Instructions for Use
Life Science Kits & Assays

RoboGene® MTB DNA Qualitative Kit

analytikjena
An Endress+Hauser Company

Order No.:
For use with ABI PRISM® 7000/7300 SDS
847-0207300502 128 reactions
847-0207300504 72 reactions
For use with Rotor-Gene™ 3000/6000
847-0207300542 120 reactions
847-0207300544 60 reactions
For use with MiniOpticon, CFX96 and 7500 Fast
847-0207300562 128 reactions
847-0207300564 72 reactions
For use with TOptical, qTOWER2&3 and LightCycler
847-0207300582 128 reactions
847-0207300584 72 reactions
For use with SmartCycler
847-0207300552 120 reactions
847-0207300554 60 reactions

Publication No.: Manual_RoboGene_MTB_DNA_Quantification_Kit_e_rev2

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Contents

1 Introduction

1.1 Intended use

The RoboGene® MTB Qualitative Kit is a real-time PCR test which specifically detects the strains *M. tuberculosis* and *M. bovis* by targeting both the multicopy target IS6110 insertion element and also a common genomic subsequence. The test is intended for rapid qualitative detection of *Mycobacterium tuberculosis* (MTB) DNA from sputum, bronchalveolar lavage, or tissue biopsies (e.g. lymph nodes). Additionally, the kit includes an internal control DNA which is simultaneously amplified with the *MTB* target. It is important to point out that the validity of PCR results depends on the use of the internal controls parallel to all PCR steps and the combination of the kit with a highly efficient DNA extraction method.

The RoboGene® MTB Qualitative Kit is intended for research use only but not for diagnostic procedures.

1.2 Pathogen information

Tuberculosis (TB) kills 3,000,000 people in the world every year, more than AIDS, malaria, and other tropical diseases combined. One third of the world's population is infected with tuberculosis. TB is the leading infectious disease cause of death and represents more than a quarter of the world's preventable deaths. The highest prevalence of TB infection and estimated annual risk of TB infection are in sub-Saharan Africa and Southeast Asia [3, 4]. Transmission of TB occurs primarily by the aerosol route but can also occur through the gastrointestinal tract. Coughing by people with active TB produces droplet nuclei containing infectious organisms which can remain suspended in the air for several hours. Infection occurs if inhalation of these droplets results in the organism reaching the alveoli of the lungs. Only 10 % of immunocompetent people infected with *M. tuberculosis* develop active disease in their lifetime - the other 90 % do not become ill and cannot transmit the organism.

However, in some groups such as infants or the immunodeficient (e.g. those with AIDS or malnutrition) the proportion who develop clinical TB is much higher. In the lung, the microorganism is taken up by alveolar macrophages and carried to lymph nodes, from where it may spread to multiple organs. Two to eight weeks after infection, cell mediated immunity and hypersensitivity develop leading to the characteristic reaction to the tuberculin test and, in immunocompetent individuals, containment of infection. Inflammatory immune responses eventually result in lung damage. An insertion sequence-like element, IS6110, was isolated from a *M. tuberculosis* cosmid library as a repetitive sequence. IS6110 shows similarities with elements of the IS3 family. This insertion sequence found to be specific to mycobacteria belonging to the *M. tuberculosis* complex 5 is, therefore, a well suited target for highly sensitive detection by methods such as real-time PCR.

CONSULT INSTRUCTION FOR USE

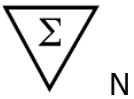
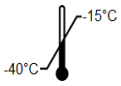
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.3 Technical assistance

In case of any problem with the RoboGene® MTB Qualitative Kit please contact our technical support team which consists of experienced scientists with long-time experience in the field of molecular diagnosis particularly of real-time PCR detection of pathogens. For technical assistance please contact us as shown inside the cover of the IFU.

1.4 Notes on the use of this instructions for use

For easy reference and orientation, the IFU uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number
	**Content** Contains sufficient reagents for <N> tests
	**Storage conditions**
	**Consult instructions for use** This information must be observed to avoid improper use of the kit and the kit components.
	**Use by**
	**Lot number** Lot number of the kit or component
	**Manufactured by**
COMP	**Component**
VOL	**Volume**
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following abbreviations are used in the IFU:

Ct	Threshold cycle value
dTTP	2′-deoxythymidine 5′-triphosphate
dUTP	2′-deoxyuracyl 5′-triphosphate
dNTP	2′-deoxynucleotide 5′-triphosphate
IC	Internal Control
IFU	Instruction For Use
IS	Insertion sequence
IU	International Units
M	Mycobacterium
MTB	*Mycobacterium tuberculosis*
NTC	Non-template control
PEI	Paul-Ehrlich-Institut, Langen, Germany
SD	Standard deviation
TB	Tuberculosis

2 Safety precautions

NOTE
Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the IFU, as well as all messages and information, which are shown.

- Human samples have to be considered as potentially infectious. Thus, always wear lab coat and gloves.
- Always use clean and nuclease-free equipment.
- Set up of template preparation, PCR reagent assembly, amplification and detection should be performed in different rooms.
- Discard sample and assay waste according to your in-house safety regulations.

ATTENTION
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personnel in a laboratory environment!

3 Test description and principle

3.1 Principle of the TaqMan® assay

TaqMan® real-time PCR is a highly sensitive assay that combines amplification with fluorescence-based online detection of the nucleic acid of interest (target, template). The assay is based on a conventional set of target-specific primers in combination with a fluorescence-labelled oligonucleotide probe, complementary to the desired target sequence. In the presence of target the probe hybridizes with its target-complementary sequence. The Taq DNA polymerase which possesses a 5`→ 3` exonuclease activity cleaves the probe and displaces the fluorescent dye. The resulting increase of fluorescence is directly proportional to the target amplification during PCR.

3.2 Explanation of the MTB Qualitative test

The RoboGene® MTB DNA Qualitative Kit is an amplification test for detection of *MTB* DNA in human sputum, bronchalveolar lavage, or e.g. lymph node samples. Both *M. tuberculosis* and *M. bovis* are amplified with equal efficiency applying primers and probe sets specific for the insertion sequence IS6110 and the *MTB* genome. The qualitative standard consists of tubes coated with a known amount of synthetic *MTB* DNA, which must be amplified in parallel with the samples.

A synthetic internal control (IC) is included via extraction tubes to control DNA extraction and to indicate for inhibitory effect on detection. Thus, the risk concerning false-negative results is drastically reduced yielding in increase of detection correctness. Amplification of *MTB* DNA in samples and standards and of IC DNA is measured independently at different wavelengths due to probes labelled with different fluorescence reporter dyes (*MTB* DNA: FAM channel; IC DNA: Yakima Yellow/VIC/JOE channel).

For sample preparation the “INSTANT Mycobacterium DNA Kit” (Analytik Jena) is recommended. DNA extraction must be performed with the respective starting sample volume strictly according to manufacturer’s instructions. Concerning final elution of filter bound DNA the use of 60 µl of elution buffer is supposed, respectively.

NOTE
The respective Internal Control is stabilized within the extraction tubes contained in the RoboGene® MTB DNA Qualitative Kit!

3.3 Restrictions

This test is validated with mucosal samples spiked with *MTB* bacterial DNA. If other than the recommended sample types are used wrong results may be obtained. The product is to be used only by educated personnel in a laboratory environment. Strict compliance with the IFU is required for optimal PCR results. The product is validated only with the mentioned real-time PCR instruments. Do not use expired components or mix with components from different batches.

For reliable results the kit has to be validated according to local lab procedures using available lab equipment e.g. real-time PCR-System, PCR consumables and deviant purification system.

4 Performance assessment

4.1 Analytical sensitivity

The analytical sensitivity is defined as the smallest amount of target that can be precisely detected in a sample. Using synthetic *MTB* DNA in different dilutions a >95 % cut-off value of at least 10 copies was calculated for the assay. Individual values below the detection limit may be plausible but with a high probability of error. To reduce this error probability 3 replicates of such samples are recommended.

4.2 Linear range

The linear range of the RoboGene® MTB DNA Qualitative Kit was found from 2x 10^2 to at least 2x 10^9 copies per run as shown in Figure 1. This evaluation was performed with serial dilutions of a synthetic *MTB* DNA specimen and 3 replicates at each level. The study was performed Rotor-Gene® 3000

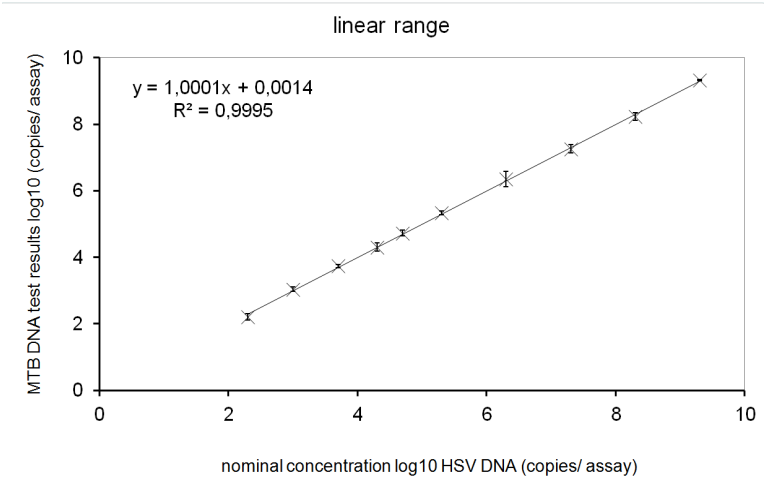


Figure 1: Linearity of the RoboGene® MTB DNA Qualitative Kit on Rotor-Gene® 3000

4.3 Analytical specificity

Sputum samples of healthy donors not diseased from TB were analyzed to determine the specificity of the RoboGene® MTB DNA Qualitative Kit, which is expressed as negative result in absence of the target. 10 donor samples were analyzed to determine the specificity (Table 1). The RoboGene® MTB DNA Qualitative Kit has a good specificity. None of the analyzed samples gave positive test results for *MTB* DNA.

Table 1: Specificity of the RoboGene® MTB DNA Qualification Kit

Analyzed samples	MTB positive	IC-DNA positive
Sputum samples of healthy donors (n=10)	0	10

5 Kit components, storage and stability

Table 2: General kit components of RoboGene® MTB DNA Qualitative Kit

General kit components				
Component	Σ 60/72	Σ 120/128	Description	Box No.
DNA_D1	50 tubes	100 tubes	Extraction tubes coated with IC DNA and carrier nucleic acid	2
MTB_D4 	2 vials	4 vials	Reagent mix lyophilized with *MTB*/IC-specific primers, -probes and dNTPs	1
Taq Polymerase FS1 	1 x 0.03 ml	2 x 0.03 ml	Taq Polymerase, 5 U/µl	separate
10x PCR buffer FS1 	1 x 0.50 ml	1 x 0.50 ml	10x PCR buffer with $MgCl_2$	separate
PCR grade water DNA 	2 x 1.50 ml	4 x 1.50 ml	PCR grade water	1
IFU	1	1		1

Table 3: Kit components for application to Rotor-Gene® 3000/6000/Q using regular profile tubes 0.2 ml (clear)

Component	Σ 60	Σ 120	Description	Box No.
REF	847-0207300544	847-0207300542		
MTB_D2_RG	50 tubes	100 tubes	Sample tubes coated with amplification enhancer	1
MTB_D3_RG	10 tubes	20 tubes	Qualitative standard tubes coated with cut-off amount of *MTB* DNA (about 250 copies per tube), IC DNA and amplification enhancer	1

Table 4: Kit components for application to TOptical, qTOWER2&3 and LightCycler® 480 II using low profile strips 0.1 ml (white)

Component	Σ 72	Σ 128	Description	Box No.
REF	847-0207300584	847-0207300582		
MTB_D2_LPW	7 strips (7x 8 tubes)	13 strips (13x 8 tubes)	Sample tubes coated with amplification enhancer	1
MTB_D3_LPW	2 strips (2x 8 tubes)	3 strips (3x 8 tubes)	Qualitative standard tubes coated with cut-off amount of *MTB* DNA (about 250 copies per tube), IC DNA and amplification enhancer	1
OT_AB	1	2	Optical tape	1

Table 5: Kit components for application to MiniOpticon, CFX96 and ABI® 7500 Fast using low profile strips 0.1 ml

Component	Σ 72	Σ 128	Description	Box No.
REF	847-0207300564	847-0207300562		
MTB_D2_LP	7 strips (7x 8 tubes)	13 strips (13x8 tubes)	Sample tubes coated with amplification enhancer	1
MTB_D3_LP	2 strips (2x 8 tubes)	3 strips (3x 8 tubes)	Qualitative standard tubes coated with cut-off amount of *MTB* DNA (about 250 copies per tube), IC DNA and amplification enhancer	1
OT_AB	1	2	Optical tape	1
10x ROX [1)]	1x 0.50 ml	1x 0.50 ml	10x ROX passive reference dye	1

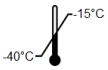
[1)] Kit component not required for use with MiniOpticon and CFX96

Table 6: Kit components for application to ABI PRISM® 7000/7300 SDS

Component	Σ 72	Σ 128	Description	Box No.
REF	847-0207300504	847-0207300502		
MTB_D2_AB	7 strips (7x 8 tubes)	13 strips (13x8 tubes)	Sample tubes coated with amplification enhancer	1
MTB_D3_AB	2 strips (2x 8 tubes)	3 strips (3x 8 tubes)	Qualitative standard tubes coated with cut-off amount of *MTB* DNA (about 250 copies per tube), IC DNA and amplification enhancer	1
OT_AB	1	2	Optical tape	1
10x ROX	1x 0.50 ml	1x 0.50 ml	10x ROX passive reference dye	1

Table 7: Kit components for application to SmartCycler

Component	Σ 60	Σ 120	Description	Box No.
REF	847-0207300554	847-0207300552		
MTB_D2_SC	50 tubes	100 tubes	Sample tubes coated with amplification enhancer	1
MTB_D3_SC	10 tubes	20 tubes	Qualitative standard tubes coated with cut-off amount of *MTB* DNA (about 250 copies per tube), IC DNA and amplification enhancer	1
10x BSA	1x 0.50 ml	1x 0.50 ml	10x BSA	1



STORAGE CONDITIONS

The RoboGene® MTB Qualitative Kit is delivered at room temperature except the Taq polymerase enzyme which is shipped on dry ice. Store the RoboGene® MTB Qualitative Kit incl. Taq polymerase enzyme at (-18°C) – (-40°C) in the dark. The kit is stable until the expiration date when stored under these conditions.

IMPORTANT

An appropriate amount of Reagent mix (MTB_D4) should be dissolved in PCR grade water shortly before use. Remaining dissolved reagent mix can be stored at 2 - 8 °C up to 14 days (do not freeze!). Always protect from light!

6 Necessary laboratory equipment and additives

- *MTB*-positive samples; provided qualitative standards (MTB_D3_xx) may be considered as positive control
- *MTB* negative control (e.g. mucosal samples free of *MTB* DNA)
- Rotor-Gene® 3000/6000, ABI PRISM® 7000/7300 SDS, MiniOpticon, CFX96, ABI® 7500 Fast, TOptical, qTOWER2&3, LightCycler® 480 II, SmartCycler
- Real-time instrument specific software for data analysis and reporting
- Suitable pipetting tools
- Micro centrifuge applicable for 0.2 ml and 1.5 ml tubes
- Vortex mixer
- 1.5 ml tubes
- Sterile pipette aerosol-barrier tips
- Applicator for optical tape and compression pad (for application to ABI PRISM® 7000/7300 SDS)
- Precision plate holder for tube strips (for application to 7500 Fast Real Time PCR System)
- Adapter Plate for tube strips (for application to LightCycler® 480)
- Gloves, lab coat

7 Procedure

7.1 Collection and handling of clinical samples

- Sputum, bronchalveolar lavage, or tissue biopsies (e.g. lymph nodes) should be collected in sterile tubes or syringes
- Analyze samples within one day or store samples frozen until use

7.2 MTB DNA purification from clinical samples

The RoboGene® MTB DNA Qualitative Kit has been verified together with the INSTANT Mycobacteria DNA Kit (Analytik Jena, 847-0259300102). The *MTB* DNA purification steps were performed according to the instructions for use.

NOTE
Include at least one replicate each of positive control plasma, negative control plasma, and NTC per run. Control plasma is not contained in the kit.

7.3 Internal DNA Control

The RoboGene® MTB Qualitative Kit is provided together with stabilized internal control DNA (IC DNA). The IC DNA is contained in the extraction tubes which are stably coated with IC DNA and carrier nucleic acid, respectively. The tubes are labelled with DNA_D1 and are contained in box 2 of the kit.

Applying the IC DNA containing extraction tubes together with the DNA extraction kit of choice always allows controlling for extraction yield, inhibitor load and judging the efficiencies of DNA extraction and subsequent PCR amplification, respectively. False-negatives due to failed

extraction or excess of inhibitors in the sample may be excluded when getting positive amplification results for the internal control.

In case of using the INSTANT Mycobacteria DNA Kit or any other bacteria DNA extraction kit replace the original lysis tubes labelled with "Extraction tubes" by the extraction tubes (DNA_D1) contained in box 2. To consider the purification successful, the Ct value of the IC DNA purified together with *MTB* negative samples should be ≤ 35.

NOTE
Please add at first the lysis solution contained in the respective DNA purification kit to the extraction tubes containing the IC DNA and subsequently the patient sample. Do not add the sample directly to the extraction tube. Since the IC DNA is already contained in the extraction tube no additional pipetting steps are required.

7.4 General procedure of qualitative analysis

The qualitative standards are provided as ready-to use standard strips which are stably coated with a defined amount of *MTB* standard DNA [1, 2].

NOTE
Please note that the standard values, and thus quantification, are dependent on the DNA purification kit as well as the used real-time PCR device and device specific consumables used together with the RoboGene® MTB DNA Qualitative Kit. For valid quantification results the whole procedure needs to be validated.

8 Protocol

8.1 Preparation of 5x Reagent Mix (MTB_D4, 5x)

1. Centrifuge the **MTB_D4** briefly at full speed to collect the lyophilized Reagent Mix on the bottom of the tube.
2. Add 200 µl **PCR grade water DNA** to **MTB_D4**; close the tube, mix by brief vortexing followed by brief centrifugation at full speed.
3. Incubate at 37 °C for 20 min using a thermal mixer (800 - 1,000 rpm), mix by brief vortexing followed by brief centrifugation at maximum speed.

NOTE
Dissolved Reagent Mix can be stored at 2-8 °C and always protected from light up to 14 days (do not freeze!)

8.2 Preparation of reaction set

1. Prepare the 1x Master Mix according to the following table. Mix by vortexing for at least 3 s followed by brief centrifugation.

Table 8: Composition of 1x Master Mix per reaction

Reagent	Volume for 1x rxn (µl)				Final concentration
	RotorGene 3000/6000/Q, TOptical, qTOWER 2&3, LightCycler® 480 II, MiniOpticon and CFX96	ABI PRISM® 7000/7300 SDS	ABI ® 7500 Fast	SmartCycler	
PCR grade water	12.1	9.6	11.85	9.6	-
10x PCR buffer FS1	2.5	2.5	2.5	2.5	1x
MTB_D4, 5x	5.0	5.0	5.0	5.0	1x
10x ROX	-	2.5	0.25	-	1x
10x BSA				2.5	
Taq Polymerase FS1 (5 U/µl)	0.4	0.4	0.4	0.4	2 U/reaction
Total	20	20	20	20	

2. Identify sample tubes (MTB_D2_xx) and standards (MTB_D3_xx) carefully and place them onto a suitable rack.

NOTE
Attention should be paid to correct orientation of standards.

3. Add 20 µl 1x Master Mix to sample tubes and each tube with standards.
4. Add 5 µl **PCR grade water** to tubes that serve as NTC and to all quantification standards containing the 1x Master Mix. Do not exceed a final reaction volume of 25 µl.

5. Add 5 µl of eluate from DNA isolation (e. g. using the INSTANT Mycobacteria DNA Kit) to the respective sample tubes containing the 1x Master Mix. Do not exceed a final reaction volume of 25 µl.

NOTE
In order to continue preparation of real-time PCR setup, please choose the corresponding real-time PCR cycler and follow the instructions.

Rotor-Gene® 3000/6000/Q

6. Cover the tubes with attached caps.
7. Program the applied real-time PCR platforms as indicated in Table 9 below and start the program.

Table 9: PCR program for Rotor-Gene® 3000/6000/Q

Cycle	Profile	Temperature	Time
1	Taq activation	95 °C	4 min
45	Denaturation	95 °C	15 sec
	Annealing/Elongation*	59 °C	1 min
* Data acquisition: Fluorescence Detection (FAM; JOE/VIC)			

ABI PRISM® 7000/7300 SDS

6. Cut optical tape (**OT_AB**) according to the required size and cover sample and quantitation standard strips carefully. Prevent cut-injuries! Use of an appropriate applicator for fixing the tape at the tube surface of the strips is recommended.
7. Centrifuge rack with loaded strips at 200x g for 1 min.
8. Program the applied real-time PCR platforms as indicated in Table 10 below and start the program.

Table 10: PCR program for ABI PRISM® 7000/7300 SDS

Cycle	Profile	Temperature	Time
1	Taq activation	95 °C	4 min
45	Denaturation	95 °C	30 sec
	Annealing/Elongation*	59 °C	1:30 min
* Data acquisition: Fluorescence Detection (FAM; JOE/VIC)			

TOptical, qTOWER 2&3 and LightCycler® 480 II

6. Cover the tubes with optical tape (**OT_AB**) according to the required size and cover sample and quantitation standard strips carefully. Use of an appropriate applicator for fixing the tape at the tube surface of the strips is recommended.

7. Centrifuge reaction plate with tubes at 200x g for 1 min.

8. Program the applied real-time PCR platforms as indicated in below Table 11 and start the program.

Table 11: PCR program for TOptical, qTOWER 2&3 and LightCycler® 480 II

Cycle	Profile	Temperature	Time	Ramping
1	Taq activation	95 °C	4 min	3.5 °C/sec
45	Denaturation	95 °C	30 sec	3.5 °C/sec
	Annealing/Elongation*	59 °C	1:50 min	2.5 °C/sec
* Data acquisition: Fluorescence Detection (FAM; JOE/VIC)				

MiniOpticon, CFX 96 and ABI® 7500 Fast

6. Cut optical tape (**OT_AB**) according to the required size and cover sample and standard strips carefully. Use of an appropriate applicator for fixing the tape at the tube surface of the strips is recommended.

7. Centrifuge reaction plate with tubes at 200x g for 1 min.
8. Program the applied real-time PCR platforms as indicated in below Table 12and start the program.

Table 12: PCR program for MiniOpticon, CFX96 and ABI® 7500 Fast

Cycle	Profile	Temperature	Time
1	Taq activation	95 °C	4 min
45	Denaturation	95 °C	20 sec
	Annealing/Elongation	59 °C	1:00 min
* Data acquisition: Fluorescence Detection (FAM; JOE)			

SmartCycler

6. Cover the tubes and centrifuge

7. Program the applied real-time PCR platforms as indicated in below Table 13 and start the program.

Table 13: PCR program for SmartCycler

Cycle	Profile	Temperature	Time
1	Taq activation	95 °C	4 min
50	Denaturation	95 °C	15 sec
	Annealing/Elongation	59 °C	1:00 min
* Data acquisition: Fluorescence Detection (FAM; JOE)			

9 Data analysis

Each DNA amplification is associated with generation of a fluorescence signal measurable in FAM channel (for *MTB* DNA) or in VIC/JOE channel (for IC) resulting in a sigmoid growth curve (log scale). The data analysis is performed according to manufacturer's instructions of the real-time PCR instrument using the respective software. Check the obtained data to ensure that the run is valid and to interpret results (Table 14). *MTB* DNA is determined based upon the Ct values for the sample *MTB* DNA. *MTB* DNA concentration is expressed as positive or negative according to the obtained Ct value.

NOTE

Setting of threshold may markedly influence Ct values

- Rotor-Gene: select Dynamic tube: Yes; Slope correction: Yes; Ignore first: 4; No template control threshold: 15 %; Threshold modus: manual, *MTB*: <0.01, IC DNA: 0.01-0.04
- ABI PRISM: select auto baseline and set threshold manually to > 0.05

Set the threshold for all not listed real-time PCR instruments in the exponential phase of the amplification curve according to the instructions of the specific real-time PCR instrument IFU.

Due to manufacturing reasons the amplification of 1 standard of the standard strip may fail and should be omitted from calculation. In such case no right for warranty of the whole product may be deduced

Table 14: Interpretation of the results

FAM channel	JOE/VIC channel [1)]	Interpretation
Interpretation of detection results		
x	x	valid, detection of sample *MTB* DNA
x	-	valid, detection of sample *MTB* DNA[1)]
-	x	valid, *MTB* negative sample
-	-	invalid, no amplification/detection at all, repeat run
x (< 10 copies/run)	x	below lower limit of detection range of test - no detection
x (> 2x 10^9 copies/run)	x	above detection range of test - dilute original sample with phosphate buffered saline and test once again

[1)] IC DNA-specific signal may be declining or missing in presence of a *MTB* DNA concentrations of >1x10^5 copies per run due to competition as tested with synthetic *MTB* DNA.

Expected Ct value for IC DNA (VIC/JOE channel) should be less than 37.

10 Troubleshooting

Problem / probable cause	Comments and suggestions
No signal at all	
▪ Fluorescence measurement not activated	Read the user guide of the real-time PCR device.
▪ False channels selected	Select FAM channel for *MTB* DNA and VIC/JOE channel for IC DNA.
▪ Incorrect cycling program	Check instrument settings, repeat run.
▪ Incorrect application of the kit	Read instruction for use.
▪ Storage conditions did not comply with instructions, expiry date of detection kit is exceeded	Check storage conditions and expiry date.
Low fluorescence signal recorded for both target and IC, target copy number underestimated	
▪ Target DNA degraded	Use DNase free consumables and reagents; store DNA immediately on ice after purification. Read instruction for use of the extraction kit.
▪ Optical lenses contaminated (Rotor-Gene)	See chapter “Maintenance” of respective instrument brochure, alternatively clean lens once per month using absolute isopropanol and cotton swabs.
▪ Thermal block and/or optics polluted (96-well format blocks)	See chapter “Maintenance” of respective instrument brochure, alternatively fill each well with isopropanol, incubate 10 min at 50 °C, remove isopropanol and rinse with ddH_2O
No or weak signal for IC DNA in MTB-negative sample	
▪ Incorrect cycling program	Check instrument settings, repeat run.

▪ Excess of inhibitors in the sample/ loss of DNA during extraction	Use the recommended extraction kit and follow exactly manufacturer's instructions.
▪ Incorrect sample material	Request for fresh material.
▪ Wrong sequence of reagent addition to extraction tube	Add lysis solution to extraction tubes prior to addition of the sample
▪ Storage conditions did not comply with instructions, expiry date of detection kit is exceeded	Check storage conditions and expiry date.
Unexpectedly low Ct values for IC DNA particularly with high standards or high viral load samples	
▪ Cross talk between target and IC recording channels (especially VIC/JOE)	Calibrate instrument using pure fluorescence dyes
Non-sigmoidal growth curves of qualitative standards, unacceptable high deviation of Ct	
▪ Frequent freezing/thawing or incorrect storage of dissolved reagent mix	Read IFU, check storage conditions, prepare new reagent mix.
▪ Storage conditions did not comply with instructions, expiry date of detection kit is exceeded	Check storage conditions and expiry date.
Different amplification behavior of sample MTB DNA and standards, non-parallel growth curves in exponential phase of reaction	
▪ Excess of inhibitors in the sample	Use the recommended extraction kit, follow exactly the manufacturer's instructions; consult attending doctor for patient medication.
▪ Incorrect sample material	Use recommended sample type.

FAM signal for MTB-negative samples / NTC recorded	
▪ Contamination with *MTB* DNA or DNA amplicons	Repeat extraction and/or PCR with new reagents; decontaminate instruments and work space.

If you have any further questions which are not answered, please contact our technical service.

11 References

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