User manual

Check-MDR CT103XL

Molecular Detection and Identification of Carbapenemase, MCR 1-2, AmpC and ESBL genes

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For Research Use Only
Not for use in diagnostic procedures

Contents

Introduction

The worldwide emergence and dissemination of resistance to 3rd and 4th generation β-lactam antibiotics and to carbapenems among gram-negative bacteria 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 this β-lactam resistance in gram-negative bacteria is the expression of Extended Spectrum β-Lactamases (ESBL's), AmpC β-Lactamases or carbapenemases. Check-MDR CT103XL is a rapid molecular test that reliably detects the presence of almost all clinically relevant AmpC β-lactamase, ESBL and carbapenemase genes in Enterobacteriaceae, Pseudomonadaceae and Acinetobacter spp. The assay is carried out with DNA isolated from cultured bacterial cells. The test results enable the confirmation and genotyping of bacterial isolates suspected of having AmpC, ESBL and/or carbapenemase genes.

Principle of the method

The principle of the Check-Points diagnostic system is based on specific molecular recognition of DNA target sequences and subsequent amplification with universal primers. Each individual DNA target is recognized by a specific probe that contains a unique ZIP code corresponding to a unique position (address) on the microarray. These ZIP codes are used for detection on the microarray after amplification. Probes consist of two segments (probe arms), which are joined by a DNA ligase when they match perfectly with the target DNA. Only connected probe arms will result in amplification products. Probes that differ from the target DNA will not give amplification products, even in the case of a single nucleotide difference. Amplification products are hybridized to the microarray and visualized by colorimetric detection. The microarrays are contained in so called CP Array Tubes, which are inserted in the Check-Points Tube Reader upon completion of the detection reaction. This generates an array image that is analyzed by dedicated software to yield a definitive and objective assay result.

Kit contents (for 24 samples)

Table 1

Components (Mat. No.)	Description	Storage conditions
Box Room Temperature		
Detection Buffer (9-0007)	1 bottle 80 ml	Room temperature
Blocking Buffer (9-0008)	1 bottle 20 ml	Room temperature
Staining Solution (9-0014)	1 bottle 5 ml	Room temperature **store in the dark**
CP Array Tubes (10-0003)	6 bags of 4 each (total 24)	Room temperature
Manual (9-0034)	Leaflet – download from website	Not critical
Box -20 °C		
Solution P (9-0035)	1 tube (purple cap ●) 100 µl	- 20°C
Solution A (9-0021)	1 tube (brown cap ●) 600 µl	- 20°C
Solution B3 (9-0019)	1 tube (white cap O) 1600 µl	- 20°C
Solution C (9-0024)	1 tube (red cap ●) 120 µl	- 20°C
Conjugate Solution (9-0027)	1 brown tube & black cap (●) 120 µl	- 20°C

Positive and negative controls are built into the system. It is, however, strongly recommended to use a positive and negative control for each series of reactions.

Materials required but not supplied with the kit

Table 2

	Pre-PCR	Post-PCR
Equipment	• Thermocycler* • Vortex Mixer • Mini-centrifuge	• Thermocycler* • Vortex Mixer • Mini-centrifuge • Thermomixer with active cooling* • Check-Points Tube Reader with E-Ads software • Computer with USB and internet connection • Barcode Reader (optional)
Supplies	• Disposable laboratory (powder-free) gloves • Pipettes & disposable (preferable filter-) tips for volumes of 1 to 1000 µl • 1.5 ml tubes ("Eppendorf tubes") • 10 ml tubes • PCR tubes (suitable for Thermocyclers used)	• Disposable laboratory (powder-free) gloves • Pipettes & disposable (preferable filter-) tips for volumes of 1 to 1000 µl • 1.5 ml tubes ("Eppendorf tubes") • 10 ml tubes

* contact your local representative for specifications.

Shelf life, Storage and Handling

Please check the individual components for optimal storage conditions immediately after delivery of the kit and store components accordingly. Reagents stored at the appropriate storage conditions can be used until the expiration date indicated on the boxes. Please visually inspect the boxes upon initial opening to ensure that their content is intact. Do not use when damaged. Please contact the Check-Points office at **support@check-points.com** if you have any questions or in case shipping has taken more than 2 days.

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:

- The test must be performed by adequately trained personnel.
- Spinning down for a few seconds is done in the various steps to ensure that all material is collected at the bottom of the tubes.
- Do not use reagents after their expiration date.
- Before use, thaw frozen reagents completely at room temperature and vortex briefly to obtain a homogeneous solution. After vortexing briefly, spin down the solution to avoid contamination when opening the lid. Avoid unnecessary freeze-thawing of the kit content.
- Periodically, verify the accuracy and precision of pipettes, as well as correct functioning of the instruments.

Prevention of contamination

PCR produces a very high quantity of DNA amplification products (amplicons) even from minute quantities of starting material. Check-MDR CT103XL may therefore yield unreliable results if samples become contaminated with amplicons from previous amplification reactions prior to the PCR (step B of the protocol). Preventive measures to minimize the risk of amplicon contamination must be taken. Please read carefully and follow the instructions outlined below.

Use separate rooms: a pre-PCR room and a post-PCR room.

- Sample preparation, DNA recognition (step A) and preparation of the amplification step (step B) is carried out in the pre-PCR room.
- Incubation in the PCR thermocycler of step B is carried out in the post-PCR room.
- The detection step is also carried out in the post-PCR room. Alternatively, the detection step is carried out in a third room separate from the other two rooms.
- Never bring the reaction products of step B to the pre-PCR room.
- Never prepare the ligation and/or amplification steps in the post-PCR 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 or samples to a reaction tube to avoid contamination.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Use separate equipment, pipettes, thermocyclers, sample holders, lab coats, gloves, disposables and reagents that are assigned to these rooms. Never transfer items from the post-PCR room to the pre-PCR room.
- Wear a clean lab coat and clean gloves during all steps of the test.
- Wear clean gloves and a clean lab coat not previously worn while handling amplified PCR products or during sample preparation.
- 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.

Users are strongly advised to read the full protocol before starting the test

Protocol

The protocol consists of the following steps:
1. DNA recognition step A
2. DNA amplification step B
3. Detection step

The procedure requires DNA of high quality. Please contact Check-Points for suitable DNA extraction methods that have been validated for use with Check-MDR CT103XL. Store DNA extracts according to the manufacturer's instructions for the DNA extraction method used.

1. DNA recognition step A

Important points before starting: step A is completely carried out in the pre-PCR room.

Procedure:
1. Thaw all reagents (i.e. Solution A, DNA samples if kept at -20˚C), mix well and keep on ice.
2. For *x* number of samples, add the following to a 1.5 ml tube: (*x* + 1)*2.5 µl Solution P (purple cap ●) and (*x* + 1)*5 µl Solution A (brown cap ●). Mix well and spin down briefly. The solution should have a uniform blue color.
3. Add 7.5 µl of this mixture to each reaction tube. Next add 10 µl DNA solution of each sample. For concentrated DNA solutions a smaller amount may be required, in that case adjust volume to 10 µl with MQ water. Please write down the sample reference for each tube.
4. Close the tubes, mix well by tapping against each tube and spin down briefly using the mini-centrifuge.
5. Place the tube(s) in the thermocycler and run the CP step A program (total sample volume 18 µl). The program will run for approximately 2.5 hours. (The step A program is outlined in Appendix 3).

Note*:*
- PCR-tubes/strips are NOT supplied with the kit.
- When closing PCR tubes, don't use excessive pressure as the cap may distort and the sample may evaporate during steps A and B.
- Refer to Appendix 3 if the settings of the thermocycler are lost.

2. DNA amplification step B

Important point before starting*:* the preparation of the step B reaction is carried out in the pre-PCR room.

Procedure:
1. Briefly spin down the reaction tubes when step A has been completed.
2. For amplification step B prepare BC-mix in a 1.5 ml or 10 ml tube. Take Solution B3 (white cap O) from the freezer. Thaw properly at room temperature, mix well, and spin down briefly before use. Then take Solution C (red cap ●) from the freezer. Prepare the required amount of BC-mix using the pipetting scheme in Appendix 4. First add the required amount of Solution B3 to the tube. Then dispense Solution C in Solution B3 by pipetting up and down 3 times. Mix very well by vortexing and spin down briefly.
3. Add 30 µl of the freshly prepared BC-mix to each reaction tube. Close the tubes, mix by tapping each tube and spin down briefly.
4. **Transfer the tubes to the post-PCR room**.
5. Place the tube(s) in the PCR thermocycler and run the CP step B program (total sample volume 48 µl). The program will run for approximately 1.5 hours. (The step B program is outlined in Appendix 3).
6. Briefly spin down the reaction tubes after amplification step B is completed.
7. After completion of step B the reaction tubes may be stored at 4˚C for maximum two hours (e.g. in the PCR thermocycler). Alternatively, store the reaction mixtures at -20°C for a maximum period of two weeks.

3. Detection step

Figure 1: *A) the CP Array Tube (AT) and B) the Check-Points Tube Reader.*

Important points before starting: the detection step is carried out in the post-PCR room.

Procedure:

1. Start preparing the required number of CP Array Tubes (ATs) for detection approximately 10 minutes before the end of step B. One AT is required for every reaction tube. Remove the ATs from their package(s) and place them in the thermomixer.
2. Add 300 µl Detection Buffer to each AT. Switch on the thermomixer and heat to 50˚C. It is not necessary to close the tubes.

Note:

- Be careful when removing or adding liquids with the pipette to or from the AT. Do not touch the microarray at the bottom of the tube at any time. Pipette all material in or out of the AT at the side of the bottom of the tube without touching the array.
- At 50˚C, remove and add solutions to one AT at a time to prevent the ATs from drying out.

3. When the thermomixer has reached 50˚C, incubate the tubes for 2 minutes (400 rpm).
4. Replace the Detection Buffer with 300 µl fresh Detection Buffer (one AT at a time), and incubate again for 2 minutes at 400 rpm.
5. Replace the Detection Buffer (one AT at a time) with 300 µl fresh Detection Buffer.
6. Take the samples from step B. Samples stored for longer than 2 hours after step B was completed should be heated in the PCR thermocycler at 98°C for 2 minutes (CP Melt program of the PCR thermocycler see Appendix 3). Briefly spin down the reaction mixture.
7. Transfer 10 µl reaction mixture from each reaction tube to the corresponding AT. Please note that <u>one</u> reaction tube corresponds to <u>one</u> AT. The total volume of the AT will be 310 µl. The lid of the AT may be labelled for reference.

Note:

- Samples may contain a white-colored precipitate. This is due to denaturation of one of the reaction components, a protein stabilizer. The presence of this precipitate has no effect on the result of the detection step and may be ignored when adding the sample.
- When adding samples to the AT <u>do not</u> remove the AT from the thermomixer, to prevent the buffer from cooling down. Add the sample directly into the Detection Buffer of the AT by pipetting up and down.

8. Close the lids of the ATs properly to prevent them from drying out and incubate the tubes for 30 minutes at 50°C (400 rpm).
9. After 30 minutes, replace the Detection Buffer with 300 µl Blocking Buffer: do this with one AT at a time! Remove the AT from the thermomixer, discard the Detection Buffer using a pipette and immediately replace with 300 µl Blocking Buffer using a new pipette tip. Place the AT back in the thermomixer at 50°C and proceed with the next AT until all the AT solutions have been replaced with Blocking Buffer. Incubate for 5 minutes at 50°C (400 rpm).

Optional:

- Evaporated water condensed in the lid of the AT may be removed with a pipette, e.g. before removing the Detection Buffer containing the sample.

Note:

- Collect the liquids removed from the ATs in a disposable tube, and dispose of it the same day with the other laboratory waste.

10. Replace the Blocking Buffer with 300 µl fresh Blocking Buffer. Set the temperature of the thermomixer to 30°C and incubate for 10 minutes (400 rpm). During this time the thermomixer containing the ATs will cool down from 50°C to 30°C.
11. During step 10, prepare a dilution of the Conjugate Solution (brown 0.5 ml tube with black cap●) with Detection Buffer using Appendix 4 at the back of this protocol. For this purpose a 1.5 ml tube or a 10 ml tube may be used depending on the amount required. Dispense the Conjugate Solution in the Detection Buffer by pipetting up and down 3 times. Mix well by vortexing for 30 seconds.

Note:

- The Conjugate Solution is stored at -20°C but is not frozen and can be used directly.
- Conjugate dilutions have to be made fresh.
- At 30˚C, remove solutions from all ATs before proceeding with adding new solutions to the ATs.

12. Remove the Blocking Buffer completely from all ATs. Then add 150 µl conjugate dilution to each AT. Incubate for 15 minutes at 30°C (400 rpm).
13. Remove the conjugate dilution from the ATs and add 600 µl Detection Buffer. Incubate the tubes for 2 minutes at 30°C (400 rpm).
14. Replace the Detection Buffer with 600 µl fresh Detection Buffer, and incubate the tubes again for 2 minutes at 30°C (400 rpm).
15. Remove the Detection Buffer from the ATs and add 150 µl Staining Solution to each AT. Incubate for 15 minutes at room temperature outside the thermomixer to complete the staining procedure. Continue with the image analysis immediately after the 15 minutes incubation time. Do not incubate with Staining Solution for more than 15 minutes, because images may become too dark.
    During the 15 minutes incubation time, complete the required sample information and relevant test data in the Check-Points software as outlined in point 16 a-g.

Note:

- Store the bottle with Staining Solution in the dark after use.

16. Filling out the experimental data:
    a. Start the computer
    b. Start software on the computer by double-clicking the Check-Points desktop icon:

    c. Double Click on **“Check-MDR** CT103XL**.arr”** in the first screen, “Array selection”, as shown in figure 2.

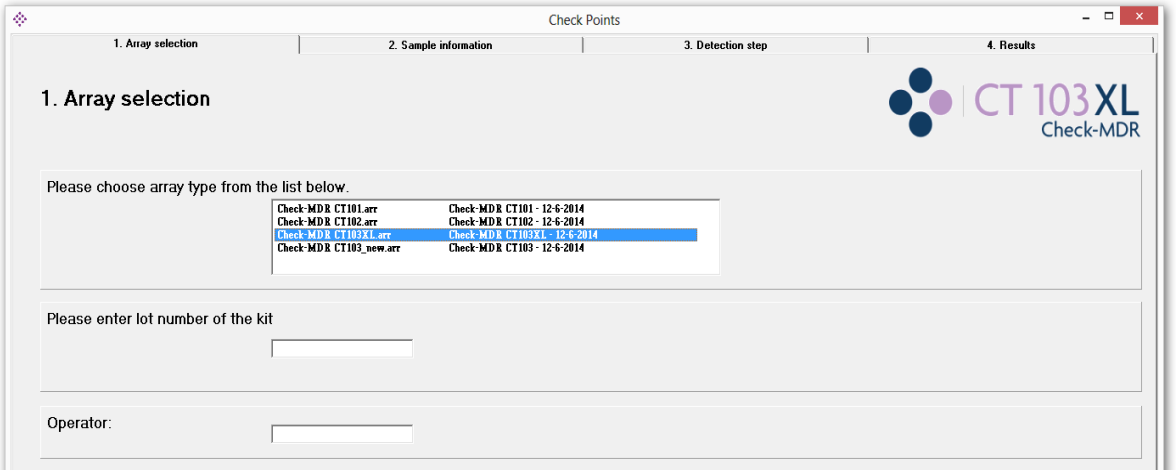

Figure 2: *“Array Selection”.*

d. Enter the lot number of the kit in the appropriate field and the name of the operator, followed by pressing the "Next Step" button.
e. Insert the sample codes in the screen "Sample information" (see figure 3).

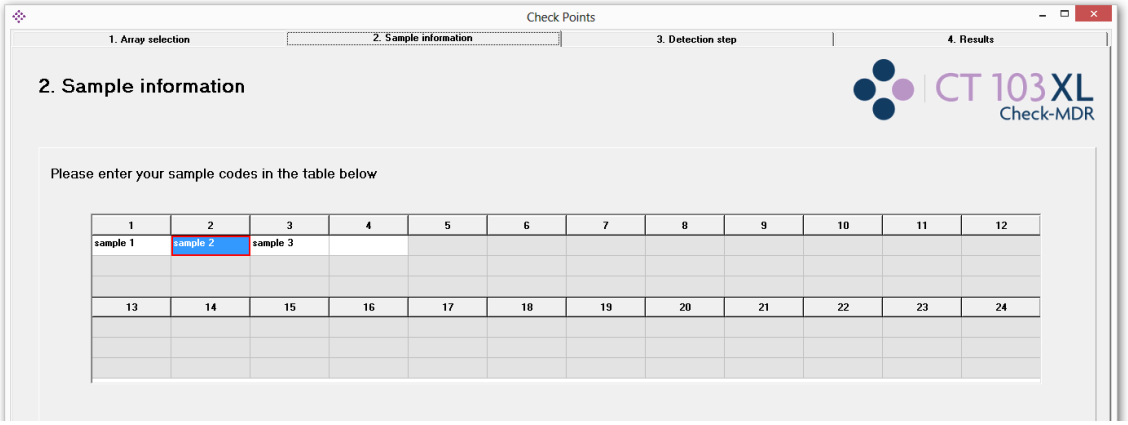

Figure 3: *"Sample information".*

Note:

- Additional remarks may be added per sample (optional), when sample references have been filled out. Double click on one of the sample reference fields. A pop-up will appear (see figure 4) allowing remarks to be added to individual or all samples by marking the box(es) of the sample(s) in question.

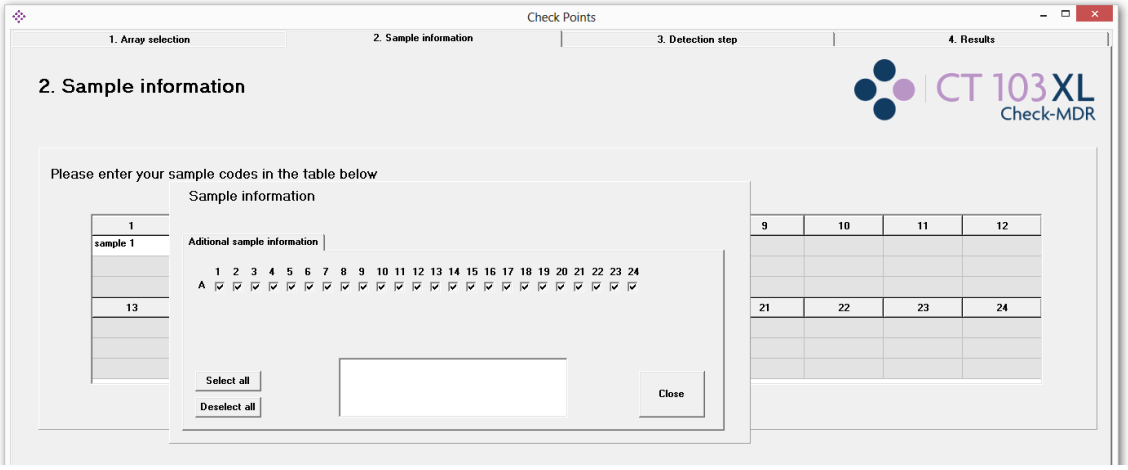

Figure 4: *Pop-up for additional sample information.*

f. Go to the "Detection step" screen (see figure 5) by clicking the "Next Step" button.
g. The software will now indicate the samples to be analyzed first.

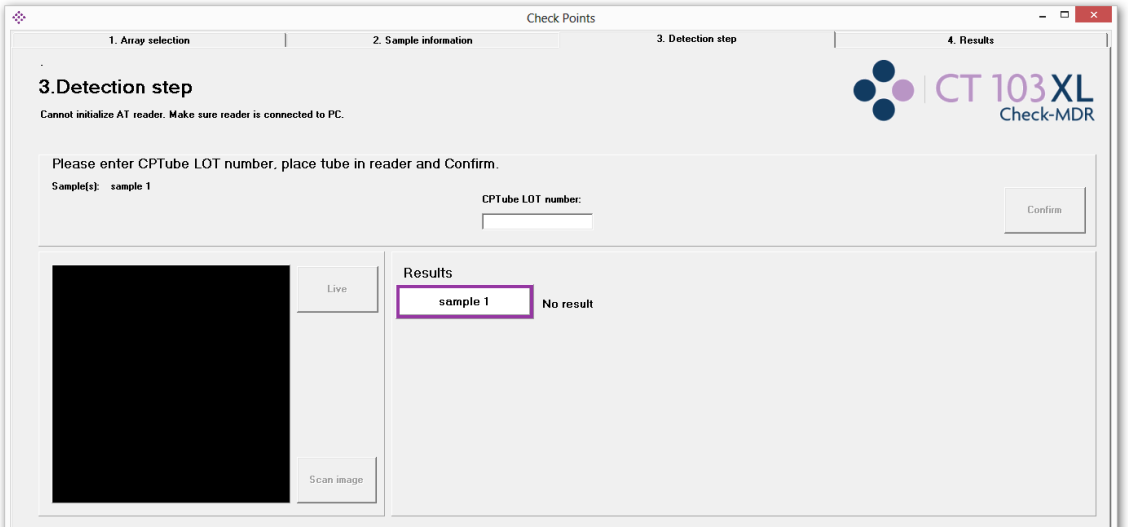

Figure 5: *"Detection step".*

17. Enter the AT lot number in the appropriate field when the 15 minutes incubation time has been completed (optional: use the Barcode Reader to scan the AT lot numbers). Next, insert the AT with open cap into the reader, close the lid of the reader, and click on the “Confirm” button in the software. Then click on “Scan image”: the results will be displayed immediately (see figure 6). Finally click on “Save Results” to save the results in the database. The software will now indicate which sample should be analyzed next. Repeat this step until all ATs are analyzed.

Figure 6: *Presentation of the final result.*

Note:

- It is important to adhere to the 15 minutes incubation time with Staining Solution as much as possible (step 15). A shorter incubation time may lead to faint images; exceeding the 15 minutes incubation time may lead to overstaining. In both cases incorrect results may be obtained.

18. When all ATs have been analyzed, a new window with the summary of the results will appear (see figure 7), which may also be printed (click on the “Print results” button). The results summary will display the sample ID and the presence/absence of carbapenemases, MCR, plasmidic AmpC and ESBL as well as combinations thereof (see table 3 for a list of the genes that can be reported). Please note that for the CTX-M-1 group, additional information regarding the subgroup will be displayed (see Appendix 2). Click on the “Quit” button to end this run of analyses.

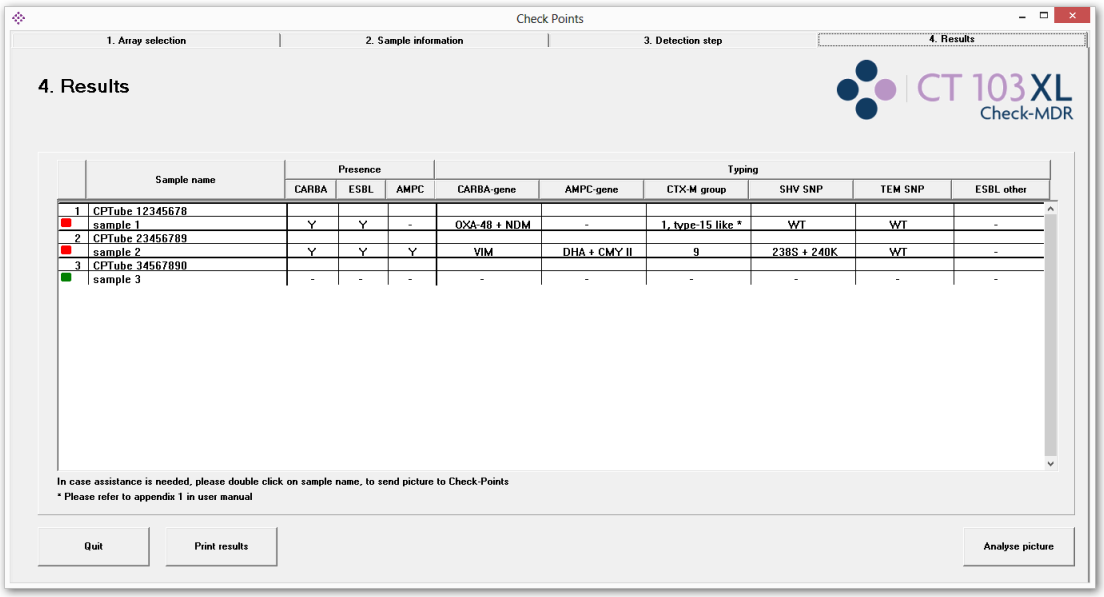

Figure 7: *Summary of the results.*

Table 3: *β-lactamase genes reported by the Check-Points software.*

Carbapenemases	ESBLs			Minor ESBLs	AmpCs	MCR
GES	CTX-M-1 group	TEM wt	SHV wt	BEL	ACC	MCR 1-2
GIM	CTX-M-1 subgroup	TEM 104K	SHV 238A	GES	ACT/MIR	
IMP	CTX-M-2 group	TEM 164C	SHV 238S	PER	CMY I/MOX	
KPC	CTX-M-3 subgroup	TEM 164H	SHV 240K	VEB	CMY II	
NDM	CTX-M-8 group	TEM 164S			DHA	
OXA-23	CTX-M-9 group	TEM 238S			FOX	
OXA-24	CTX-M-15 subgroup					
OXA-48	CTX-M-25 group					
OXA-58	CTX-M-32 subgroup					
VIM						
SPM						

Note:

- For support concerning results please send the result image along with the desired information to **support@check-points.com**. For this purpose, double click on the result(s) in question in the result summary window. A pop-up will appear (see figure 8) in which your comments may be added to the file.

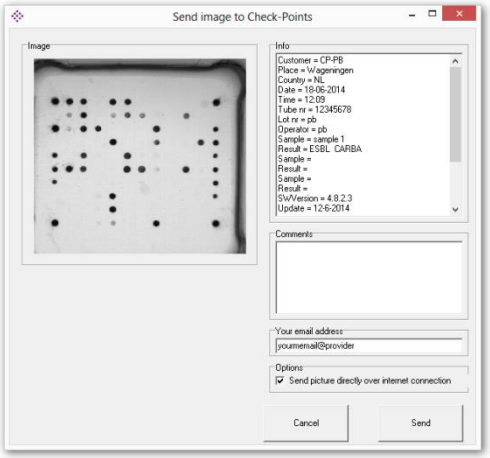

Figure 8: *"Send image to Check-Points" pop-up.*

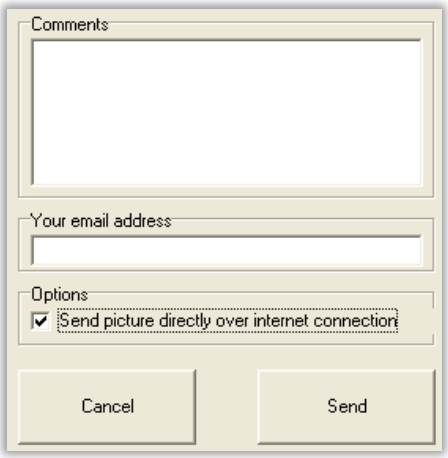

Figure 9: *Check the box to send pictures directly over the internet.*

Click on the "Save (Send later)" button to store the file, which will have a .cpfe extension on the computer and send it by e-mail. Alternatively, if the computer has internet access, check the box "Send picture directly over internet connection" and enter the reply e-mail address followed by clicking on send. In both cases feedback should be expected within two working days.

Support may also be requested at a later stage when the program has been closed. For this purpose, the "Send image to Check-Points" pop-up (see figure 9) may also be accessed from the database viewer ("DBview" shortcut that is located on the desktop). Open the database by clicking on "File" followed by "Open" (the database is located in C:\Images by default) and scroll down to the results which require support. By clicking on "Save", the "Send image to Check-Points" pop-up will appear just like in the result summary window.

Frequently asked questions (FAQ) & Troubleshooting

1. **The thermocycler states an error in step A or B.**
   Please contact Check-Points Technical Support: **support@check-points.com**
2. **During the PCR programs (step A or B) sample(s) have (partly) evaporated.**
   Tubes may not have been closed properly. Please restart the procedure from step A.
3. **I have left Solutions A, B and/or C out of the -20°C (-4˚F) storage.**
   These reagents must be stored at -20°C (-4°F) for proper performance of the test. The performance of the product cannot be fully guaranteed if these solutions were left out of -20°C (-4˚F) for longer than 24 hours.
4. **Staining Solution turned blue after adding it to the AT.**
   Conjugate dilution was not properly removed by washing steps 13 and 14 (detection step). Continue incubation with Staining Solution for 15 minutes and take the image as described in the protocol. If the image is too dark, please refer to question 5.
5. **The picture of the array is very dark.**
   The conjugate dilution (detection step 12) was not properly removed by washing steps 13 and 14 (detection step). Please replace the Staining Solution with Detection Buffer and take image immediately. If the image is still too dark, please repeat detection step with new AT.
6. **Software indicates: "Background intensity too high".**
   See question 5.
7. **The software indicates: "Image too weak".**
   The intensity of the spots is too low. Causes may be:
   7.1. The conjugate dilution was not prepared and/or added properly. Please repeat detection step with new AT.
   7.2. Incubation time with the Staining Solution was shorter than 15 minutes. Continue incubation up to 15 minutes.
   7.3. Detection Buffer was not removed properly before adding Staining Solution. Please repeat detection step with new AT.
8. **The software indicates: "Reference spots not found".**
   The software did not find the reference spots on the AT. Causes may be:
   8.1. An air bubble is interfering with the result. Tap the AT gently or pipette the liquid gently up and down and then retry to take the image.
   8.2. The picture is very dark: conjugate dilution was not removed properly. Please refer to question 5.
   8.3. The picture is completely white.
   Staining Solution was not added. Please add Staining Solution again and proceed from detection step 15.
   If the results do not improve, then the staining has failed. Most likely no conjugate dilution was added, or the conjugate dilution was not prepared properly. Please repeat detection step with new AT.
9. **The software indicates: "Hybridisation not OK".**
   The software did not find the hybridisation control spots on the AT. The hybridisation control is used to check if the hybridisation (at 50°C) of the PCR product with the AT has been performed properly. Causes may be:
   9.1. The picture is completely dark: conjugate dilution was not removed properly. Please refer to question 5.
   9.2. The reaction mixture was not added to the AT after completion of step B. Please repeat detection step with new AT.
   9.3. Hybridisation temperature too high. Please verify that the thermomixer temperature was 50°C when the ATs were hybridized.
   9.4. The BC-mix was not prepared properly or was not added to the assay: please repeat the test.
10. **The software indicates: "Reaction not OK", please contact Check-Points".**
    This message will be displayed in the following two scenarios:

    a) The software did not find the reaction control spots on the AT. These amplification controls are used to check the performance of the assay in steps A and B. Possible explanations are:
    10.1. The sample DNA was not added to the assay in step A.
    10.2. The sample DNA contains contaminants inhibiting the reactions. These may originate from the growth medium or the DNA isolation method. This may be remedied by diluting the DNA extract 10-fold with distilled water. Repeat the test with the diluted DNA.
    10.3. The A mix was not added in step A. Please repeat the test.
    10.4. The BC-mix was not prepared properly or was not added to the assay: please repeat the test.

    b) The amount of bacterial DNA added in step A was probably insufficient: the DNA extraction may have failed or the DNA concentration may have been too low. Please repeat the test with a new DNA extract.

11. **The software indicates: "ESBL suspected".**
    The software did not find sufficient spots to give a conclusive result. Please inspect the picture visually: an air bubble or dust particles may interfere with the result. Tap the AT gently or pipette the liquid gently up and down and retry to take the image. Please repeat the test if the results do not change.
12. **The software indicates: "Picture not found" or "Image capture error".**
    Check if the Check-Points Tube Reader is properly installed. Please contact Check-Points Technical Support at **support@check-points.com** if reinstallation of the reader does not solve this problem.
13. **The AT image is covered with small spots.**
    There may be various causes for this phenomenon. Most likely the array dried out during the detection step. Please make sure that the AT always contains sufficient amounts of reagents (Detection Buffer, Blocking Buffer, conjugate dilution or Staining Solution). This is particularly critical during the incubation steps at 50°C. In most cases the software will be able to handle these small spots. If not, repeat the detection step with a new AT.
14. **The AT was incubated for more than 15 minutes with Staining Solution before taking the image.**
    Results may be unreliable due to overstaining. Inspect image: if spots are very dark, please repeat detection step with a new AT.
15. **Duplicate DNA samples tested with Check-MDR CT103XL do not yield identical results.**
    15.1. Inspect images for dust particles. Tap or gently shake the tubes to try to remove the particles from the array and rescan the images.
    15.2. In case of extra spots, repeat the test to confirm result.
16. **May the assay be interrupted and continued at a later stage?**
    Reaction mixtures from Step A can be kept at hold at +4˚C for a maximum period of two hours. Reaction mixtures from Step B can be kept at -20˚C for a maximum period of two weeks. For further inquiries, please contact Check-Points Technical Support: **support@check-points.com**
17. **The spot intensities on various ATs are weak.**
    Please repeat the test. Contact Check-Points Technical Support at **support@check-points.com** if results do not improve.
18. **The AT image contains dust particles.**
    The software will correct this in most cases. To prevent any interference with the results, please take the AT out of the reader and shake it gently until the dust particles have moved to the side of the AT.

Limitations

Check-MDR CT103XL uses a range of specific DNA markers to identify the presence or absence of ESBL, AmpC, MCR 1/2 and carbapenemase genes. The test detects the presence of the ESBL genes TEM, SHV and CTX-M, PER, VEB, BEL and GES and it also detects Single Nucleotide Polymorphisms (SNPs) corresponding to amino acid positions 104, 164 and 238 in TEM, 238 and 240 in SHV and 170 in GES. The nature of the above SNPs in TEM and SHV determines the ESBL phenotype (ESBL yes or no) for the majority of TEM and SHV types. (For a detailed explanation see **www.lahey.org/studies** and M. Gniadkowski, Clin. Microbiol. Infect. 2008; 14 [Suppl. 1]: 11–32). Check-MDR CT103XL also detects the AmpC genes CMY, DHA, FOX, MOX, ACC, ACT and MIR, which presently represent the clinically most prevalent AmpC genes. It should be noted that other AmpC genes are not detected. Finally, Check-MDR CT103XL detects the carbapenemase genes KPC, NDM, OXA-48, VIM, IMP, GES, GIM, SPM, OXA-23, OXA-24 and OXA-58 , which presently represent the clinically most prevalent carbapenemase genes. Check-MDR CT103XL requires DNA purified from a colony or bacterial culture. Clinical specimens cannot be tested directly. The assay has been tested extensively with purified DNA from gram-negative bacteria, such as *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Pseudomonas* and *Acinetobacter,* 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-MDR CT103XL cannot and does not make any representation or warranty that it is capable of correctly detecting the ESBL, AmpC MCR1/2 and carbapenemase genes in all gram-negative species, subspecies or type or in any clinical sample source. 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 sub-types might not be detected. The test reflects the state of knowledge of Check-Points Health B.V. The quality of the input DNA is an important factor in obtaining reliable results from Check-MDR CT103XL. DNA must be extracted from cultured bacteria using a suitable method such as commercially available extraction methods based on columns or beads. Methods should be used that extract and purify total DNA, i.e. genomic as well as plasmid DNA. The presence of multiple bacterial species in a sample may hamper the interpretation of the test.
The Check-Points' software is continuously improving thanks to the expanding global database of available results. In order to further improve the software, original result images (including specific data*) are shared with this database

through a default setting in the software by means of an encrypted file. This data will be used solely for software improvements. All information will be kept strictly confidential and will not be shared with any third parties.

*The specific data included are: the number identifying the CP Array Tube, the kit lot number, the software version used, the operator and the customer name. All patient data and sample information is removed in the encrypted file. 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-MDR CT103*XL.

Key to symbols used

Symbol	Definition
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

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Check-Points
rapid molecular detection

Appendix 1: Performance Characteristics

In silico Specificity

The specificity of Check-MDR CT103XL is ensured by the design of optimal target-specific probes, as well as the selection of stringent reaction conditions. Probe sequences were designed to specifically identify the gene variants listed in the Table below. A 100% sequence match with the target-DNA segment of the probes by *in silico* analysis was assumed to warrant reliable detection of each of the depicted variants. Single mismatches 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.

Carbapenemase gene	Variants detected
GES-Carba	2,4-6,13-15,17,18,20,21
GIM	1
IMP	1,3-8,10,13,19,20,24-26,30
KPC	1 – 17
NDM	1 – 10
OXA-23 like	23,27,49,73,146,165-171,225,239
OXA-24 like	24-26,33,40,72,139,207
OXA-48 like	48,162,163,181,204,232,244,245,247,370
OXA-58 like	58,96,164
SPM	1
VIM	1 – 6 , 8 – 38
ESBL gene	**Variants detected**
BEL	1 – 3
CTX-M1 group	See appendix 2
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
CTX-M8/25 group	8,25,26,39-41,63,78,89,91,94
GES-ESBL	1,3,7-12,16,19,22
PER	1-7
SHV-ESBL	2-5,7,9,10,12,13,15,16,18-23,25,29,30,31,34,39,45,46,55,64,66,86,90,91,102,105,106,115,120,123,124, 126,128,129,134,141,152,153,160,163-165
TEM-ESBL	3-12,15,17,18,21,22,24,26-29,42,43,46-50,52,53,56,60,61,63,66,68,71,72,75,85-89,91-94,101,102,106, 107,109,111,113-115,118,120,121,123-125,129-134,136-138,139,142-144,147,149,151-155,158,165,167, 177,187,188,193,195,197,199,205,211
VEB	1-8
MCR gene	**Variants detected**
MCR	1,2
AmpC gene	**Variants detected**
ACC	1,2,4
ACT	1,3,6,7,9,10
CMY	1,2-12,14,38,43-45,49-57,59-63,71,73,80,94,95,99,110
DHA	1-3,5-7
FOX	1-10
MIR	1-6
MOX	1,2,4

Appendix 2: CTX-M1 group subtyping

The Check-Points software reports four subgroups belonging to the CTX-M1 group: CTX-M1-like, CTX-M15-like, CTX-M3-like and CTX-M32-like.

CTX-M1 group			
CTX-M1 subgroup	**CTX-M15 subgroup**	**CTX-M3 subgroup**	**CTX-M32 subgroup**
CTX-M-1	CTX-M-15	CTX-M-3	CTX-M-32
CTX-M-23	CTX-M-28	CTX-M-10	CTX-M-53
CTX-M-52	CTX-M-29	CTX-M-11	CTX-M-55
CTX-M-58	CTX-M-33	CTX-M-12	CTX-M-57
CTX-M-60	CTX-M-71	CTX-M-22	CTX-M-69
CTX-M-61	CTX-M-72	CTX-M-30	CTX-M-79
CTX-M-62	CTX-M-96	CTX-M-34	CTX-M-114
CTX-M-68	CTX-M-101	CTX-M-36	
CTX-M-116	CTX-M-103	CTX-M-37	
CTX-M-136	CTX-M-107	CTX-M-42	
CTX-M-139	CTX-M-108	CTX-M-54	
	CTX-M-109	CTX-M-66	
	CTX-M-117	CTX-M-88	
	CTX-M-132	CTX-M-133	
	CTX-M-142		

Appendix 3: Thermocycler settings

Step A

Step	Temperature / Time	Cycles
1	95°C / 3 min	(1x)
2	95°C / 30 sec	(24x)
	65°C / 5 min	
3	98°C / 2 min	(1x)
Hold	4°C	

Step B

Step	Temperature / Time	Cycles
1	95°C / 10 min	(1x)
2	95°C / 5 sec	(35x)
	55°C / 30 sec	
	72°C / 30 sec	
3	98°C / 2 min	(1x)
Hold	4°C	

Step Melt

Step	Temperature / Time	Cycles
1	98°C / 2 min	(1x)
Hold	4°C	

Appendix 4: Pipetting schemes

Pipetting scheme for BC-mix:

samples	µl B3	µl C
1	60	2
2	90	3
3	120	4
4	210	7
5	210	7
6	210	7
7	300	10
8	300	10
9	300	10
10	390	13
11	390	13
12	390	13
13	510	17
14	510	17
15	510	17
16	600	20
17	600	20
18	600	20
19	690	23
20	690	23
21	690	23
22	780	26
23	780	26
24	780	26

Pipetting scheme for conjugate dilution:

ATs	µl Conjugate Solution	µl Det. Buf.
1	3	295
2	4	395
3	5	495
4	10	990
5	10	990
6	10	990
7	15	1485
8	15	1485
9	15	1485
10	20	1980
11	20	1980
12	20	1980
13	25	2475
14	25	2475
15	25	2475
16	30	2970
17	30	2970
18	30	2970
19	35	3465
20	35	3465
21	35	3465
22	40	3960
23	40	3960
24	40	3960