User manual

Check-Direct ESBL for BD MAX™

Real time PCR kit for the detection of ESBL-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 ESBL for BD MAX is a qualitative *in vitro* diagnostic test for the rapid detection of Extended Spectrum Beta-Lactamase (ESBL) genes in *Enterobacteriaceae*. The assay is performed on the BD MAX™ system using bacterial cell suspensions from clinical specimens of individuals at risk of colonization with ESBL-producing *Enterobacteriaceae*. Check-Direct ESBL detects the presence of the ESBL gene families CTX-M1, CTX-M2, CTX-M9 and SHV-ESBL, presently the primary cause of extended spectrum β-lactam resistance 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 ESBL for BD MAX can be used as an aid to identify, prevent and control ESBL-producing *Enterobacteriaceae* that colonize patients in healthcare settings. Check-Direct ESBL for BD MAX is not intended to diagnose infections with ESBL-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 extended spectrum β-lactam resistance in *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 extended spectrum β-lactam resistance in *Enterobacteriaceae* is the production of Extended Spectrum Beta-Lactamases, i.e. ESBL's. Most Enterobacteriaceae producing ESBL's have resistance to all β-lactam antibiotics except carbapenems. Presently, the vast majority of clinically relevant ESBL's are expressed from one of the following plasmid-encoded gene families: CTX-M1, CTX-M2, CTX-M9 and SHV-ESBL.

Check-Direct ESBL is a multiplex real-time PCR assay for detection of the CTX-M1, CTX-M2, CTX-M9 and SHV-ESBL gene families. 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. The CTX-M1, CTX-M2, CTX-M9 and SHV-ESBL gene families have many gene variants and Check-Direct ESBL has been designed to reliably detect all variants. Check-Direct ESBL for BD MAX employs five different fluorescent probes and enables detection and discrimination of the 4 ESBL gene families and the control target SPC, that monitors DNA extraction and PCR amplification.

Kit contents (for 24 reactions)

Components (Mat. No.)	Description	Storage conditions
ESBL reagent tubes (9-0065)	24 sealed tubes (red seal)	+ 4°C, **store in the dark**
ESBL positive control (9-0064)	1 tube (red cap)	+ 4°C
User Manual (9-0109)	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 • 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 ESBL 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 access 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 of McFarland 0.5 (≈1 x 10^8 CFU/ml) from the bacterial cells grown on the agar plate. Cell suspension buffer (*e.g.* PBS or 10mM Tris.HCl pH8.0) or Milli-Q water or aqua bidest may be used.
3. Pipette 500 of Milli-Q water or aqua bidest and 10 µL of the bacterial cell suspension (≈1 x 10^6 CFU) 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 Tube 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 ESBL PCR run. The positive control is supplied with the kit.

- **Positive control:**
  Pipette 10 µL of the positive control and 500 µL of Milli-Q water or aqua bidest into one Sample Buffer Tube. Vortex for 10 seconds.
- **Negative control:**
  Pipette 500 µL of 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	CTX-M1	CTX-M2	SHV-ESBL	CTX-M9	SPC*

*SPC: Sample Processing Control

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

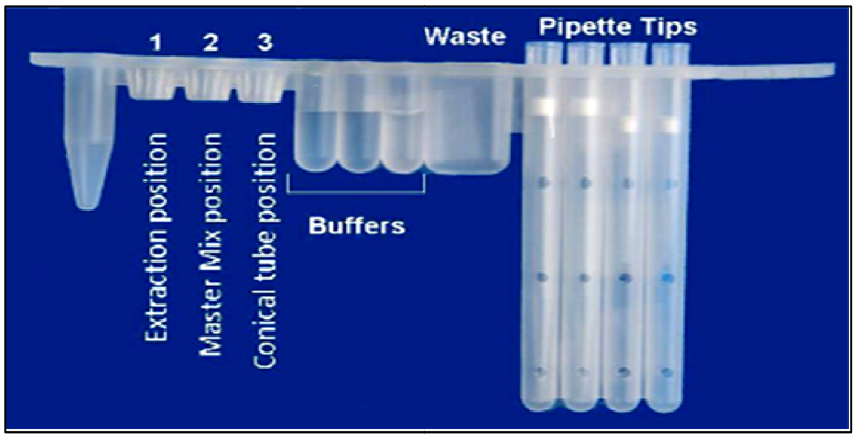

Figure 1: *DNA Unitized Reagent Strip setup.*

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 ESBL reagent tube (red seal) into position 3 of the DNA Strip, see Figure 1.

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 ESBL*.
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 ESBL 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 ESBL test.*

Sample Type*	C_T 475/520 CTX-M1	C_T 530/565 CTX-M2	C_T 585/630 SHV-ESBL	C_T 630/665 CTX-M9	C_T 680/715 SPC
Positive controls	33 ±3	30 ±3	30 ±3	30 ±3	29 ±3
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 of bacterial cells will be in a specific Ct-window for each target because of the well-defined amount of cells used as input material for the test. However, Ct-values will differ between individual strains. Ct-values out of this Ct-window are regarded as invalid and should be retested. A higher Ct-value above the Ct-window suggest contamination of the sample or a strain that is not pure. A lower Ct-value suggests an aberrant amplification plot. Please check the amplification plot in such a case to confirm this.

Table 3: *Data interpretation guidelines for bacterial cells*

C_T 475/520 CTX-M1	C_T 530/565 CTX-M2	C_T 585/630 SHV-ESBL	C_T 630/665 CTX-M9	C_T 680/715 SPC	Interpretation
≤ 28	≤ 28	≤ 30	≤ 29	29 ±3	Positive
-1	-1	-1	-1	29 ±3	Negative
> 28	> 28	> 30	> 29	29 ±3	Invalid
-1 or YES	-1 or YES	-1 or YES	-1 or YES	<26 or >32	Invalid
-1 or YES	-1 or YES	-1 or YES	-1 or YES	-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 ESBL reagent tubes containing the fluorescent probes and primers may be degraded. Please check expiration date and make sure that the ESBL 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 ESBL 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 ESBL uses a range of specific DNA markers to detect the presence of the CTX-M1, CTX-M2, CTX-M9 and SHV-ESBL genes, which currently represent the clinically most prevalent ESBL's. The test detects almost all clinically relevant variants of CTX-M1, CTX-M2, CTX-M9 and SHV-ESBL. It should be noted that some more rare variants of CTX-M1, CTX-M2, CTX-M9 and SHV-ESBL are not detected and that other ESBL gene families like e.g. TEM, GES, VEB and PER are also not detected. Bacterial cell suspensions should be used as input material for the test.

The quality of the input DNA is an important factor for obtaining reliable results with Check-Direct ESBL. 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 ESBL cannot and does not make any representation or warranty that it is capable of correctly detecting the ESBL 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 ESBL	ESBL 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

support@check-points.com
+31 317 453 908

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 ESBL*.
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Check-Points Health BV
Binnenhaven 5
6709 PD Wageningen
The Netherlands

Tel: +31 317 453 908
Fax: +31 317 210 147
info@check-points.com
www.check-points.com

Check-Points

Appendix 1: Creating the Check-Direct ESBL 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 ESBL*
- 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	80	100
530/565	80	100
585/630	30	200
630/665	80	100
680/715	30	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	45	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		1.6	0.0	0.0	0.0
	530/565	1.2		1.2	0.0	0.0
	585/630	0.0	0.0		10.7	0.0
	630/665	0.0	0.0	6.3		5.6
	680/715	0.0	0.0	0.0	7.8	

Appendix 2: Performance Characteristics

Limit of Detection with control DNA targets

The analytical limit of detection (LoD) of Check-Direct CPE was determined using linearized control plasmids containing target sequences for CTX-M1, CTX-M3 & M15, CTX-M10, CTX-M2, CTX-M9, SHV-2 and SHV-5 & -12. Of each target DNA 6 concentrations were tested, i.e. 10^5, 10^4, 10^3, 10^2, 10, 1 DNA copies/µl. Samples were tested on the BD MAX system in the PCR only mode. First Check-Direct ESBL Snap tubes were rehydrated with 12.5µl of a solution containing Tris EDTA buffer, MMK PCR diluent and SPC target DNA. Next 12.5 µl of target DNA solution was added. Subsequently, the resulting 25µl of each reaction mix this mix was transferred to the MMK tube to rehydrate the master mix. Finally, 12µl of each solution was manually transferred into the individuals well of a BD MAX PCR cartridge. The cartridge was then placed into the BD MAX instrument and the PCR only protocol was started using the Check-Direct ESBL test program.

The assays with 10^5, 10^4, 10^3, 10^2 DNA copies/µl were always positive. The assays with 10 and 1 DNA copies/µl reached the true LoD of the test: results are presented in the table below.

Target	Input DNA copies	DNA copies/PCR	Success rate
CTXM-1	12.5	2	4 out of 4
CTXM-2	12.5	2	3 out of 4
CTXM-3/15	12.5	2	2 out of 2
CTXM-10	125	20	2 out of 2
CTXM-9	12.5	2	2 out of 2
SHV-2	12.5	2	2 out of 2
SHV-5/12	12.5	2	2 out of 2

In silico Specificity

The specificity of the Check-Direct ESBL 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.

ESBL gene	Variants detected
CTX-M1 group	1,3,10-12,15,22,23,28-30, 32-34,36,37,42,52-55,57,58,60-62,66,68,69,71,72,88,96,101,107-109,114,116,117,132 133,136
CTX-M2 group	2,4-7,20,31,35,43,44,56,59,76,77,95,124,131
CTX-M9 group	9,13,14,16-19,21,24,27,38,45-51,55,57,64,65,67,81,93,102,104,105,106,110,111,112,113,121,122,123,126,134
SHV-ESBL	2,2a,3, 4,5,7,9,10,12,15,20-23,30,34,39, 45,46,55,64,66,86,90,105,106,123,124,128,129,134,141,152,153,152,160, 163-165