Instructions for use

eazyplex® *C. difficile*

Molecular biological rapid test for direct detection of toxigenic *Clostridium difficile*

for use with Genie® II Mk2 devices

For in vitro diagnostic use

CE

eazyplex® *C. difficile* complete		REF: 7635
eazyplex® *C. difficile* classic		REF: 7636
eazyplex® *C. difficile* basic		REF: 7637
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, eazyplex *C. difficile* basic duo (REF 7638) removed, page numbers inserted, centrifuge added (4.2), 4000 rpm replaced by 1000 x g (4.2), 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., 11. Troubleshooting amended, Toxin A and B interchanged under 12B), general improvement of phrases.

1. Intended Use

The **eazyplex®** ***C. difficile*** test system is a qualitative in vitro diagnostic medical device for the detection of toxigenic *Clostridium difficile* in stool samples or bacterial colonies.

eazyplex® ***C. difficile*** **complete (Cat. No. 7635)** determines toxin A, toxin B, binary toxin and GDH.

The following variants are available:
eazyplex® ***C. difficile*** **classic (Cat. No. 7636)** determines toxin B and GDH

eazyplex® ***C. difficile*** **basic (Cat. No. 7637)** determines toxin B

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

- screening of patients suspected of having *C. difficile* associated diarrhea (CDAD) via stool samples
- 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 stool samples or bacterial colonies.

2. Clostridium difficile

The toxin-producing *Clostridium difficile* is one of the most important pathogens of antibiotic-associated diarrhoea and colitis and the trigger of practically all cases of pseudomembranous colitis. Although approximately 2% of all healthy adults are colonised with *C. difficile*, many patients acquire this organism via nosocomial infections. Most antibiotics are considered to be responsible for promoting the proliferation of the toxin-producing *C.difficile* by destroying the natural intestinal flora. Two toxins, toxin A and toxin B, are associated with the disease triggered by *C. difficile*. These toxins differ from each other immunochemically and biologically. The antiserum against pure toxin A shows no cross reaction with toxin B, and the antiserum against pure toxin B shows no cross reaction with toxin A.
Toxin A is described as an enterotoxin which triggers an increase in intestinal permeability with a subsequent accumulation of intestinal fluid and diarrhoea. Toxin B is a powerful cytotoxin which causes a rounding of cells in cell cultures. Toxin B is lethal to hamsters if administered either singly intravenously or intragastrically in combination with sublethal doses of toxin A. The role of toxin B in the development of the intestinal disease is unclear. However, the hypothesis has been advanced that both proteins act *in vivo* synergistically.
Some strains additionally express the binary toxin (encoded by the genes cdtA/B) which damages human cells by repression of actin polymerization.

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. 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 **LPTV-RS** without addition of sample material. In this case, only the inhibition control is allowed to create a positive signal.

eazyplex® *C. difficile* complete:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Inhibition control	Inhibition control	**red**
2	GDH	gluD	**orange**
3	toxin A	tcdA	**yellow**
4	toxin B	tcdB	**light green**
5	binary toxin	cdtA	**dark green**
6-8	-	-	

eazyplex® *C. difficile* classic:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Inhibition control	Inhibition control	**red**
2	GDH	gluD	**orange**
3	toxin B	tcdB	**yellow**
4-8	-	-	

eazyplex® *C. difficile* basic:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Inhibition control	Inhibition control	**red**
2	toxin B	tcdB	**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 5* 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
LPTV	2 ml –tube with 500 µl „LPTV“	24 x 500 µl
RS	reddish 2 ml –tubes with 125 µl „RS - Resuspension Solution”	24 x 125 µl

*in the variants: 3 (eazyplex® *C.difficile* classic) or 2 (eazyplex® *C.difficile* basic).

4.2 Additional accessories required

- GENIE® II Mk 2 with eazyReport™ - software version 2.34 or higher (including Instructions for Use in PDF format)
- Heating block for 2 ml tubes
- If necessary: Centrifuge for 2 ml tubes
- Inoculation needle
- 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**® ***C. difficile*** 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 stool. Optimally, the sample is not older than 12 hours.

Alternatively, a bacterial colony from agar plate can be used.

Any swabs, liquid culture or blood 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

Stool samples:
Transfer 10 µg of stool into the reaction tube which contains 500 µl LPTV and mix.

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

Incubate this **LPTV**-suspension at 99°C for 3 minutes for cell lysis.
If the suspension is strongly turbid or contains large (stool) particles, LPTV-suspension should be centrifuged for 1 min at 1000 x g.

Afterwards, transfer 125 µl of the lysed LPTV-suspension (or rather supernatant) into 125 µl RS (reddish 2 ml "SafeLock" tube) and mix (please do not vortex).

Carefully remove the protective foil from the test strip.
Pipette 25 µl of the **LPTV-RS**-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).

Bacterial colony:
Suspend a small part of a single bacterial colony in 500 µl **LPTV** 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 **LPTV** -suspension at 99°C for 3 minutes for cell lysis.
If you wish further microbiological testing, transfer 50 µl of the cell suspension in a sterile tube **before** cell lysis.

Afterwards, transfer 125 µl of the lysed **LPTV**-suspension into 125 µl **RS** (reddish 2 ml "SafeLock" tube) and mix (please do not vortex).

Carefully remove the protective foil from the test strip.
Pipette 25 µl of the **LPTV-RS**-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®** ***C. difficile*** system is a rapid test for the specific detection of toxigenic *Clostridium difficile* directly from stool or bacterial colonies.
Positive results of the **eazyplex®** ***C. difficile*** system demonstrate the presence of specific genes of the infectious agents in a sample.

The **eazyplex®** ***C. difficile*** system is neither intended to diagnose an infection with infectious agents 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.

The **eazyplex®** ***C. difficile*** system is able to detect some few genome copies within 25 minutes under optimal conditions. But it is not possible to detect one single copy of the infectious agents. Therefore, a negative test result can be generated despite a weak colonization of the patient´s intestinal tract.

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 LPTV or dilute already suspended cell material with LPTV.
- If assay with diluted sample is invalid again ➔ perform assay with LPTV without sample.
- If inhibition control is negative again ➔ device may be damaged, please contact our support team.

- In case of testing stool samples: Too much sample material was used for amplification ➔ dilute 50 µl of remaining **LPTV**-suspension in 500 µl LPTV.
- If assay with diluted sample is invalid again ➔ perform assay with LPTV without sample; if this assay with diluted sample is valid, further dilution of the sample is necessary.
- If inhibition control is negative again ➔ device may be damaged, please contact our support team.

12. Performance data

A) As part of a prospective evaluation study at the „Institut für medizinische Mikrobiologie des Universitätsklinikum Jena" (Head of Department: Prof. Dr. Pfister; study coordinator: PD Dr. Rödel), 38 stool samples from patients suspected of having *C.difficile* associated diarrhea were tested in comparison to immunological detection of GDH (miniVIDAS® GDH, Biomerieux) and molecular biological routine methods (Detection of toxin gene tcdB via BD MAX Cdiff, Becton-Dickinson):

	mini VIDAS® GDH:	
GDH	**positive**	**negative**
eazyplex® *C.difficile* positive	15	0
eazyplex® *C.difficile* negative	4*	18
invalid	0	1
total	19	19

* for all four samples with a positive immunological result for GDH, Toxin B-PCR was negative (in accordance with the eazyplex® *C.difficile* results). The miniVIDAS® GDH test previously showed a specificity of 90% in comparison to *C. difficile* culture and a specificity of 89,4% in comparison to GDH PCR (Davies *et al.* 2015).

The following performance data for GDH detection result (in comparison to miniVIDAS® GDH):

Sensitivity:	79,0 %
Specificity:	100 %
PPV:	100 %
NPV:	82,8 %
Inhibition rate:	2,6 %

	BD MAX Cdiff:	
cdtB (toxin B)	**positive**	**negative**
eazyplex® *C.difficile* positive	15	0
eazyplex® *C.difficile* negative	0	22
invalid	0	1
total	15	23

The following performance data for **toxin B** detection result (in comparison to BD MAX Cdiff):

Sensitivity:	100 %
Specificity:	100 %
PPV:	100 %
NPV:	100 %
Inhibition rate:	2,6 %

All 15 consistently toxin B positive samples also showed positive results for **toxin A** in the eazyplex® *C.difficile* complete; 7 out of the 15 toxA/B positive samples showed an additional positive result for the **binary toxin** in the eazyplex® *C.difficile* complete.

B) As part of our internal evaluation study, 29 different reference *C. difficile* strains were tested, which belong to 20 different ribotypes (001, 002, 005, 010, 012, 013, 014, 023, 027, 029, 049, 053, 064, 072, 078, 081, 087, 090, 135, 149):

- GDH was detected in all 29 strains
- Toxin B was detected in all 29 strains
- Toxin A was detected in 28 out of 29 strains; one strain was known to be Toxin A negative.
- Binary toxin was detected in all 6 included highly pathogenic ribotypes 027 as well as in 4 further strains.

13. References

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