User manual

Check-Direct CPE Screen for BD MAX™

For detection and differentiation of carbapenemase genes from *Enterobacteriaceae* in rectal swabs

Version 1.1
Date of issue: 10.03.2017

REF **18-0057** Σ **24**

For Research Use Only (RUO)
Not for use in diagnostic procedures

Contents

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 and OXA-48 like variants). Moreover, CPE often have other non–β-lactam resistance determinants resulting in multidrug- and pandrug-resistant isolates.

Check-Direct CPE Screen for BD MAX™ 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 Screen has been designed to reliably detect most of the variants. The variants detected and predicted to be detected for each resistance gene are presented in the *in silico* specificity paragraph in Appendix 2. Check-Direct CPE Screen 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
CPE Screen reagent tubes (9-0121)	24 sealed tubes (blue seal)
CPE positive control (9-0061)	1 tube (purple cap)
CP Mastermix (9-0122)	1 tube (green cap) 330 µl
User Manual (9-0126)	Leaflet – download from website

Materials required but not supplied with the kit

Supplies	Equipment
• BD MAX™ ExK™ DNA-1 Extraction Kit (Ref:442818) • BD MAX™ PCR Cartridges (Ref: 437519) • Disposable laboratory (powder-free) gloves • Pipettes & disposable (filter-) tips for volumes of 10 and 25 µl • PCR-grade water (e.g. Milli-Q or aqua bidest) • Swabs and transport media compatible with rectal specimen collection. Recommended swab collection device: Copan ESwab, Cat.No. 480CE	• Real-time PCR instrument: BD MAX™ System, software version 4.30B or higher • Vortex mixer

Storage and stability

The Check-Direct CPE Screen for BD MAX™ kit is shipped at ambient temperature, should be stored in the dark and at 2 to 8 °C upon receipt. Reagents are stable at 2 to 8 °C through the stated expiration date. Do not use expired components.

Check-Direct CPE Screen for BD MAX™ reagent tubes, PCR Mastermix and positive control are supplied in a sealed pouch. To protect reagents from humidity, immediately re-seal pouch after opening. Reagent Tubes are stable for up to 14 days at 2 to 8 °C after initial opening and re-sealing of the pouch.

Warnings and Precautions

- The Check-Direct CPE Screen for BD MAX™ Assay is for research use only. Not for use in diagnostic procedures. Performance characteristics have not been established and potential interfering substances have not been evaluated.
- This product can only be used on the BD MAX™ System.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Check reagent strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes).
- Check reagent strips to ensure that all pipette tips are present .
- Do not remove desiccant from reagent pouches.
- Do not use reagents if desiccant is not present or is broken inside reagent pouches.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not interchange or reuse caps, as contamination may occur and compromise test results.
- Proceed with caution when using chemical solutions as Master Mix and Extraction Tube barcode readability may be altered.
- Do not use expired reagents and/or materials.
- Good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all materials and reagents.
- To avoid contamination by amplicons, do not break apart the BD MAX™ PCR Cartridges after use. The seals of the BD MAX™ PCR Cartridges are designed to prevent contamination.
- BD MAX™ PCR Cartridges may be used for up to two runs.
- Performing the Check-Direct CPE Screen for BD MAX™ Assay outside the recommended time ranges can produce invalid results. Assays not performed within the specified time ranges should be repeated with a new specimen.
- Additional controls may be tested according to guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
- In cases where culture or other PCR tests are conducted in the laboratory, care must be taken to ensure that the Check-Direct CPE Screen for BD MAX™ Assay components, any additional reagents required for testing, and the BD MAX™ System are not contaminated. Avoid microbial and deoxyribonuclease (DNase) contamination of reagents at all times. Gloves must be changed before manipulating reagents and cartridges.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in the CLSI Document M2911 and in Biosafety in Microbiological and Biomedical Laboratories.
- Wear protective clothing and disposable gloves while handling all reagents.
- Wash hands thoroughly after performing the test.
- Do not smoke, drink, chew or eat in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with local, state, provincial and/or federal regulations.
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.

Please read the full protocol before starting the test

Instruction for Use

Sample preparation procedures

Test preparation for rectal swabs

Note: The procedure for specimen collection and storage must be followed carefully using adequate specimen collection devices (see section *Materials required but not supplied with the kit)*. Rectal swabs will contain varying amounts of faecal material depending on the procedure for specimen collection.

1. Collect rectal specimen according to local guidelines and swab manufacturer recommendations.
2. Transfer the swabs to the tubes containing liquid transport medium.
3. Transfer rectal swab samples to be analyzed to the PCR room or store until further use according to the swab manufacturer recommendation and/or local regulations.
4. Mix each tube with rectal specimen briefly and pipette 25 µl of the transport medium into one DNA Sample Buffer Tube SB-1.
5. Close the Sample Buffer Tube with a septum cap and vortex 10 seconds at medium speed.

Preparation of control reactions

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

- **Positive control:**
  Pipette 10 µL of the positive control into one Sample Buffer Tube. Vortex for 10 seconds.
- **Negative control:**
  Pipette 10 µL of PCR-grade water 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	NDM	SPC*

*SPC: Sample Processing Control

When the test is performed for the first time create the PCR test program “C-D CPE Screen” 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 Put the Unitized Reagents Strips in their positions in the BD MAX™ rack. Do not “click in” the Strips yet.
2.2.b. Snap a DNA extraction BD Exk-1 Reagent tube (white seal) into position **1** of the DNA Strip, see Figure 1.
2.2.c. Snap a CPE Screen reagent tube (blue seal) into position **3** of the DNA Strip, see Figure 1.
2.2.d. Pierce the blue seal of the CPE Screen reagent tube in position **3**, *e.g.* with a disposable pipette tip. Next, carefully dispense 12.5µl of CP Mastermix at the bottom of the tube making sure not to create air bubbles.
2.2.e. Click the Unitized Reagents Strips into their rack positions when strips preparation is finished.

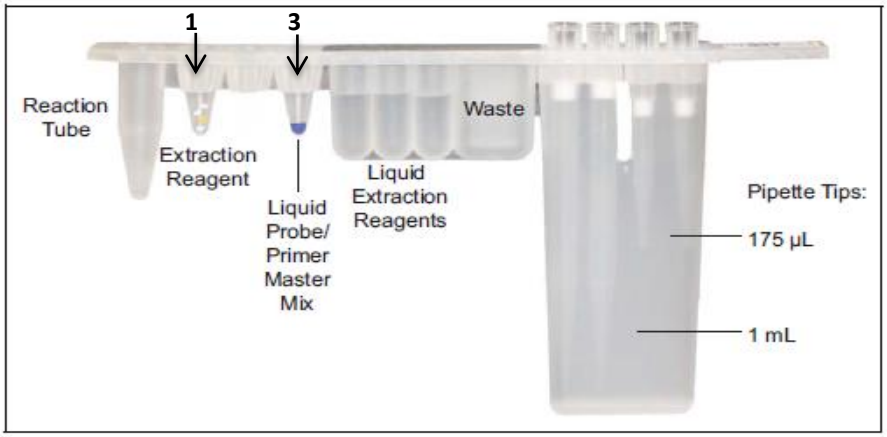

Figure 1: *DNA Unitized Reagent Strip setup.*

3. BD MAX™ instrument set-up

3.1 Open the **Run** tab of the BD MAX™ System **software v4.30B** or higher and fill in the **Worklist**.
3.2 Select the **Test** "*C-D CPE Screen"*. See Appendix 1 to create the "*C-D CPE Screen"* test if not yet in the Test menu.
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. Place each of the Sample Buffer Tubes in their corresponding position in the BD MAX™ racks (with septum cap).
3.4 Enter the specimen identification information into the **Accession** line of the work list. Check that each specimen information corresponds to its specific Sample Buffer Tube in the Rack.
3.5 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).
3.6 Load the BD MAX™ PCR cartridge(s).
3.7 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 with the specified Threshold (see Appendix 1). 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 and according to the interpretation guidelines 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. If applicable, check the positive and negative control amplification curves. Table 2 shows criteria for a valid Check-Direct CPE Screen 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 Screen test.*

Sample Type*	C_T 475/520 KPC	C_T 530/565 VIM	C_T 585/630 OXA-48	C_T 630/665 NDM	C_T 680/715 SPC
Positive controls	32 ±3	29 ±3	28 ±3	31 ±3	28 ±3
Negative sample	-1	-1	-1	-1	28 ±3

2.2 Results interpretation

If the run has been validated, interpret results as positive, negative or unresolved with the C_T values obtained for the samples following the guidelines summarized in Table 3. Please always check that the amplification curve of each sample is in an agreement with the C_T values and results interpretation given by the software. Unresolved runs should be retested.

Table 3: *Data interpretation guidelines for rectal swabs.*

KPC, VIM, OXA, NDM C_T values	SPC C_T values	Interpretation
YES	YES	Positive sample
-1	28 ± 3	Negative sample
-1	< 25 or > 32	Unresolved
-1 or YES	-1	Unresolved

IMPORTANT NOTES:

- YES means that a C_T value is observed and given in the results table.
- A positive test result does not necessarily indicate the presence of viable organisms in the sample tested.
- C_T-values of rectal swabs may vary widely due to differences in faecal material and "bacterial load" of rectal swabs in transport medium.
- If the BD MAX™ system gives an Indeterminate or Incomplete results (IND or INC) due to BD MAX™ System failure, please contact your local BD representative.

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 unresolved.** 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 CPE Screen reagent or CP Mastermix 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 unresolved results.**
   For unresolved 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 unresolved?**
   Possible causes and troubleshooting:
   - The positive control solution was not added.
   - The CPE Screen reagent or CP Mastermix 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 Screen reagent tubes containing the fluorescent probes and primers may be degraded. Please check expiration date and make sure that the CPE Screen 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 Screen assay 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 Screen for BD MAX™ 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 test is only intended to be used with rectal swabs in transport medium as input material.

The quality of the input DNA is an important factor for obtaining reliable results with Check-Direct CPE Screen for BD MAX™. DNA must be extracted from rectal swabs using the devices and procedures described in this manual. 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 Screen 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.

A positive test result does not necessarily indicate the presence of viable organisms in the sample tested. Carbapenemase DNA may have been detected from nonviable organisms.

The presence of multiple bacterial species in a sample may hamper the interpretation of the test.

Key to symbols used

Symbol	Definition
CP Mastermix	CP Mastermix
Control CPE	CPE control
RUO	For Research Use Only (RUO) Not for use in diagnostic procedures
REF	Catalog number
LOT	Batch code
	Use before YYYY-MM
	Consult instructions for use
	Manufacturer
	Temperature limitation
Σ	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 Screen.*
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Check-Points

Appendix 1: Creating the Check-Direct CPE Screen test program v.4.30B or higher

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 4.30B or higher.**

To create a new Test, in the **Test Editor** tab, select **Create**, and apply the following instructions:

1. In the **Basic Information** tab enter the following parameters:

- Test Name: *C-D CPE Screen.*
- Extraction Type: Select *Exk DNA-1 (Plasma/Serum).*
- Master Mix Format: select *Type 3: Liquid MM with Primers and Probes.*
- Sample Extraction Parameters: select *User defined* and adjust *sample volume* to 600µl, see Table A.
- Ct Calculation: select *Call Ct at inflection point.*

Save parameters

2. In the **PCR Settings** tab enter the following parameters:

- Alias, PCR Gain, and Threshold: for each channel detector enter the correct parameters specified in Table B.
- Color compensation: enter the correct parameters specified in Table C.

Save parameters

3. In the **Test Steps** enter the PCR steps as specified in Table D.

Save parameters

Table A: *Sample Extraction Parameters.*

Parameters	Value
Lysis Heat Time	10
Lysis Temperature	37
Sample Tip Height	1600
Sample Volume	**600**
Wash Volume	500
Neutralization Volume	----
DNase Heat Time	----

Table B: *Alias, PCR Gain,Threshold parameters.*

Detector	Alias	Gain	Threshold
475/520	KPC	80	100
530/565	VIM	80	100
585/630	OXA-48	30	100
630/665	NDM	80	100
680/715	SPC	40	100

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	

Table D: *Test PCR Steps parameters.*

Step Name	Profile Type	Cycles	Time (s)	Temp(°C)	Detect
Denaturation	Hold	1	600	98	NO
Amplification &Detection	2 - temperature	50	15	98	NO
			62	60	YES