

Instructions for use

eazyplex[®] SuperBug complete C

**Molecular biological rapid test for the detection of
carbapenemases producing bacteria**

for use with Genie[®] II Mk2 devices

For in vitro diagnostic use



eazyplex [®] SuperBug complete C	REF: 7608
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: -

1. Intended Use

The **eazyplex[®] SuperBug complete C** test system (Cat. No. 7608) is a qualitative in vitro diagnostic medical device for the detection of bacteria that are capable, from a genetic stand point, of producing certain carbapenemases.

eazyplex[®] SuperBug complete C detects all described variants of metallo- β -lactamases of the **VIM-**, **NDM-**, **KPC-** and **IMP-**type, as well the **OXA-48** family. In addition, carbapenemases genes **OXA-181/-232** can be detected.

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

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 β -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 bacterial infections, 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 β -lactamase, and/or carbapenem-hydrolyzing β -lactamases. Two types of carbapenem-hydrolyzing enzymes have been described: serine enzymes possessing a serine moiety at the active site, and metallo- β -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 β -lactamases, can be divided into those that are normally chromosomally mediated and those that are encoded by transferable genes. However, in the past 5 years many new transferable types of MBLs have been studied and appear to have rapidly spread. In some countries, *P. aeruginosa* possessing MBLs constitute nearly 20 % of all nosocomial isolates, whereas in other countries the number is still comparatively small. In recent years MBL genes have spread from *P. aeruginosa* to *Enterobacteriaceae*, and a clinical scenario appears to be developing that could simulate the global spread of extended-spectrum β -lactamases. Moreover, given that MBLs will hydrolyze virtually all

classes of β -lactams and that we are several years away from the implementation of a therapeutic inhibitor, their continued spread would be a clinical catastrophe.

Metallo- β -lactamases (MBLs):

All MBLs hydrolyze imipenem, but their ability to achieve this varies considerably and the rate of hydrolysis may or may not correlate with the bacterium's level of resistance to carbapenems. However, these enzymes possess the characteristic hallmark of being universally inhibited by EDTA as well as other chelating agents of divalent cations, a quintessential feature of MBLs that correlates with their mechanistic function.

Acquired metallo- β -lactamases (MBLs) are emerging resistance determinants in *Pseudomonas aeruginosa* and other gram-negative pathogens. These enzymes can hydrolyze most β -lactams, including carbapenems, and can confer a broad-spectrum β -lactam resistance phenotype to the bacterial host, which is not reversible by conventional β -lactamase inhibitors. MBLs are commonly encoded by genes carried on mobile elements (integron-borne gene cassettes) that can spread horizontally among different replicons and strains.

Originally thought to be uncommon and restricted to some geographical areas, acquired MBLs are presently known to be widespread, and at least four different types of these enzymes, IMP, VIM, SPM, and GIM, have been identified, with the **VIM**- and **IMP**-types being prevalent.

The VIM-type MBLs were originally detected in Europe, where these enzymes apparently prevail over IMP-type enzymes. Subsequently, VIM-type enzymes were also reported in Asia and America. MBLs of the VIM type are more common among non-fermenting gram-negative bacteria. However, during the last few years, studies have reported on the dissemination of VIM-type MBLs in members of the family *Enterobacteriaceae*, suggesting the ongoing spread of these resistance determinants among pathogens with higher infectivities.

NDM-1 was first identified in 2008 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* and *Klebsiella pneumoniae*, but the gene for NDM-1 can spread from one strain of bacteria to another by horizontal gene transfer and was found in *Morganella morganii*, *Providencia* spp., *Citrobacter freundii* and *Acinetobacter baumannii*, too.

***Klebsiella pneumoniae* carbapenemase (KPC):**

The initial report of a KPC β -lactamase which is capable of hydrolysing carbapenems, penicillins, cephalosporins and aztreonam, was from a carbapenem-resistant *K. pneumoniae* strain isolated in USA, and subsequently KPC producers have also been detected in other regions all over the world. There are currently nine recognized variants, with KPC-2 and KPC-3 being more commonly isolated.

Recently, isolates producing KPC-2 /-3 enzyme have been detected in European countries such as Greece, the UK, France and Germany. In addition, the spread of KPC producers may be underestimated, as the detection of KPC- and also MBL-producing *K. pneumoniae* may be unsuccessful because these enzymes do not always confer obvious carbapenem resistance.

OXA-48-group carbapenemase:

The OXA-48-carbapenemase was first described in 2004 in a *K. pneumoniae*-isolate from Turkey. Recently, isolates producing such carbapenemase were detected in various countries like Belgium, Israel, India, UK and Greece. In Germany it was found the first time 2008 in the south-western part of the country. Like in KPC-producing bacteria, the phenotypic detection of this resistance type seems to be difficult and unreliable. In the meanwhile a lot of variants of OXA-48-like carbapenemases are described with OXA-181 as an often isolated type.

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. 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	KPC	KPC-2 to -15	red
2	NDM	NDM-1 to -9,-16	orange
3	OXA-48	e.g. OXA-48, -162, -204, -244	yellow
4	VIM	VIM-1 to -37 (without -7)	light green
5	OXA-181	OXA-181,-232	dark green
6	IMP	IMP-1 to -7 ; -12, -13, -22	turquoise
7	Inhibition control	Inhibition control	purple
8	--	--	

4. Reagents

4.1 Content

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

Each kit contains:

TESTSTRIP	Test strips with 7 filled tubes, each containing lyophilized, ready-to-use mix for isothermal amplification. The mix contains DNA-polymerase, buffer components, Mg ₂ SO ₄ , dNTPs, oligonucleotide primers and a fluorescence dye.	24 strips
RALF	2ml . tubes with 500 µl sRALF - Resuspension and Lysis Fluid%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 complete C** 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

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 sRun%
- 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 **sStart%**
- Select block A or B
- Place the test strip into the selected block
- Close the lid
- Start test run by selecting **sYes%**
- 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 **sAmplification%**). 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 **bResults :**

Valid test run:

Positive results are colored red.

Invalid test run:

The result of **sInhibition Control%** is colored red (invalid). In this case, data interpretation of the eazyReport™ software is displayed above the result table as follows:
sInvalid control+(colored red)

Warning messages:

sWARNING! Kit expired!%

A kit has been used which is out of date (see 6.).

sWARNING! 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 **sprint%**
- or
- create a PDF result report: select **sPDF%**, the generated PDF file is stored in the folder **sReport%** 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 **sCSV%**, the generated CSV file is stored in the folder **sReport%** on the device.

The stored run file can be reviewed at any time:

- select symbol **sFolder%**
- select **sLOG%** and confirm with **s✓ %**
- the data files are numbered consecutively and archived according to creation date

10. Interpretation of the test results

The **eazyplex[®] SuperBug complete C** system is a rapid confirmatory test for the detection of seven different genes encoding carbapenemases directly from bacterial colonies.

Positive results of the **eazyplex[®] SuperBug complete C** system demonstrate the presence of resistance genes in a sample. This does not mean that the 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 complete C** 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

A) As part of an evaluation study at the German national reference center for Gram-negative nosocomial pathogens (Ruhr-Universität Bochum: Prof. Gatermann; Dr. Pfennigwerth), 32 different carbapenem-resistant and genotypically characterized isolates were tested with **eazyplex® SuperBug complete C** with the following results:

		Result of reference method						
		KPC	NDM	OXA-48	VIM	OXA-181	IMP	other* resistance genes
eazyplex® positive	KPC	4	0	0	0	0	0	0
	NDM	0	3	0	0	0	0	0
	OXA-48	0	0	3	0	0	0	0
	VIM	0	0	0	3	0	0	0
	OXA-181	0	0	0	0	2	0	0
	IMP	0	0	0	0	0	4	0

*The following carbapenem resistance genes, which are not covered by test, have been included in the study panel:

OXA-23 (2x); OXA-40 (2x); OXA-58 (2x); GIM (2x); GES (3x) und IMI (2x).

B) As part of a retrospective evaluation study (Prof. Dr. Neil Woodford, 2014) at the Antibiotic Resistance Monitoring and Reference Laboratory (Health Protection Agency Centre for Infections, London, United Kingdom), 450 different isolates of *Enterobacteriaceae* were tested in comparison to in-house PCR with the following results*:

Carbapenemase	n	eazyplex® SuperBug complete C Sensitivity
KPC	100	100 %
NDM	100	100 %
VIM	100	100 %
OXA-48 (exklusive OXA-181)	83	100 %
OXA-181	17	100 %
NDM + OXA-48 (exklusive OXA-181)	1	100 %
NDM + OXA-181	1	100 %
	n	Specificity
Carbapenemase negative (but Carbapenem-resistant)	24	100 %

*The study has been conducted with eazyplex® SuperBug complete B and is also valid for eazyplex® SuperBug complete C, due to the identical composition of the concerned parameters.

C) The following IMP variants have been detected in an internal evaluation study using eazyplex[®] SuperBug complete C:
 IMP-1, -2, -4, -5, -7, -12, -13 und -22.
 IMP-31 was not detected.

13. References

1. Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. Clin Microbiol Infect 2002;8:321. 331.
2. Cornaglia G, Mazzariol A, Lauretti L, Rossolini GM, Fontana R. Hospital outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-1, a novel transferable metallo- β -lactamase. Clin Infect Dis 2000;31:1119. 1125.
3. Rossolini GM, Riccio ML, Cornaglia G, et al. Carbapenem resistant *Pseudomonas aeruginosa* with acquired blaVIM metallo- β -lactamase determinants, Italy. Emerg Infect Dis 2000;6:312. 313.
4. Lauretti L, Riccio ML, Mazzariol A, et al. Cloning and characterization of blaVIM, a new integron-borne metallo- β -lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob Agents Chemother 1999;43:1584. 1590.
5. Fiett J, Baraniak A, Mrowka A, et al. Molecular epidemiology of acquired-metallo- β -lactamase-producing bacteria in Poland. Antimicrob Agents Chemother 2006;50:880. 886.
6. Pournaras S, Ikonomidis A, Tzouvelekis LS, et al. VIM-12, a novel plasmid-mediated metallo- β -lactamase from *Klebsiella pneumoniae* that resembles a VIM-1/VIM-2 hybrid. Antimicrob Agents Chemother 2005;49:5153. 5156.
7. Cardoso O, Leitao R, Figueiredo A, Sousa JC, Duarte A, Peixe LV. Metallo- β -lactamase VIM-2 in clinical isolates of *Pseudomonas aeruginosa* from Portugal. Microb Drug Resist 2002;8:93. 97.
8. Ikonomidis A, Tokatlidou D, Kristo I, et al. Outbreaks in distinct regions due to a single *Klebsiella pneumoniae* clone carrying a blaVIM-1 metallo- β -lactamase gene. J Clin Microbiol 2005;43:5344. 5347.
9. Henrichfreise B, Wiegand I, Sherwood KJ, Wiedemann B. Detection of VIM-2 metallo- β -lactamase in *Pseudomonas aeruginosa* from Germany. Antimicrob Agents Chemother 2005;49:1668. 1669.
10. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo- β -lactamases: the quiet before the storm? Clin Microbiol Rev 2005;18:306. 325.
11. Rossolini GM. Acquired metallo- β -lactamases: an increasing clinical threat. Clin Infect Dis 2005;41:1557. 1558.
12. Giakkoupi P, Tzouvelekis LS, Daikos GL, et al. Discrepancies and interpretation problems in susceptibility testing of VIM-1-producing *Klebsiella pneumoniae* isolates. J Clin Microbiol 2005;43:494. 496.
13. Luzzaro F, Docquier JD, Colinon C, et al. Emergence in *Klebsiella pneumoniae* and *Enterobacter cloacae* clinical isolates of the VIM-4 metallo- β -lactamase encoded by a conjugative plasmid. Antimicrob Agents Chemother 2004;48:648. 650.
14. Crespo MP, Woodford N, Sinclair A, et al. Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-8, a novel metallo- β -lactamase, in a tertiary care center in Cali, Colombia. J Clin Microbiol 2004;42:5094. 5101.
15. Li J, Nation RL, Turnidge JD, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis 2006;6:589. 601.
16. Thiolas A, Bollet C, La Scola B, Raoult D, Pages JM. Successive emergence of *Enterobacter aerogenes* strains resistant to imipenem and colistin in a patient. Antimicrob Agents Chemother 2005;49:1354. 1358.

17. Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*. 2004 Jan;48(1):15-22.
18. Carrër A, Poirel L, Yilmaz M, Akan OA, Feriha C, Cuzon G, Matar G, Honderlick P, Nordmann P. Spread of OXA-48-encoding plasmid in Turkey and beyond. *Antimicrob Agents Chemother*. 2010 Mar;54(3): 1369-73.
19. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother*. 2009 Dec;53(12):5046-54.
20. Queenan AM and Bush K. Carbapenemases: the versatile β -Lactamases. *Clin Microb Rev* 2007 Vol. 20: 3, 440. 458
21. Hanlon, G. W. 2005. The emergence of multidrug resistant *Acinetobacter* species: a major concern in the hospital setting. *Lett. Appl. Microbiol.*41:375. 378.
22. Vergara A, Zboromyrska Y, Mosqueda N, Morosini MI, García-Fernández S, Roca I, Cantón R, Marco F, Vila J. Evaluation of a Loop-mediated isothermal amplification-based methodology to detect carbapenemase carriage in *Acinetobacter* Clinical isolates. *Antimicrob Agents Chemother*. Dec. 2014, Vol. 58 no. 12, pp. 7538-7540.
23. Findlay J, Hopkins KL, Meunier D, Woodford N.: Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. *J Antimicrob Chemother*. 2015 May;70(5):1338-42.

The purchase price of this product includes a non-transferable license under European Patents EP1020534, EP1873260, EP2045337, EP2287338, EP1275715, EP2253717, EP1327679, EP2031058 or their foreign counterparts, owned by Eiken Chemical Co., Ltd.



AmplexDiagnostics GmbH
Werkstraße 2
83555 Gars-Bahnhof
Germany

Support:
Tel.: +49 (0) 8073 / 9 16 93-50
Fax: +49 (0) 8073 / 9 16 93-55
support@eazyplex.com