Instructions for use

eazyplex® SuperBug Acineto

Molecular biological rapid test for the detection of carbapenemases producing bacteria

for use with Genie® II Mk2 devices

in vitro diagnostic medical device

CE

eazyplex® SuperBug Acineto		REF: 7607
language: english	valid from:	May, 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:
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1. Intended Use

eazyplex® SuperBug Acineto (Cat. No. 7607) is a qualitative in vitro diagnostic medical device for the detection of *Acinetobacter baumannii*. In addition, **eazyplex® SuperBug Acineto** detects all described variants of carbapenemases of the **OXA-23, OXA-40, OXA-58** groups, as well as **NDM**.

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. Carbapenemases in *Acinetobacter* spec.

The increase in antibiotic resistance among gram-negative bacteria is a notable example of how bacteria can procure, maintain, and express new genetic information that can confer resistance to one or several antibiotics. Reports of resistance vary, but a general consensus appears to prevail that quinolone and broad-spectrum ß-lactam resistance is increasing in members of the family *Enterobacteriaceae* and *Acinetobacter* spp. and that treatment regime for the eradication of *Pseudomonas aeruginosa* infections are becoming increasingly limited. While the advent of carbapenems in the 1980s heralded a new treatment option for serious bacterialinfections, carbapenem resistance can now be observed in *Enterobacteriaceae* and *Acinetobacter* spp. and is becoming commonplace in *P. aeruginosa*. The common form of resistance is either through lack of drug penetration (i.e., outer membrane protein (OMP) mutations and efflux pumps), hyperproduction of an AmpC-type ß-lactamase, and/or carbapenem-hydrolyzing ß-lactamases.
Two types of carbapenem-hydrolyzing enzymes have been described: serine enzymes possessing a serine moiety at the active site, and metallo-ß-lactamases (MBLs), requiring divalent cations, usually zinc, as metal cofactors for enzyme activity. The serine carbapenemases are invariably derivatives of class A or class D enzymes and usually mediate carbapenem resistance in *Enterobacteriaceae* or *Acinetobacter* spp. Despite the avidity of these enzymes for carbapenems, they do not always mediate high-level resistance and not all are inhibited by clavulanic acid.
MBLs, like all ß-lactamases, can be divided into those that are normally chromosomally mediated and those that are encoded by transferable genes.

NDM-1 was first identified in December 2009 and was named after New Delhi, as it was first described by Yong et al. in a Swedish national who fell ill with an antibiotic-resistant bacterial infection that he acquired in India. The infection was unsuccessfully treated in a New Delhi hospital and after the patient's repatriation to Sweden; a carbapenem-resistant *Klebsiella pneumoniae* strain bearing the novel gene was identified. It was later detected in bacteria in India, Pakistan, the United Kingdom, the United States, Canada, Japan and several European countries. The most common bacteria that make this enzyme are

Escherichia coli , *Klebsiella pneumoniae* and *Acinetobacter baumannii*, but the gene for NDM can spread from one strain of bacteria to another by horizontal gene transfer and was found *in Morganella morganii*, *Providencia* spp.and *Citrobacter freundii*, too.

OXA-type carbapenemases in *Acinetobacter*:
The carbapenem-hydrolysing OXA enzymes can be subclassified into eight distinct branches or subgroups. These eight groups are only remotely related to class D oxacillinases that do not possess carbapenem-hydrolysing properties. Four of the eight clusters have been identified in *A. baumannii*, and the isolates were found in Europe, South America, Asia and French Polynesia. The first cluster is formed by OXA-23 also named ARI-1 (an acronym of *Acinetobacter* resistant to imipenem), together with OXA-27 and OXA-49.
The second family encompasses the OXA-24, -25, -26, -40 and -72 ß-lactamases. The third group consists of the OXA-51 family enzymes, while OXA-58 and OXA-92 represents the fourth group.

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 one of the corresponding genes in the sample to be investigated. Therefore, a clear genotypic determination of present resistances is possible in addition to species identification for A. baumannii.
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	Acinetobacter baumannii	*Acinetobacter baumannii* OXA-51	**red**
2	Inhibition control	Inhibition control	**orange**
3	OXA-23	e.g. OXA-23,-27,-49,-73	**yellow**
4	OXA-40	e.g. OXA-24,-40,-25,-26,-72	**light green**
5	OXA-58	e.g OXA-58,-96,-97	**dark green**
6-8	NDM	NDM-1 to -9, -16	**turquoise**
7-8	-	-	

4. Reagents

4.1 Content

The reagents contained in one kit are sufficient for 12 determinations.
Each kit contains:

TESTSTRIP	Test strips with 6 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.	12 strips
RALF	2ml –tubes with 500 µl „RALF - **R**esuspension **a**nd **L**ysis **F**luid“ each (ready for use)	12 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 Acineto** 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 colony from agar plate, selective media recommended

Any swabs, liquid culture, 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; then select test profile which matches the respective sample material.
- 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

eazyplex® SuperBug Acineto is a rapid confirmatory test for the specific detection of *Acinetobacter baumanii* and four different *g*enes encoding carbapenemases from bacterial isolates.

Positive results of **eazyplex® SuperBug Acineto** demonstrate the presence of the species *Acinetobacter baumanii* in a sample and of the resistance genes, respectively. This does not mean that the resistance genes are also actually expressed.
The pathogens found in the tested sample, however, possess the potential to express the genes. In addition, no conclusions on, for example, the minimal inhibiting concentration of certain β-lactam antibiotics, and in special carbapenems, for the pathogen can be made based on the results of this test system.

The **eazyplex® SuperBug Acineto** system is neither intended to diagnose an infection with carbapenem 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 decision about diagnosis or treatment of a patient or taking hygienic measures.

11. Troubleshooting

All signals negative (incl. inhibition control):

- Reaction is inhibited due to inhibitory substances in the sample and must not be interpreted (invalid test).

- In case of testing bacterial colonies: 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 this assay is valid, further dilution of the sample is necessary.
- If inhibition control is negative again ➔ device may be damaged, please contact our support team.

All signals positive (incl. inhibition control):

Very occasionally cell lysis in a sample will not complete within the first 3 minutes of the test run (signals are not displayed at the beginning of the run). If cell lysis occurs later on during the amplification reaction, newly appearing dsDNA will lead to unspecific amplification signals. This phenomenon can be identified easily because it will happen simultaneously in all wells (but the IC curve or correct positive amplification curves could appear earlier). ➔It is recommended to repeat the test run with fresh sample material with an extended incubation time of 10 minutes for complete cell lysis.
➔If the phenomenon reoccurs, the sample should be interpreted as "invalid“ or "not valid".

12. Performance data

As part of an evaluation study in the german national reference center for gram negative nosocomial pathogens (Ruhr-Universität Bochum: Prof. Gatermann; Dr. Pfennigwerth), 71 different genotypically characterised carbapenem resistant isolates of diverse *Acinetobacter* species (14 strains) and some *Enterobacteriaceae* and Pseudomonades (14 strains) were tested using the **eazyplex® SuperBug Acineto** with the following results:

Acinetobacter baumannii	**Reference method positive**	**Reference method negative**	Σ	
eazyplex® positive	50	0	50	**sensitivity: 100 %**
eazyplex® negative	0	21	21	**specificity: 100%**
Σ	50	21	**71**	

The following Acinetobacter species, which are not covered by test, have been included in the study panel:
Acinetobacter pittii (6x); *Acinetobacter ursingii* (1x)

OXA-23 group	**Reference method positive**	**Reference method negative**	Σ	
eazyplex® positive	12	0	12	**sensitivity: 100 %**
eazyplex® negative	0	59	59	**specificity: 100%**
Σ	12	59	**71**	

OXA-40 group	**Reference method positive**	**Reference method negative**	Σ	
eazyplex® positive	12	0	12	**sensitivity: 100 %**
eazyplex® negative	0	59	59	**specificity: 100%**
Σ	12	59	**71**	

OXA-58 group	**Reference method positive**	**Reference method negative**	Σ	
eazyplex® positive	10	0	10	**sensitivity: 100 %**
eazyplex® negative	0	61	61	**specificity: 100 %**
Σ	10	61	**71**	

NDM	Reference method positive	Reference method negative	Σ	
eazyplex® positive	15	0	15	**sensitivity: 100 %**
eazyplex® negative	0	56	56	**specificity: 100%**
Σ	15	56	**71**	

The following carbapeneme resistance genes, which are not covered by test, have been included in the study panel:
VIM (3x); KPC (2x); OXA-48/181-Gruppe (5x); IMP (4x); GIM (6x); GES (4x) und IMI (2x).

13. References

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