User manual

Check-Direct CPE for BD MAX™

Real time PCR kit for the detection of carbapenemase-producing *Enterobacteriaceae*

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EU: CE IVD

U.S.: For Research Use Only
Not for use in diagnostic procedures

Contents

Intended use

Check-Direct CPE for BD MAX is a qualitative *in vitro* diagnostic test for the rapid detection of carbapenemase genes in *Enterobacteriaceae*. The test is intended to be used for bacteria cultured from clinical specimens. Check-Direct CPE detects the presence of the carbapenemase genes KPC, NDM, VIM and OXA-48, presently the primary cause of carbapenemase production in *Enterobacteriaceae*. The assay uses the BD MAX system for extraction of DNA and subsequent real-time PCR employing the reagents provided combined with universal reagents and disposables for the BD MAX system. Check-Direct CPE for BD MAX can be used as an aid to identify, prevent and control carbapenemase-producing *Enterobacteriaceae* that colonize patients in healthcare settings. Check-Direct CPE for BD MAX is not intended to diagnose infections with carbapenemase-producing *Enterobacteriaceae* nor to guide or monitor treatment for these infections. Parallel cultures are necessary to recover organisms for epidemiological typing, susceptibility testing and for further confirmatory identification.

Introduction and principle of the method

The worldwide emergence and dissemination of carbapenem resistance among *Enterobacteriaceae* is a serious threat to public health. These organisms are associated with high mortality rates and have the potential to spread widely. The most common cause of carbapenem resistance in *Enterobacteriaceae* is the expression of carbapenemases, *i.e.* Carbapenemase-Producing *Enterobacteriaceae* or CPE. CPE have elevated or complete resistance to carbapenems and most other β-lactam antibiotics. Presently, the vast majority of CPE are associated with the presence of one of the following plasmid-encoded carbapenemases: KPC (*Klebsiella pneumoniae* carbapenemase), VIM (Verona integron–encoded metallo-β-lactamase), NDM (New Delhi metallo-β-lactamase) or OXA-48 (Oxacillinase-48). Moreover, CPE often have other non–β-lactam resistance determinants resulting in multidrug- and pandrug-resistant isolates.

Check-Direct CPE is a multiplex real-time PCR assay for detection of the KPC, OXA-48, NDM and VIM carbapenemase genes. The assay is based on specific recognition and amplification of target sequences by PCR, and the simultaneous detection of the accumulation of PCR amplification products by fluorescent DNA probes. For KPC, VIM, OXA-48 and NDM many gene variants exist, and Check-Direct CPE has been designed to reliably detect all variants. Check-Direct CPE for BD MAX employs five different fluorescent probes and enables detection and discrimination of the 4 carbapenemase genes and the control target SPC, that monitors DNA extraction and PCR amplification.

Kit contents (for 24 reactions)

Components (Mat. No.)	Description	Storage conditions
CPE reagent tubes (9-0062)	24 sealed tubes (purple seal)	+ 4°C, **store in the dark**
CPE positive control (9-0061)	1 tube (purple cap)	+ 4°C
User Manual (9-0079)	Leaflet – download from website	Not critical

Materials required but not supplied with the kit

Supplies	Equipment
• BD MAX ExK™ DNA-1 Extraction Kit (Ref:442818) • BD MAX DNA MMK Master Mix (Ref: 442848) • BD MAX PCR Cartridges (Ref: 437519) • Disposable laboratory (powder-free) gloves/Lab coat • Pipettes & disposable (filter-) tips for volumes of 10 to 1000 µl • Saline (150 mM NaCl or 0,9% w/v NaCl) • Milli-Q water or aqua bidest	• Real-time PCR instrument: BD MAX System, software version 2.96A • Densitometer suitable for bacterial suspensions • Vortex mixer

Storage, handling and stability

The Check-Direct CPE kit is shipped at ambient temperature and should be stored at + 4°C upon receipt. Please visually inspect the product upon initial opening to ensure that its contents are intact. Do not use this product if the packaging is damaged upon arrival and do not use reagents if their protective pouches are open or broken upon arrival. Do not use reagents if desiccant is not present or broken inside and do not remove desiccant from protective pouches. Store all opened reagents at + 4°C until expiration date. Store in the dark. Close protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing. Please contact the Check-Points office at *support@check-points.com* if you have any further questions.

Good laboratory practices

Recommendations for best results

The quality of the results depends on strict compliance with the following good laboratory practices, especially concerning PCR practices.

- The test must be performed by adequately trained personnel.
- Do not use reagents after their expiration date
- Follow recommendations for storage and handling to preserve the quality of the kit's reagents.
- Protect reagents from light to avoid photo-bleaching of the dyes.
- Periodically, verify the accuracy and precision of pipettes, as well as correct functioning and calibration of the instruments.

Prevention of contaminations

Use separate rooms: a sample preparation room and a PCR room with the BD MAX system.
Never transfer items from the PCR room to the sample preparation room.

To keep laboratory free of PCR product contamination:

- Use pipettes with hydrophobic filter tips.
- Make sure to always use a new pipette tip when adding solutions, test samples, and controls to a reaction tube to avoid contamination.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean disposable gloves and clean lab coats for the different steps of the test.
- Change gloves whenever you suspect that they are contaminated.
- Keep the tubes of all kit components and samples closed as much as possible.
- Clean the lab benches and all equipment regularly with a 0,5% sodium hypochlorite solution.

Please read the full protocol before starting the test

Sample preparation procedures

Test preparation for bacteria from culture

1. Inoculate nutrient agar plates with the clinical samples or the bacterial strains to be tested and incubate overnight at 37°C. Typical growth media include blood agar, MacConkey agar and Tryptic Soy agar.
2. Prepare a bacterial cell suspension in Saline of McFarland 0.5 – 1.0 (≈1 – 2 x 10^8 CFU/ml) from one or more colonies of each plate using a 1 or 10 µl öse.
3. Pipette 10 µL of the bacterial cell suspension (≈1 – 2 x 10^6 CFU/ml) and 500 µl Milli-Q water or aqua bidest into one DNA Sample Buffer Tube SB-1. (supplied by BD with the DNA extraction kit, refer to *Materials required but not supplied with the kit*).
4. Close the Sample Buffer Tube with a septum cap and vortex 10 second at low speed.
5. Transfer the Sample Buffer Tubes with the bacterial cell suspensions to be analyzed to the PCR room.

Preparation of control reactions

To validate the run, perform positive and negative control reactions for each Check-Direct CPE PCR run. The positive control is supplied with the kit.

- **Positive control:**
  Pipette 10 µL of the positive control and 500 µl Milli-Q water or aqua bidest into one Sample Buffer Tube. Vortex for 10 seconds.
- **Negative control:**
  Pipette 500 µl Milli-Q water or aqua bidest into one Sample Buffer Tube. Vortex for 10 seconds.

BD MAX operation

1. Multiplex real-time PCR setup

Table 1 presents the multiplex real-time PCR setup with the targets detected in each detector channel of the BD MAX System.

Table 1: *Multiplex qPCR setup*

Detector	475/520	530/565	585/630	630/665	680/715
Channel	1	2	3	4	5
Target	KPC	VIM	OXA-48-like	NDM	SPC*

*SPC: Sample Processing Control

When the test is performed for the first time create the PCR test program “Check-Direct CPE” as described in Appendix 1.

2. BD MAX Rack set-up

2.1. Load the BD MAX system racks with the number of DNA Unitized Reagents Strips necessary for the number of samples to test. Gently tap each strip to make sure all liquids are at the bottom of their container.

2.2. Prepare Unitized Reagents Strips:
2.2.a. Snap a DNA extraction BD Exk-1 Reagent tube (white seal) into position 1 of the DNA Strip, see Figure 1.
2.2.b. Snap a DNA MMK Master Mix tube (green/yellow seal) into position 2 of the DNA Strip, see Figure 1.
2.2.c. Snap a CPE reagent tube (blue/purple seal) into position 3 of the DNA Strip, see Figure 1.

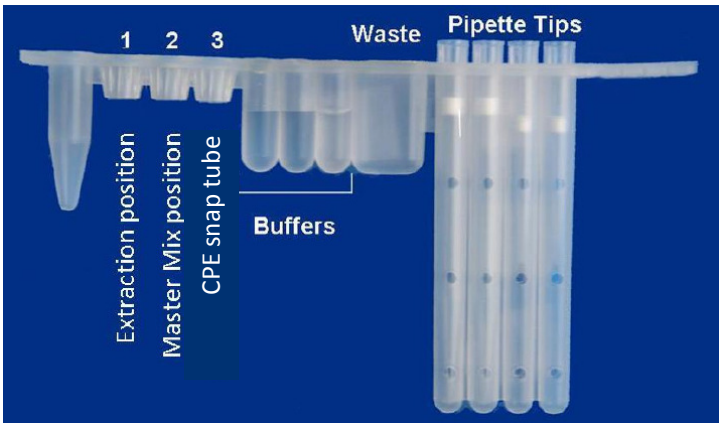

Figure 1: *DNA Unitized Reagent Strip setup.*

3. BD MAX instrument set-up

3.1 Open the **Run** screen of the BD MAX System software v2.96A.
3.2 In the **Assay** menu select *Check-Direct CPE*. (see Appendix 1 if not specified)
3.3 Enter the Sample Buffer Tube barcode using the barcode scanner (you can also enter the barcode manually). Start with position 1 of rack A.
3.4 Place each of the Sample Buffer Tubes in their corresponding position in the BD MAX racks (with septum cap).
3.5 Enter the specimen or patient identification information into the work list. Check that each specimen or patient information correspond to its specific Sample Buffer Tubes in the Rack.
3.6 Load the Rack(s) into the BD MAX System. (Rack A is positioned on the left side of the instrument and Rack B on the right side).
3.7 Load the BD MAX PCR cartridge(s).
3.8 Close the instrument door and select **Start Run.**

Results Interpretation

Important points before starting: For a detailed description on how to analyze data, refer to *BD MAX™ System User's manual*.
Always visually inspect the amplification plot for each sample tested versus C_T values obtained with the software.

1. Reported results

The BD MAX™ software reports C_T values and amplification curves for each detector channel of each specimen tested in the following way:

- C_T value of **0** indicates that there was no C_T value calculated by the software. Amplification curve of the sample showing a "0" C_T value must be checked manually.
- C_T value of **-1** indicates that no valid amplification process has occurred. Check that there is no amplification curve for the sample with a C_T value of -1 on the graphical results.
- Any other C_T value should be interpreted in correlation with the amplification curve (**PCR Analysis** tab) and according to the interpretation method outlined in Tables 2 and 3.

2. Interpretation

2.1 Run validation

Verify that the real-time PCR run is valid before data interpretation of the results. Check that there is no report of BD MAX System failure. Check the positive and negative control amplification curves. Table 2 shows criteria for a valid real-time Check-Direct CPE run on the BD MAX™ System. If the C_T values of the controls are not as expected refer to FAQ and Troubleshooting **"3"**.

Table 2: *Criteria for a valid run with Check-Direct CPE test. (N.R. = not relevant)*

Sample Type*	C_T 475/520 KPC	C_T 530/565 VIM	C_T 585/630 OXA-48 like	C_T 630/665 NDM	C_T 680/715 SPC
Positive controls	32 ±3	30 ±3	29 ±3	31 ±3	N.R.
Negative sample	-1	-1	-1	-1	29 ±3

2.2 Results interpretation

If the run has been validated, interpret results as positive, negative or invalid with the C_T values obtained for the samples following the guidelines summarized in Table 3. Invalid runs should be retested.

Ct-values obtained with bacterial cells will generally be in a specific Ct-window for each target because of the well-defined amount of cells used as input material for the test. Note however that Ct-values may differ significantly between individual strains. **Table 3** specifies the upper limit of this C_T-window, a higher C_T-value suggests contamination of the sample or a strain that is not pure. Therefore, this will be regarded as an "Invalid" result.

Table 3: *Data interpretation guidelines for bacterial cells (N.R. = not relevant)*

C_T 475/520 KPC	C_T 530/565 VIM	C_T 585/630 OXA-48 like	C_T 630/665 NDM	C_T 680/715 SPC	Interpretation
≤33	≤27	≤26	≤32	N.R.	Positive
-1	-1	-1	-1	29 ±3	Negative
> 33	>27	>26	>32	N.R.	Invalid
-1	-1	-1	-1	-1	Invalid

Frequently asked questions (FAQ) & Troubleshooting

Refer to "the troubleshooting" section of the BD MAX™ System User's Manual for additional information

1. **Real-time results show no C_T values or interpretation indicates that the sample is invalid.** Possible causes and troubleshooting:
   - The PCR reaction has been inhibited by exogenous or endogenous substances. Please repeat sample testing. When still inhibited a lower amount of input sample may improve the results.
   - The DNA extraction failed since the SPC was not detected.
   - The BD DNA MMK may have expired.
   - An error in liquid handling has occurred: check unitized reagent strips and PCR cartridge to determine where liquid handling problem has occurred (example: air bubble in the cartridge) and re-run the sample. If the problem persists, contact your local BD representative.
2. **Troubleshooting for invalid results.**
   For Invalid results: Repeat test with the original specimen by preparing a new Sample Buffer Tube. Alternatively, test newly collected specimen or use a lower amount of specimen.
3. **Real-time results show no C_T values for the positive control or interpretation indicating that sample is invalid?**
   Possible causes and troubleshooting:
   - The positive control solution was not added.
   - The BD DNA MMK may have expired.
   - Air bubbles have occurred in the PCR reaction chamber of the positive control.
4. **Real-time results show very low fluorescent signals in all samples and detector channels including the SPC signal.**
   Possible causes and troubleshooting:
   - The CPE reagent tubes containing the fluorescent probes and primers may be degraded. Please check expiration date and make sure that the CPE tubes have been stored correctly.
   - The BD MAX™ System can be responsible for these results. Please refer to BD MAX™ User's manual or contact your BD local representative.
5. **The BD MAX™ System states an error or failure.**
   Refer to the BD MAX™ instrument user manual or contact your BD local representative.
6. **Duplicate samples tested with Check-Direct CPE test do not yield identical results.**
   C_T values of identical samples may vary between individual reactions. Large variations, > 2 C_T values, suggest pipetting errors or other differences between the duplicate samples.

Limitations

Check-Direct CPE uses a range of specific DNA markers to detect the presence of the carbapenemase genes KPC, NDM, OXA-48, and VIM, which currently represent the clinically most prevalent carbapenemases. The test detects all presently known variants of KPC, NDM, OXA-48 and VIM, except VIM-7, a rare variant only found in *Pseudomonas aeruginosa*. It should be noted that other rare carbapenemase gene families are not detected.

The quality of the input DNA is an important factor for obtaining reliable results with Check-Direct CPE. For cell suspensions the correct cell densities are an important factor to obtain reliable results and the procedure described in this manual must be strictly followed. The assay has been tested extensively with DNA purified from gram-negative bacteria, such as *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Pseudomonas*, with excellent results. However, it may never be excluded that other Gram-negative bacteria or certain strains of the above species will yield poor results. Check-Direct CPE cannot and does not make any representation or warranty that it is capable of correctly detecting the carbapenemase genes in all gram-negative species, subspecies or types or in all clinical samples. Results may need to be confirmed by additional methodologies in specific cases (e.g. for regulatory samples). Due to the high variability of bacterial genomes it is possible that certain subtypes might not be detected. The test reflects the state of knowledge of Check-Points Health B.V.
The presence of multiple bacterial species in a sample may hamper the interpretation of the test. As with other diagnostic assays, the results of this test may only be interpreted in combination with additional laboratory and clinical data available to the responsible person. Use of this assay is limited to appropriately qualified personnel, well-trained in performing DNA-based molecular detection methods.

Key to symbols used

Symbol	Definition
Control CPE	CPE control
IVD	For *In Vitro* Diagnostic Use
REF	Catalog number
LOT	Batch code
IFU	IFU number
	Use before YYYY-MM
	Consult instructions for use
	Manufacturer
	Temperature limitation
	Contains sufficient for < n > tests

Technical assistance

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Despite the utmost care in the development and preparation of the protocol Check-Points cannot take any responsibility for errors, omissions and/or future changes herein.

Literature Citation: When describing a procedure for publication using this product, please refer to it as the *Check-Direct CPE.*
Notice to Purchaser:
This product is sold under license from PHRI Properties and may be used under PHRI Properties patent rights only for human in vitro diagnostics, food testing, veterinary testing, or research.
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Appendix 1: Creating the Check-Direct CPE test program

Important points before starting: Refer to BD MAX System User's Manual for detailed instructions on how to operate the BD MAX System and software version 2.96A.

1. Create a new Test, select **Create test**, and enter the following parameters:

- Test Name: type *Check-Direct CPE*
- Extraction Type: Select *Exk DNA-1 (Plasma/Serum)*
- Master Mix Format: choose *Type 1: BD MMK or MMK(SPC) and Dried Primers & Probes*
- Channel detector Settings: set *Gain* and *Threshold* with parameters presented in Table A
- GardRail: select Default
- Test details: enter the PCR profile, see Table B
- Spectral Cross Talk tab: enter parameters presented in Table C

2 Select **Save Test.**

Table A: *Gain parameters.*

Detector	Gain	Threshold
475/520	40	100
530/565	80	150
585/630	30	150
630/665	80	150
680/715	40	150

Table B: *Real-time protocol parameters.*

Step Name	Profile Type	Cycles	Time (s)	Temp(°C)	Detect
Denaturation	Hold	1	600	98	NO
Amplification &Detection	2 - temperature	40	15	98	NO
			62	60	YES

Table C: *Spectral cross-talk parameters.*

		False Receiving Channel				
		475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520		0.0	0.0	0.0	0.0
	530/565	0.0		0.0	0.0	0.0
	585/630	0.0	0.0		7.4	0.0
	630/665	0.0	0.0	0.0		0.0
	680/715	0.0	0.0	0.0	4.4	

Appendix 2: Performance Characteristics

Limit of Detection

The analytical limit of detection (LoD) of Check-Direct CPE was determined using the individual positive controls supplied with the test. These positive controls contain the target DNA at 10^3 copies per µl. Serial dilutions were made of each of the positive controls and 10^4, 10^3 and 10^2 copies were added in triplicate to a sample buffer tube: a total of 36 reactions processed in 2 BD MAX runs following the protocol as described on pages 4 and 5 of this User Manual (10 µl target DNA + 500 µl PCR-grade water added to SB-1). The whole procedure was executed twice.

The 10^4 and 10^3 DNA copies were always detected regardless of the target DNA used. Only at 100 copies added as input DNA for the test a difference in LoD was visible, which is depicted in the table below.

Target	Input DNA copies	DNA copies/PCR	Success rate
KPC	100	12	3 out of 6
VIM	100	12	6 out of 6
OXA-48	100	12	6 out of 6
NDM	100	12	3 out of 6

In silico Specificity

The specificity of the Check-Direct CPE real-time diagnostic test is ensured by the selection of the correct primers and probes, as well as the selection of stringent reaction conditions. Primers and Probes sequences were designed to specifically identify the gene variants listed in the Table below. A 100% sequence match with the primers and probes by *in silico* analysis was assumed to warrant reliable detection of each of the depicted variants. Single mismatches with the primers and probes exist in some variants, of which we expected that detection would not be compromised. This was confirmed by testing such variants in comparison with variants which were 100% homologous.

Primers and Probes sequences were tested for potential homologies with genes from other organisms using all gene sequences present in the international gene bank on April 1st, 2014. (GenBank®, NIH genetic sequence database). using sequence comparison analysis. No cross homology was found with other organisms for the selected primers and probes.

Carbapenemase gene	Variants detected
KPC	1 – 17
NDM	1 – 10
VIM	1 – 6 & 8 – 38
OXA-48 like	48, 162, 163, 181, 204, 232, 244, 245, 247, 370

Analytical Specificity

The analytical specificity of the Check-Direct CPE real-time diagnostic test was determined by testing the cross-reactivity with samples containing a high amount of non-target organisms. 132 carbapenemase-negative strains were used to test the specificity of the Check-Direct CPE real-time test. An overview of these strains is outlined in the table below. All isolates tested negative with the Check-Direct CPE assay and the internal control was reliably detected in all samples. The Check-Direct CPE test showed 100% specificity based on the reference strains tested.

Species	Strains tested
Campylobacter jejuni	2
Citrobacter freundii	5
Enterobacter aerogenes	1
Enterobacter cloacae	42
Enterococcus casseliflavus	1
Enterococcus faecalis	2
Escherichia coli	51
Klebsiella oxytoca	1
Klebsiella pneumoniae	16
Pseudomonas aeruginosa	2
Salmonella typhimurium	1
Pseudomonas mirabilis	3
Staphylococcus aureus	2
Serratia marcescens	1
Stenotrophomonas maltophilia	2

Analytical Inclusivity

A retrospective study was performed with 93 bacterial strains of 13 different gram-negative species, that were previously identified carbapenemase-positive with the Check-Points micro-array diagnostics test Check-MDR CT103 (Check-Points Health). All 93 bacterial strains were typed correctly for the targeted carbapenemase genes with the Check-Direct CPE test. Results are depicted in the table below.

Number of strains tested	Check-MDR CT103 result	Check-Direct CPE result
19	KPC	KPC
16	NDM	NDM
33	VIM	VIM
23	OXA-48	OXA-48
1	NDM + OXA-48	NDM + OXA-48
1	VIM + OXA-48	VIM + OXA-48