-1

Molecular biological rapid test
for the detection of the mcr-1 gene

for use with Genie® II Mk2 devices

For in vitro diagnostic use

CE

eazyplex® SuperBug mcr-1		REF: 7604
language: english	valid from:	April 2019

Explanation of symbols

IVD	in vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use by
	Temperature limitation
TESTSTRIP	Teststrip
	Contains sufficient for <n> tests
	Consult instructions for use
	No re-use
	Manufacturer

Document Revision Information:
Actualization of symbols according to EN ISO 15223-1, page numbers inserted, software version actualised (4.2), „select the test manually“deleted (8.1), Documentation under 9. amended by PDF report and CSV file, Warning messages inserted under 9., general improvement of phrases.

1. Intended Use

The **eazyplex®** **SuperBug mcr-1** test system (Cat. No. 7604) is a qualitative in vitro diagnostic medical device for the detection of the mcr-1 gene, which confers a resistance in gram negative bacteria to Colistin (Polymyxin B).

The test can be performed at any time by qualified professional staff in a medical laboratory. The intended use includes:

- diagnosis (confirmatory assay to verify results of previous testing) and aid to diagnosis (providing additional information to assist in the determination or verification of a patient´s clinical status, test is not the sole determinant) of all kind of patients via testing bacterial colonies.

2. Colistin and mcr-1

Due to the increase of carbapenem resistant gram-negative bacteria (caused by the spread of plasmid coded carbapenemases), Colistin, which was discovered in the early 1950s, has gained more importance in treatment of infections. This last-resort antibiotic was not used in human medicine for decades because of its serious side-effects.
Resistances against Colistin were described soon after it was reintroduced. These were inducible and caused by point mutations in several genes (e.g. mgrB, phoPQ or crrB) so they could not spread by horizontal transfer.
By the end of 2015, Liu et al. discovered an *E. coli* isolate that possessed a gene called “mcr-1” localized on a plasmid. This gene encodes a phosphoethanolamine-transferase and enables the carrier resistance against Colistin.
Soon, further mcr-1 positive bacteria were found worldwide, especially in veterinary medical isolates and in food, but also in human isolates or in environmental samples.

In addition, and the cause of great concern, is the fact that carbapenem-resistant isolates carrying mcr-1 were found, making a successful therapy nearly impossible. mcr-1 has also been detected in species other than *E. coli,* such as *Klebsiella pneumoniae* and *Enterobacter* spec.

3. Principle of the test

A single **eazyplex®** test strip contains six oligonucleotide primers in each filled cap and these provide the means for simultaneous, specific amplification of different genes in a single isothermal amplification reaction. In the presence of relevant DNA sequences, specific amplification products are generated and visualised by real-time fluorescence measurement of a fluorescence dye bound to double-stranded DNA. Thus, positive signals indicate the presence of the mcr-1 gene in the sample to be investigated and a clear genotype determination of a (potential) resistance to Colistin is possible.
Data interpretation is based on an algorithm in the eazyReport™ software.

The following protective mechanisms prevent the use of false results:
Performance of “inhibition control” with each sample prevents the use of false negative test results due to inhibition of the amplification reaction and simultaneously serves as reagent control.
As required, a test strip can be processed as negative / contamination control by testing **RALF** without addition of sample material. In this case, only the inhibition control is allowed to create a positive signal.

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	mcr-1	mcr-1	**red**
2	Inhibition control	Inhibition control	**orange**
3-8	--	--	--

4. Reagents

4.1 Content

The reagents contained in one kit are sufficient for 24 determinations.
Each kit contains:

TESTSTRIP	Test strips with 2 filled tubes, each containing lyophilized, ready-to-use mix for isothermal amplification. The mix contains DNA-polymerase, buffer components, Mg_2SO_4, dNTPs, oligonucleotide primers and a fluorescence dye.	24 strips
RALF	2ml –tubes with 500 µl „RALF - **R**esuspension **a**nd **L**ysis **F**luid“ each (ready for use)	24 x 500 µl

4.2 Additional accessories required

- GENIE® II Mk 2 with eazyReport™ - software version 2.34 or higher (including Instructions for Use in PDF format)
- Inoculation needle
- Heating block for 2 ml tubes
- Pipettes with sterile disposable filter tips
- optional: USB bar code scanner
- optional: printer DYMO® Labelwriter 450 (Dymo) with labels 54x101mm

5. Warnings and precautions

- All reagents and materials which come into contact with potentially infectious samples must be treated with suitable disinfectant or autoclaved.

- Suitable disposable gloves must be worn during the entire test.

- **Never** open a test strip after use! Autoclave used test strips!

6. Handling notes – preparation of assay realization

The components of **eazyplex® SuperBug mcr-1** have to be stored from 15°C to 30°C.
The kits have an expiry date. Quality cannot be guaranteed after this date.
Before beginning the test, remove a test strip from the bag. The test strip may only be used if the white pellets in the filled tubes are visible.

As with any test procedure, good laboratory practice is essential to the proper performance of this assay.

7. Sample material

The sample material has to be:

-bacterial colonies from agar plates, selective media recommended

Any swabs, blood or stool samples **CANNOT** be used.

8. Test procedure

8.1 Preparation of the system

- Turn on GENIE® II Mk2
- Touch the Screen
- Enter user name and password
- Select „Run“
- Scan test barcode via barcode scanner or enter test barcode manually
- Check display if the right test profile is selected
- Enter patient´s / sample ID (via barcode scanner or keyboard)
- Confirm the selection with “Enter”

8.2 Preparing the amplification reaction

Suspend a small part of a single bacterial colony in 500 µl **RALF** by an inoculation needle. As soon as a little amount of cell material is visible on the inoculation needle, it is sufficient sample material for the test.

☝ **Caution! Too much cell material may considerably reduce the effectiveness of the reaction and lead to invalid test runs.**

Incubate this **RALF** -suspension at 99°C for 2 minutes for cell lysis.
If you wish further microbiological testing, transfer 50 µl of the cell suspension in a sterile tube **before** cell lysis.

Carefully remove the protective foil from the test strip.

Pipette 25 µl of the **RALF**-suspension onto the ready-to-use mix in each filled tube of the test strip, taking care not to allow the pipette tip to make contact with the pellet. Do not vortex, shake heavily or pipette up and down. Remove any air bubbles by tapping the test strip gently.

Once the pellets have dissolved, place the test strip immediately into the GENIE® II Mk2 device and start the run (8.3).

8.3 Realization of the amplification reaction

- Select „Start“
- Select block A or B
- Place the test strip into the selected block
- Close the lid
- Start test run by selecting „Yes“.
- If the second block is not in use, a second test run can be initiated by the button “start test run” (see 8.1)
- Note: the directory path and number of test run data file can be seen on the display
- Once the run is complete, open the lid and remove the test strip, taking care as the block could still be hot!
- **Never** open a test strip after use! Danger of contamination!

9. Evaluation

The test run can be monitored in real time mode (choose „Amplification“). Positive results are indicated by a strong rise in fluorescence signal (in the form of a typical amplification curve). Unambiguous assignment of the curves to the test parameters takes place by coloring.

After completion of the test run select „Results“:

Valid test run:
Positive results are colored red.

Invalid test run:
The result of „Inhibition Control“ is colored red (invalid). In this case, data interpretation of the eazyReport™ software is displayed above the result table as follows:
„Invalid control” (colored red)

Warning messages:
„WARNING! Kit expired!“:
A kit has been used which is out of date (see 6.).

„WARNING! Too much sample material!“:
Too much sample material was used; this could have been the reason for an invalid test run (see 11.)

Documentation:

- Result printout via Dymo® Labelwriter 450 printer: select „print“

or

- create a PDF result report: select „PDF“; the generated PDF file is stored in the folder „Report“ on the device and can be exported via USB stick (according to Instructions for Use Genie® II Mk2).

or

- create a result file in CSV format: select „CSV“; the generated CSV file is stored in the folder „Report“ on the device.

The stored run file can be reviewed at any time:

- select symbol „Folder“
- select „LOG“ and confirm with „✓ “
- the data files are numbered consecutively and archived according to creation date

10. Interpretation of the test results

The **eazyplex® SuperBug mcr-1** system is a rapid, universally applicable confirmatory test for the specific detection of the mcr-1 gene in bacterial isolates.

Positive results in the **eazyplex® SuperBug mcr-1** system demonstrate the presence of the resistance gene in an isolate. This does not mean that the genes are also actually expressed. The pathogen, however, possesses the potential to express the gene. No conclusions on, for example, the minimal inhibiting concentration of Colistin can be made based on the results of this test system.

The **eazyplex® SuperBug mcr-1** system is neither intended to diagnose an infection with Colistin resistant bacteria (it cannot be distinguished between colonisation and infection) nor to guide or monitor medical treatment.
The test solely generates a test result. The attending doctor is responsible for achieving a diagnosis and a decision about the treatment of the patient and / or appropriate hygienic measures.

11. Troubleshooting

All signals negative (incl. inhibition control):

- Too much cell material was used for amplification ➔ suspend just a small part of one colony in 500 µl RALF or dilute already suspended cell material with RALF.
- If assay with diluted sample is invalid again ➔ perform assay with RALF without sample.
- If inhibition control is negative again ➔ device may be damaged, please contact our support team.

12. Performance data

A) As part of a retrospective evaluation study at the Institut für Medizinische Mikrobiologie der Justus Liebig Universität Gießen (Gießen, Germany: Prof. Dr. Trinad Chakraborty), 30 different isolates (*Enterobacteriaceae* and *Acinetobacter baumannii*) were tested in comparison to mcr-1 specific PCR according to Liu et al. with the following results:

	PCR according to Liu et al.			
	positive	negative		
eazyplex® mcr-1 positive	8	0	analyt. sensitivity	100,0%
eazyplex® mcr-1 negative	0	22	analyt. specificity	100,0%
inhibited	0	0	inhibition rate	0,0%

B) As part of a retrospective evaluation study at the Antibiotic Resistance Monitoring and Reference Laboratory (Health Protection Agency Centre for Infections, London, United Kingdom: Prof. Dr. Neil Woodford), 58 different phenotypically (MIC >2 µg/ml) Colistin-resistant isolates (*Enterobacteriaceae*, *Pseudomonas* spec. and *Acinetobacter* spec.) were tested in comparison to mcr-1 specific PCR according to Liu et al. with the following results:

	PCR according to Liu et al.			
	positive	negative		
eazyplex® mcr-1 positive	11	0	analyt. sensitivity	100,0%
eazyplex® mcr-1 negative	0	47	analyt. specificity	100,0%
inhibited	0	0	inhibition rate	0,0%

C) As part of a retrospective evaluation study at the CHU Dinant Godinne (Yvoir, Belgium: Prof. Youri Glupczynski and Dr. Pierre Bogaerts), 129 different phenotypically (MIC >4 µg/ml) Colistin-resistant isolates (*Enterobacteriaceae*, *Pseudomonas* spec. and *Acinetobacter* spec.) were tested in comparison to mcr-1 specific PCR according to Liu et al. with the following results:

	PCR according to Liu et al.			
	positive	negative		
eazyplex® mcr-1 positive	2	0	analyt. sensitivity	100,0%
eazyplex® mcr-1 negative	0	127	analyt. specificity	100,0%
inhibited	0	0	inhibition rate	0,0%

13. References

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