

User manual

Check-Direct ESBL Screen for BD MAX™

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

Version 1.0

Date of issue: 15.01.2015

REF 18-0066  24 IFU 066-01

EU:  IVD

U.S.: For Research Use Only

Not for use in diagnostic procedures

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Intended use

Check-Direct ESB Screen for BD MAX™ is a qualitative *in vitro* diagnostic test for the rapid detection in rectal swabs of Extended Spectrum β -Lactamase (ESBL) genes from *Enterobacteriaceae*. Check-Direct ESB Screen detects the presence of the ESB gene families CTX-M1, CTX-M2, CTX-M9 and SHV-ESBL, presently the primary cause of ESB 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 ESB Screen for BD MAX™ can be used as an aid to identify, prevent and control ESB-producing *Enterobacteriaceae* that colonize patients in healthcare settings. Check-Direct ESB Screen for BD MAX™ is not intended to diagnose infections with ESB-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. ESB's. Most *Enterobacteriaceae* producing ESB's have resistance to all β -lactam antibiotics except carbapenems. Presently, the vast majority of clinically relevant ESB's are expressed from one of the following plasmid-encoded gene families: CTX-M1, CTX-M2, CTX-M9 and SHV-ESBL.

Check-Direct ESB Screen for BD MAX™ 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 ESB has been designed to reliably detect almost all variants. Check-Direct ESB for BD MAX™ employs five different fluorescent probes and enables detection and discrimination of the 4 ESB 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 Screen reagent tubes (9-0131)	24 sealed tubes (orange seal)	+ 4°C, store in the dark
ESBL positive control (9-0064)	1 tube (orange cap)	+ 4°C
CP Mastermix (9-0122)	1 tube (green cap) 330 μ l	+ 4°C
User Manual (9-0134)	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™ PCR Cartridges (Ref: 437519) - Disposable laboratory (powder-free) gloves - Pipettes & disposable (filter-) tips for volumes of 10 and 25 μl - Milli-Q water or aqua bidest - Swabs and transport media compatible with rectal specimen collection. Recommended swab collection device: Copan ESwab, Cat.No. 480CE or 490CE	- Real-time PCR instrument: BD MAX™ System, software version 2.96A or 4.3 or higher - Vortex mixer

Storage, handling and stability

The Check-Direct ESB Screen 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 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 fecal material depending on the procedure for specimen collection. Check-Points advises to validate your specimen collection and processing method with Check-Direct ESB Screen prior to routine use of the test.

1. Collect rectal specimen according to local guidelines and swab manufacturer recommendations.
2. Transfer the swabs to the tubes containing liquid transport medium. Proceed with testing the samples or store according to local guidelines and swab manufacturer recommendations until further use.
3. Vortex each tube with rectal specimen briefly and pipette 25 µl of the transport medium into one DNA Sample Buffer Tube SB-1 from the BD MAX™ ExK™ DNA-1 Extraction Kit (Ref: 442818)
4. Close the Sample Buffer Tube with a septum cap and vortex for 10 seconds.
5. Transfer the DNA Sample Buffer Tubes to the PCR room.

Preparation of control reactions

Quality Control requirements must be performed according to local, state and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures. Quality control procedures are intended to monitor reagent and assay performance. The positive control and a negative control should be run regularly until adequate process validation is achieved on the BD MAX™ System. Reduced frequency of control testing should be based on adequate data and determined by the individual laboratory.

To validate the test on a regular basis, perform positive and negative control reactions with the Check-Direct ESB 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 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

If the test is performed for the first time create the PCR test program "C-D ESB 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. Put the unitized Reagents Strips in their positions in the BD MAX™ rack. Do not "click in" the Strips yet.
 - 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 ESB Screen reagent tube (orange seal) into position **3** of the DNA Strip, see Figure 1.
 - 2.2.c. Pierce the orange seal of the ESB 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.d. Click the Unitized Reagents Strips into their rack positions.
- 2.3. Place the Sample Buffer Tubes with septum caps in the BD MAX™ rack.

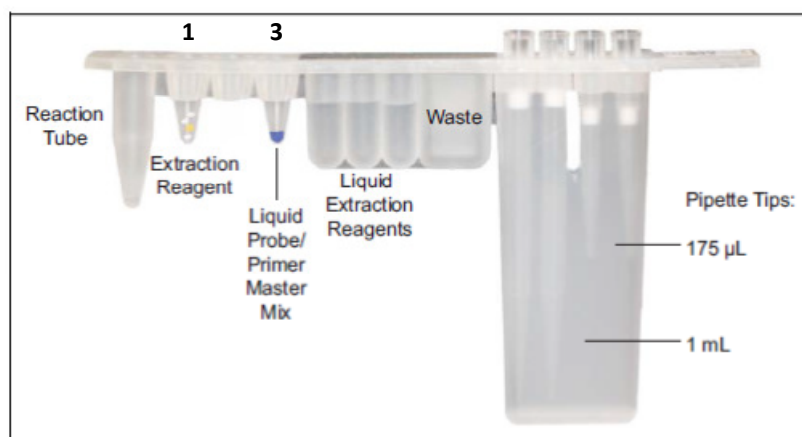


Figure 1: DNA Unitized Reagent Strip setup.

3. BD MAX™ instrument set-up

- 3.1 Open the **Run** tab of the BD MAX™ System software and fill in the **Worklist**.
- 3.2 Select the **Test “C-D ESBL Screen”**. (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 Enter the specimen or patient identification information into the **Accession** line of the work list. Check that each specimen or patient information correspond to its specific Sample Buffer Tubes 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 described below.

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 ESBL 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 ESBL Screen 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	29 ±3	29 ±3	29 ±3	29 ±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. Please always check that the amplification curve of each sample is in a 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.

CTX-M1 CTX-M2 SHV-ESBL CTX-M9 C_T values	SPC C_T values	Interpretation
YES	29 ± 3	Positive sample
-1	29 ± 3	Negative sample
-1 or YES	< 26 or > 32	Unresolved
-1 or YES	-1	Unresolved

Note:

- YES means that a C_T value is observed and given in the Results table.
- C_T values of rectal swabs may vary widely due to differences in fecal material and “bacterial load” of rectal swabs in transport medium.

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 ESBL 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 or CP Mastermix was not added.
 - The ESBL Screen reagent or CP Mastermix may have expired.
 - Air bubbles have occurred in the PCR 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 Screen reagent tubes containing the fluorescent probes and primers may be degraded. Please check expiration date and make sure that the ESBL 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 ESBL 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 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. 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 ESBL 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 ESBL Screen 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
	CP MasterMix
	ESBL control
	For <i>In Vitro</i> Diagnostic Use
	Catalog number
	Batch code
	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 ESBL Screen*.

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. Dye & quencher compounds in this product are sold under license from Biosearch Technologies, Inc. and protected by U.S. and world-wide patents either issued or in application. The license grant covers human *in vitro* diagnostic (IVD) applications

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Appendix 1a: Creating the Check-Direct ESBL Screen test program (v2.96A and up)

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 or higher.

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

- Test Name: type *C-D ESBL Screen*.
- Extraction Type: Select *Exk DNA-1 (Plasma/Serum)*.
- Master Mix Format: choose *Type 3: Liquid MM with Primers and Probes*.
- Channel detector Settings: set *Gain* and *Threshold* with parameters specified in Table A.
- GardRail: select "User" and enter values specified in Table B. Only the "Sample Volume" differs from the default settings.
- Test details: enter the PCR profile as outlined in Table C.
- Spectral Cross Talk tab: enter parameters specified in Table D.

2. Select **Save Test**.

Table A: Gain parameters.

Detector	Alias	Gain	Threshold
475/520	CTX-M1 group	80	100
530/565	CTX-M2 group	80	100
585/630	SHV-ESBL	30	100
630/665	CTX-M9 group	80	100
680/715	SPC	40	100

Table B: GardRail 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 C: Real-time protocol 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

Table D: 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 1b: Creating the Check-Direct ESBL Screen test program (v 4.30B and up)

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:

- In the **Basic Information** tab enter the following parameters:
 - Test Name:** *C-D ESBL 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

- In the **Channel Settings** tab:
 - 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

- In the **Test Steps** tab: 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	CTX-M1 group	80	100
530/565	CTX-M2 group	80	100
585/630	SHV-ESBL	30	100
630/665	CTX-M9 group	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		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	

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

Appendix 2: Performance Characteristics

1. Limit of Detection with Rectal Swabs

The analytical limit of detection (LoD) of Check-Direct ESBL Screen for BD MAX™ was determined with ESBL-producing bacteria spiked into ESBL-negative natural human pooled rectal swabs matrix. The LoD was determined for each gene analyte, i.e., the genes encoding CTX-M1 group, CTX-M2 group, CTX-M9 group, and SHV-ESBL. ESwab Amies transport medium (Copan) was “sampled” with approximately 10 mg/ml of human feces. Strains containing the target ESBL genes were grown o/n and a minimum of 5 different concentrations of cell suspensions were prepared in Milli-Q water starting from a suspension with a density of 0.5 McFarland. Bacteria were counted by plating on non-selective media and diluted into the rectal swab matrix to create specimens with a well-defined amount of fecal material and target bacteria.

Specimens created as described above were used to assess the analytical limit of detection (LoD) following the protocol described on pages 4 and 5 of this User Manual. Results are depicted in the Table below. SBT refers to the BD MAX™ Sample Buffer Tube.

ESBL group	Organism Name	Strain ID	CFU/SBT
CTX-M1 group	<i>Enterobacter cloacae</i>	CP0159	2
CTX-M1 group	<i>Klebsiella pneumoniae</i>	CP0160	11
CTX-M2 group	<i>Escherichia coli</i>	CP0187	5
CTX-M2 group	<i>Escherichia coli</i>	CP0086	50
CTX-M9 group	<i>Escherichia coli</i>	CP0158	5
CTX-M9 group	<i>Enterobacter cloacae</i>	CP0028	45
SHV-ESBL	<i>Escherichia coli</i>	CP0232	30
SHV-ESBL	<i>Klebsiella pneumoniae</i>	CP0128	53
SHV-ESBL; CTX-M9 group	<i>Escherichia coli</i>	CP0456	24

2. In silico Specificity

The specificity of the Check-Direct ESBL Screen 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, 32-34,36,37,42,52-55,57,58,60-62,66,68,69,71,72,79,80,82,88,96,103,107,109,114,116, 117,133,139,142
CTX-M2 group	2,4-7,20,31,35,43,44,56,59,76,77,92,97,124,131
CTX-M9 group	9,13,14,16-19,21,24,27,38,45-51,55,57,64,65,67,81,83-86,90,98,102,104,105,106,110-113,121-123,126,134,147
SHV-ESBL	2,5,12,15,39,45,46,48,56,64,66,71

3. Analytical Inclusivity

The analytical inclusivity of the Check-Direct ESBL Screen real-time diagnostic test was evaluated by testing a panel of 17 bacterial strains, 5 for CTX-M1 group, 3 for CTX-M2 group, 5 for CTX-M9 group and 7 for SHV-ESBL. See Table below. A similar protocol as for the LoD study was used (see above section 1). Bacteria were tested in duplicates or triplicates in pooled negative rectal swab matrix near the analytical limit of detection (LoD) and concentration were confirmed by plating bacterial cell suspension on non-selective media in duplicates. The 17 bacterial strains used in this retrospective study were previously well characterized with the Check-Points microarray diagnostics test Check-MDR CT103 (Check-Points Health). Under the conditions of this study, all 17 bacterial strains were detected with the Check-Direct ESBL Screen for BD MAX™ assay. Inclusivity was 100%. Results are depicted in the Table below.

Organism Name	Strain ID	Multidrug resistant genotype (Check MDR CT103)					CFU/SBT*
		TEM	SHV	CTX-M group	Carba*	AmpC	
<i>Escherichia coli</i>	CP0255	WT*		1-3			4
<i>Klebsiella pneumoniae</i>	CP0160			1-3			30
<i>Enterobacter cloacae</i>	CP0159	WT		1-15	OXA-48		2
<i>Escherichia coli</i>	CP0060	WT		1-1			27
<i>Escherichia coli</i>	CP0085	WT+104K+238S		1-32			41
<i>Klebsiella pneumoniae</i>	CP0189		WT	2			19
<i>Escherichia coli</i>	CP0087	WT		2			117
<i>Escherichia coli</i>	CP0086	WT		2			91
<i>Enterobacter cloacae</i>	CP0028			9			45
<i>Escherichia coli</i>	CP0158	WT		9	OXA-48		5
<i>Escherichia coli</i>	CP0152	WT	WT+238S+240K		IMP-8		9
<i>Klebsiella pneumoniae</i>	CP0232	WT	WT+238S/240K		KPC-2		47
<i>Klebsiella pneumoniae</i>	CP0149		238S		IMP-1		52
<i>Escherichia coli</i>	CP0128	WT	238S			ACC	53
<i>Enterobacter cloacae</i>	CP0066		238S+240K	9			74
<i>Escherichia coli</i>	CP0456		238S+240K	9			24
<i>Escherichia coli</i>	CP0308	WT	WT+238S/240K	9	VIM-1		58

*SBT: sample buffer tube (BD); Carba: carbapenemase gene; WT = wild type = non-ESBL TEM or SHV

4. Analytical Exclusivity

The analytical exclusivity of the Check-Direct ESBL Screen real-time diagnostic test was determined by testing the cross-reactivity with samples containing a high amount of non-target organisms. 73 ESBL-negative strains were used to test the specificity of the Check-Direct ESBL Screen real-time test, that were previously well characterized with the Check-Points microarray diagnostics test Check-MDR CT103 (Check-Points Health). An overview of these strains is outlined in the Table below. Strains were tested in replicates at concentrations $\geq 10^6$ CFU/ml. Bacteria were spiked into pooled negative rectal swab matrix. All isolates tested negative with the Check-Direct ESBL Screen for BD MAX™ and the internal control was reliably detected in all samples. The analytical exclusivity was 100% for the specified strains tested.

Organism	Strain ID	Related β -lactamase present
<i>Acinetobacter baumannii</i>	CP0281	TEM-wt
<i>Acinetobacter baumannii</i>	CP0284	---
<i>Acinetobacter baumannii</i>	CP0327	PER
<i>Acinetobacter baumannii</i>	CP0439	OXA-58
<i>Acinetobacter baumannii</i>	CP0441	OXA-23+ -58
<i>Acinetobacter baumannii</i>	CP0444	OXA-23
<i>Acinetobacter baumannii</i>	CP0445	OXA-23; TEM-wt
<i>Aeromonas caviae</i>	CP0374	VIM
<i>Aeromonas genomospecies</i>	CP0169	VIM-4
<i>Citrobacter braakii</i>	CP0338	GES; CMY-2
<i>Citrobacter braakii</i>	CP0375	VIM
<i>Citrobacter freundii</i>	CP0035	---
<i>Citrobacter freundii</i>	CP0288	---
<i>Citrobacter freundii</i>	CP0337	GES; ACT
<i>Enterobacter aerogenes</i>	CP0398	OXA-48
<i>Enterobacter cloacae</i>	CP0006	ACT
<i>Enterobacter cloacae</i>	CP0068	ACT
<i>Enterobacter cloacae</i>	CP0161	VIM-1; ACC
<i>Enterobacter cloacae</i>	CP0236	KPC; TEM-wt
<i>Enterobacter cloacae</i>	CP0272	TEM-wt
<i>Enterobacter cloacae</i>	CP0339	GES; ACT
<i>Enterobacter cloacae</i>	CP0371	GIM; ACT

TEM-wt and SHV-wt refer to wild type = non-ESBL TEM & SHV

Organism	Strain ID	Related β -lactamase present
<i>Escherichia coli</i>	CP0016	TEM-wt
<i>Escherichia coli</i>	CP0021	CMY-2
<i>Escherichia coli</i>	CP0027	GES
<i>Escherichia coli</i>	CP0095	IMP
<i>Escherichia coli</i>	CP0096	KPC
<i>Escherichia coli</i>	CP0097	OXA-48
<i>Escherichia coli</i>	CP0139	ACC; TEM-wt
<i>Escherichia coli</i>	CP0131	FOX; TEM-wt
<i>Escherichia coli</i>	CP0134	DHA
<i>Escherichia coli</i>	CP0136	ACT; TEM-wt
<i>Escherichia coli</i>	CP0196	KPC; TEM-wt; SHV-wt
<i>Escherichia coli</i>	CP0228	NDM-1; CMY-2
<i>Escherichia coli</i>	CP0457	---
<i>Klebsiella oxytoca</i>	CP0352	GES
<i>Klebsiella oxytoca</i>	CP0402	OXA-48
<i>Klebsiella pneumoniae</i>	CP0056	SHV-wt
<i>Klebsiella pneumoniae</i>	CP0101	SHV-wt; DHA
<i>Klebsiella pneumoniae</i>	CP0104	SHV-wt; TEM-wt+164S; DHA)
<i>Klebsiella pneumoniae</i>	CP0107	SHV-wt; DHA
<i>Klebsiella pneumoniae</i>	CP0108	SHV-wt; DHA
<i>Klebsiella pneumoniae</i>	CP0135	SHV-wt; TEM-wt; DHA
<i>Klebsiella pneumoniae</i>	CP0141	SHV-wt; TEM-wt+164S; ACT
<i>Klebsiella pneumoniae</i>	CP0142	SHV-wt; TEM-wt+164S; ACT
<i>Klebsiella pneumoniae</i>	CP0143	CMY-2; SHV-wt
<i>Klebsiella pneumoniae</i>	CP0233	KPC; TEM-wt; SHV-wt
<i>Klebsiella pneumoniae</i>	CP0243	VIM; SHV-wt
<i>Klebsiella pneumoniae</i>	CP0314	SHV-wt
<i>Klebsiella pneumoniae</i>	CP0316	SHV-wt
<i>Klebsiella pneumoniae</i>	CP0342	GES; SHV-wt
<i>Klebsiella pneumoniae</i>	CP0349	GES; SHV-wt
<i>Klebsiella pneumoniae</i>	CP0363	OXA-48; SHV-wt
<i>Klebsiella pneumoniae</i>	CP0365	KPC; SHV-wt
<i>Klebsiella pneumoniae</i>	CP0379	VIM; SHV-wt
<i>Klebsiella pneumoniae</i>	CP0392	KPC; TEM-wt; SHV-wt
<i>Klebsiella pneumoniae</i>	CP0393	KPC; TEM-WT; SHV-WT
<i>Klebsiella pneumoniae</i>	CP0418	OXA-48; TEM-WT; SHV-WT
<i>Proteus mirabilis</i>	CP0015	CMY-2; TEM-wt
<i>Proteus mirabilis</i>	CP0068	CMY-2; TEM-wt
<i>Proteus mirabilis</i>	CP0317	VEB; TEM-wt
<i>Providencia stuartii</i>	CP0319	VEB; TEM-wt
<i>Providencia rettgeri</i>	CP0358	VEB; NDM
<i>Pseudomonas aeruginosa</i>	CP0245	VIM
<i>Pseudomonas aeruginosa</i>	CP0246	VIM
<i>Pseudomonas aeruginosa</i>	CP0336	BEL; VIM
<i>Pseudomonas aeruginosa</i>	CP0352	PER; VIM
<i>Pseudomonas aeruginosa</i>	CP0358	VEB
<i>Salmonella barreilly</i>	CP0125	ACC
<i>Salmonella barreilly</i>	CP0127	ACC
<i>Salmonella heidelberg</i>	CP0133	CMY-2
<i>Salmonella heidelberg</i>	CP0139	CMY-2
<i>Stenotrophomonas maltophilia</i>	CP0062	---

TEM-wt and SHV-wt refer to wild type = non-ESBL TEM & SHV