FluoroType® MTBDR

VER 2.0

Instructions for Use

IFU-628-07

CE

IVD for in vitro diagnostic use only

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HAIN LIFESCIENCE

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FluoroType® MTBDR VER 2.0
Molecular Genetic Assay for Automated Detection of the *M. tuberculosis* Complex and its Resistance to Rifampicin and Isoniazid from Sputum Specimens and Cultivated Samples

Please read the instructions on hand and the operator's manual of the **FluoroCycler® XT** completely and carefully before using the kit. Strictly adhere to the established procedure to obtain correct test results.
If possible, register a new kit lot in the **FluoroSoftware® XT-IVD** immediately upon receipt (see chapter 4.4).

1 Intended Use

The **FluoroType® MTBDR** VER 2.0[1)] is a qualitative in vitro test for automated detection of the *Mycobacterium tuberculosis* (MTB) complex and its resistance to rifampicin (RMP) and/or isoniazid (INH) from sputum specimens and cultivated samples.
The test is indicated as an aid for diagnosis and intended for use in medical laboratories.
Use of this assay is limited to qualified personnel well trained in the test procedure and familiar with molecular biological methods.

1) This kit is sold under license from the Brandeis University and may be used under its patent rights only for human in vitro diagnostics.

2 Summary and Explanation

Tuberculosis (TB) is a bacterial infectious disease passed on by droplet infection. In 2019, there were an estimated 10.0 million incident cases of TB globally, and an estimated 1.4 million TB deaths [1].
The TB-causing MTB complex comprises the following species: *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium bovis* BCG, *Mycobacterium caprae*, *"Mycobacterium canettii"*, *Mycobacterium microti*, and *Mycobacterium pinnipedii*. TB treatment requires a therapy over several months. Emergence and spread of multidrug-resistant TB (MDR-TB) is a major medical and public problem threatening global health. MDR-TB is defined as TB that is resistant at least to RMP and INH, the two most important first-line anti-TB drugs [2]. MDR-TB is a challenge to TB control due to its complex diagnosis and obstacles in treatment. In 2019, there were an estimated 390,000 new cases of MDR-TB and another 110,000 patients with RMP resistance [1].
While resistance to RMP is mainly caused by mutations in the *rpoB* gene, resistance to INH can be caused by at least two different genes [3]; mutations at codon 315 in the *katG* gene are associated with a high-level INH resistance, mutations in the *inhA* promoter region are associated with a low-level INH resistance [4].
As long as MDR-TB is not verified, use of inadequate and hence ineffective antibiotics may lead to further spread of drug-resistant strains and further development of resistance. Therefore, rapid diagnosis and identification of MDR-TB is a prerequisite for appropriate treatment.

3 Principles of the Procedure

For automated DNA extraction from NALC-NaOH-decontaminated sputum specimens or mycobacteria grown in liquid medium (MGIT; BD Diagnostics, Franklin Lakes, USA), the **GenoXtract® 96** in combination with the **GXT96 X2 Extraction Kit** and the **Inactivation Set** or the **GenoXtract®** in combination with the **X2 Cartridge** and the **Inactivation Set** can be used. Alternatively, the **FluoroLyse** can be used for manual DNA extraction. Amplification and subsequent detection are performed with the **FluoroCycler® XT**.
With the **FluoroType® MTBDR** VER 2.0, resistance to RMP is identified by detection of the most significant associated mutations of the *rpoB* gene (coding for the β-subunit of the RNA polymerase). For detection of high-level INH resistance, the *katG* gene (coding for the catalase peroxidase) and for detection of low-level INH resistance, the promoter region of the *inhA* gene (coding for the NADH-enoyl-ACP reductase) are examined.
The **FluoroType® MTBDR** VER 2.0 is based on the **LiquidArray** technology. This technology combines asymmetric PCR [5] resulting in excess single-stranded amplicons with Lights-On/Lights-Off probes [6]. In a melting curve analysis, a characteristic fluorescence signature is detected in the **FluoroCycler® XT**. The interpretation of the fluorescence signature is displayed in the **FluoroCycler® XT Report** created by the **FluoroSoftware® XT-IVD**.

4 Reagents and Instruments

4.1 Kit contents

	Σ 24 [2]	Σ 96
Catalog number	**62824**	**62896**
Amplification Mix A (AM-A FT MTBDR VER 2.0) contains buffer, Taq polymerase, bovine serum albumin	144 µl	4x 144 µl
Amplification Mix B (AM-B FT MTBDR VER 2.0) contains buffer, specific oligonucleotides, nucleotides, bovine serum albumin, salts	336 µl	4x 336 µl
Universal Internal Control DNA 6 (U-IC6) contains specific polynucleotides	1.14 ml	2x 1.14 ml
Control DNA (C+ FT MTBDR VER 2.0) contains specific polynucleotides	220 µl	2x 220 µl
Instructions for use	1	1
Barcode label for Analyzer software	1	1
Lot label	2	2

[2] Available on request.

4.2 Storage, handling, and disposal of kit constituents

Kit constituent	Storage area	Storage temperature	Handling
AM-A, AM-B	Pre-PCR	–20°C to –18°C	Refreeze immediately after use. Avoid repeated freezing and thawing (> 4x); if necessary, aliquot using suitable screw cap tubes (recommendation: see chapter 4.5).
U-IC6	DNA extraction and detection	–20°C to –18°C	Refreeze immediately after use. Avoid repeated freezing and thawing (> 4x); if necessary, aliquot using suitable screw cap tubes (recommendation: see chapter 4.5).
C+	DNA extraction and detection	–20°C to –18°C	Manual extraction: Refreeze immediately after use. Avoid repeated freezing and thawing (> 4x); if necessary, aliquot using suitable screw cap tubes (recommendation: see chapter 4.5). Automated extraction: Do not reuse. Discard after first use.

Do not use the reagents beyond their expiry date.
Dispose of unused reagents and waste in accordance with federal, state, and local regulations.

4.3 Precautions for handling kit constituents

The kit constituents are not classified as hazardous substances. However, suitable protective clothing and gloves should always be worn. Observe all federal, state, and local safety and environmental regulations. AM-A and AM-B contain biological material. Hence, they must be considered as potentially infectious and must be handled accordingly (e.g. see [7] or [8]).

4.4 Registration of kit lots

The Analyzer is loaded into the **FluoroSoftware® XT-IVD** and contains all information required for processing and evaluating the assay. Each kit lot must be registered in the **FluoroSoftware® XT-IVD** prior to its first use to ensure that the required Analyzer version is loaded and correct interpretations are generated.

If possible, register a new kit lot immediately upon receipt. You can find the required information on the barcode label included in the kit. The information can either be scanned with a barcode reader or entered manually (see operator's manual of the **FluoroCycler® XT**); select "MTBDR 2.0".



Analyzer version

If a new Analyzer version is required, it can be accessed through: www.hain-lifescience.de/en/analyzer.html

4.5 Materials required but not included in the kit

General equipment

	Product	Description	Available from	Order number
	Disposable gloves			
	Pipettes	Calibrated, adjustable (10 to 1000 µl)		
	Sterile pipette tips	With filter, free from DNases		
	Table top centrifuge for 1.5 and 2 ml reaction tubes	Applicable for spinning down droplets and condensate		
	Table top centrifuge for PCR plates	Applicable for spinning down droplets and condensate		
	Vortexer			
	Reagents for NALC-NaOH sample decontamination	The necessary equipment is also required		
	Reagents for cultivation of mycobacteria	When cultivated samples (MGIT; BD Diagnostics, Franklin Lakes, USA) are to be used; the necessary equipment is also required		
Choose from	0.5 ml screw cap tube	For preparation of aliquots (see chapter 4.2); with skirted base, with assembled cap, sterile	Sarstedt, Nümbrecht, Germany	72.730.105
	1.5 ml screw cap tube	For preparation of aliquots (see chapter 4.2); without skirted base, with assembled cap, sterile	Sarstedt, Nümbrecht, Germany	72.692.005

Specific equipment

	Product		Description	Available from	Order number
GXT96 X2 Extraction Kit	**Inactivation Set**		For inactivation of mycobacteria in decontaminated patient specimens and cultured material	Hain Lifescience	522960
	Disposable transfer pipettes				
	Choose from	Copan Tube	Copan tube, 80 mm x 12 mm	Hain Lifescience	PFPM913S
		5 ml Tube	Screw cap tube 5 ml, 75 x 13 mm Screw cap	Sarstedt, Nümbrecht, Germany	60.504.010 65.163
		7 ml Tube	Screw cap tube 7 ml, with assembled cap, 82 x 13 mm	Sarstedt, Nümbrecht, Germany	60.550.109
	GXT96 X2 Extraction Kit		Kit for automated nucleic acid extraction of up to 960 samples using the **GenoXtract® 96**	Hain Lifescience	520960
	GenoXtract® 96		Instrument for nucleic acid extraction of up to 96 samples in one run	Hain Lifescience	900044
Automated PCR setup and amplification	0.5 ml screw cap tube		For preparation of C+ aliquots; with skirted base, with assembled cap, sterile	Hain Lifescience	72.730.005
	2 ml screw cap tube		For preparation of AM-A and AM-B; without skirted base, with assembled cap, sterile	Hain Lifescience	72.693.005
	5 ml screw cap tube		For automated master mix preparation; with skirted base, with assembled cap, sterile	Hain Lifescience	62.558.201
X2 Cartridge	**Inactivation Set**		For inactivation of mycobacteria in decontaminated patient specimens and cultured material	Hain Lifescience	522960
	Disposable transfer pipettes				
	1.5 ml screw cap tube		For eluate; without skirted base, with assembled cap, sterile	Sarstedt, Nümbrecht, Germany	72.692.005
	2 ml screw cap tube		For samples; without skirted base, with assembled cap, sterile	Sarstedt, Nümbrecht, Germany	72.693.005
	X2 Cartridge		Kit for automated nucleic acid extraction of up to 96 samples using the **GenoXtract®**	Hain Lifescience	12.02.02
	GenoXtract®		Instrument for nucleic acid extraction of up to 12 samples in one run	Hain Lifescience	8.31.01

	Product	Description	Available from	Order number
FluoroLyse	**FluoroLyse**	Kit for manual DNA extraction of 24 samples	Hain Lifescience	51624
		Kit for manual DNA extraction of 96 samples	Hain Lifescience	51696
	Disposable transfer pipettes			
	1.5 ml screw cap tube	For preparation of samples; without skirted base, with assembled cap, sterile	Sarstedt, Nümbrecht, Germany	72.692.005
	Water bath	Precision +/- 1°C		
Amplification	**FluoroCycler® XT**	Instrument for amplification and analysis of up to 96 samples in one run	Hain Lifescience	2017
	FrameStar® 96well PCR Plate Roche Style[3)]	96-well PCR plates, semi-skirted, white wells, clear frame, barcoded; 4titude	Hain Lifescience	1876705
	Heat sealer for 96 well PCR plates	For information on appropriate sheet heat sealer please refer to the operator's manual of the **FluoroCycler® XT**.		
	Clear Weld Seal Mark II	Sealing foils; 4titude	Hain Lifescience	1877137

3) *Disclaimer:* FrameStar® is covered by one or more of the following US patents or their foreign counterparts, owned by Eppendorf AG: US Patent Nos. 7,347,977 and 6,340,589. FrameStar® is a registered trademark owned by 4titude®Ltd.

5 Quality Control

Observe the usual precautions for amplification setup. It is essential that all materials (such as pipette tips) coming in contact with the reagents are free from DNases. Do not interchange or pool reagents from different kits unless the lots are identical (exception: U-IC6, see chapter 5.1). You can find the kit lot and the corresponding lots of the kit constituents on the lot labels included in the kit.

5.1 Internal control

The kit includes the Universal Internal Control DNA 6 (U-IC6) which serves as an extraction control, as an inhibition control for the amplification, and as a control of the correct performance of the test and the proper functioning of kit constituents. The U-IC6 is added to each sample during DNA extraction.
Isolates containing the U-IC6 can be used for all **FluoroType**® tests that are processed with the U-IC6 and are designed for the used sample material and DNA extraction method. There is no need for a new DNA extraction or for adding the U-IC6 again. A multiple addition of the U-IC6 can have a negative effect on the test evaluation and/or test performance.
The AM-B contains an internal marker to monitor sample quality and melting conditions. It is used by the **FluoroSoftware® XT-IVD** to adjust the fluorescence signatures to a standardized level.

5.2 External controls

A positive control sample containing the provided Control DNA (C+) is required for quality control and must be included for each run during PCR setup (see chapter 7). The C+ contains polynucleotides with specific sequences for mutations in the target regions (*rpoB:* S531L / S531W, *katG:* S315T1, and *inhA:* C-15T). In case of manual PCR setup, each of the C+ tubes provided is sufficient for 11 positive control samples. In case of automated PCR setup, each of the C+ tubes provided is sufficient for one positive control sample.

Note: Do not reuse the C+ tube after the **GenoXtract® 96** run. Discard the C+ tube (closed cap) after first use. Alternatively, transfer 40 µl C+ to a 0.5 ml screw cap tube (see chapter 4.5, Automated PCR setup) and place this in the **GenoXtract® 96** instead of the original C+ tube (refreeze original C+ tube immediately after use).

A negative control sample for detection of possible contamination events should be part of each run and is included during DNA extraction. In the negative control, only the U-IC6 must be detected.

6 Specimen Requirements

NALC-NaOH-decontaminated sputum specimens as well as mycobacteria grown in liquid medium (MGIT; BD Diagnostics, Franklin Lakes, USA) are used as starting material for DNA extraction. The performance of the test has not been validated with other sample materials.

6.1 Precautions for handling specimens

Patient specimens and cultures made from patient specimens must always be considered as infectious and must be handled accordingly (e.g. see [7] or [8]). Always wear suitable protective clothing and gloves. Samples from patients at risk (infected by pathogenic microorganisms or viruses including Hepatitis B and Human Immunodeficiency Virus (HIV)) and cultures made from those samples must always be labeled and handled under suitable safety conditions according to institutional guidelines.
All specimens that may contain mycobacteria should be handled applying Biosafety Level 2 practices or, when indicated, Biosafety Level 3 practices (e.g. see [7]). Observe all federal, state, and local safety regulations.
Discard used pipette tips immediately after use in a container for biohazardous waste. After finishing the assay, discard all used disposables in a container for biohazardous waste.

6.2 Storage and transport

All specimens should be collected and transported as recommended in the CDC publication "Public Health Mycobacteriology: A Guide for the Level III Laboratory"[9], the "Clinical Microbiology Procedures Handbook" [10], or your laboratory procedure manual.
It must be ensured that until decontamination, specimens are kept in sterile plastic containers at a temperature of 2°C to 8°C. The transport of specimens at room temperature must be carried out as soon as possible and should be done within 1-2 days [11,12]. Specimens used for decontamination must not be older than 4 days.
After decontamination and subsequent resuspension of the bacteria pellet with phosphate buffer, samples can be stored at –20°C or –80°C for a maximum of 5 days until performing DNA extraction.

6.3 Preparation

NALC-NaOH decontamination
Clinical specimens must be processed using the NALC-NaOH method according to the CDC publication "Public Health Mycobacteriology: A Guide for the Level III Laboratory" [9].
After decontamination, the cell pellet must be resuspended in 1 to 2 ml phosphate buffer. Due to the potential inhomogeneity of the specimen, the decontaminated sample must be mixed before removing the aliquot to be analyzed; otherwise the sensitivity of the test might be influenced.

Cultivation
If the sample is to be cultivated, cultivation must be performed in liquid medium (MGIT; BD Diagnostics, Franklin Lakes, USA).
Due to the potential inhomogeneity of the cultured sample, it must be mixed before removing the aliquot to be analyzed; otherwise the sensitivity of the test might be influenced.

Inactivation using the **Inactivation Set**
Prior to automated extraction, NALC-NaOH decontaminated clinical specimens or liquid culture samples must be processed using the **Inactivation Reagent** (included in the **Inactivation Set**, see respective instructions for use).

7 DNA Extraction and PCR Setup

The methods mentioned here were used for performance evaluation of the **FluoroType® MTBDR** VER 2.0. For correct evaluation it is mandatory that only these DNA extraction methods are applied.

Isolates containing the U-IC6 can be used for all **FluoroType®** tests that are processed with the U-IC6 and are designed for the used sample material and DNA extraction method. There is no need for a new DNA extraction or for adding the U-IC6 again. A multiple addition of the U-IC6 can have a negative effect on the test evaluation and/or test performance.

7.1 Automated DNA extraction with the GXT96 X2 Extraction Kit and automated PCR setup

The PCR setup can be performed automatically in the **GenoXtract® 96** directly following DNA extraction.

Please note that DNA extracted from sputum specimens and DNA extracted from cultivated samples may not be amplified in one PCR plate since different PCR protocols are required.

For details and options, please also refer to the instructions for use of the **GXT96 X2 Extraction Kit**, the instructions for use of the **Inactivation Set**, the operator's manual of the **GenoXtract® 96**, and the operator's manual of the **GenoXtract® 96 Software**.

7.1.1 Preparation of samples

Make sure that the sample tubes are compatible to the **GenoXtract® 96**. Respect the minimum and maximum filling levels (see instructions for use of the **GXT96 X2 Extraction Kit**).

1. **Mix sample material carefully but thoroughly by pipetting up and down with a disposable transfer pipette** (see chapter 4.5).
2. **Transfer 500 µl of sample material to a new sample tube** (see chapter 4.5)**, add 167 µl Inactivation Reagent (included in the Inactivation Set) and close tube properly.** Mix thoroughly by vortexing (5 s to 10 s) and incubate the sample for 30 min at room temperature (15°C to 25°C).
3. **A negative control sample for detection of possible contamination events should be part of each run.**

   Negative control for NALC-NaOH-decontaminated sputum specimens:

   Transfer 500 µl phosphate buffer (pH 6.8; same phosphate buffer as used for decontamination of patient specimens) to an empty sample tube (see chapter 4.5) and add 167 µl **Inactivation Reagent**.

   Negative control for liquid culture samples:

   Transfer 500 µl water (molecular biology grade) or phosphate buffer (pH 6.8; same phosphate buffer as used for decontamination of patient specimens) to an empty sample tube (see chapter 4.5) and add 167 µl **Inactivation Reagent**.

   The negative control sample is processed like regular samples.
4. **Starting at position 1, place the sample tubes in the sample tube racks.** If applicable, make sure that the barcode on the sample tube is facing the right side of the instrument where the barcode reader is located (for details on barcode specifications, please refer to the **GenoXtract® 96** operator's manual).
5. **Remove caps of sample tubes.**

7.1.2 Preparation of the Internal Control DNA

The U-IC6 is added to each sample during DNA extraction.

1. **Determine the total number of samples** (number of samples to be analyzed plus control samples).
2. **Thaw U-IC6 and equilibrate to room temperature** (15°C to 25°C)**, vortex for 5 s and spin down briefly.**
3. **Prepare a mix of X2-B6** (included in the **GXT96 X2 Extraction Kit**) **and U-IC6:**

   Prepare a 30 ml container ("Container 06") as described in the instructions for use of the **GXT96 X2 Extraction Kit**. Label container and lid.

   Prepare a mix of X2-B6 and U-IC6 as described in the instructions for use of the **GXT96 X2 Extraction Kit**.

   The mix contains 50 µl X2-B6 and 1 µl U-IC6 for each sample.

   Consider an additional dead volume of 16 reactions per run of the **GenoXtract® 96** for both X2-B6 and the U-IC6.

Please proceed as described in the instructions for use of the **GXT96 X2 Extraction Kit** to equip the **GenoXtract® 96** with reservoirs, reagents, and labware.

7.1.3 Preparation of automated PCR setup

Please note that DNA extracted from sputum specimens and DNA extracted from cultivated samples may not be amplified in one PCR plate since different PCR protocols are required.

To avoid contamination, the following steps must be performed in separate working areas.

<u>Pre-PCR area (free from contaminating DNA)</u>

1. **Determine the total number of samples** (number of samples to be analyzed plus control samples).
2. **Prepare a PCR plate. Only use FrameStar® 96well PCR Plates Roche Style** (see chapter 4.5). Please note that due to the fluorescence measurement, the PCR plate may only be labeled on its frame.
3. **Thaw AM-A and AM-B, spin down briefly, and mix carefully by pipetting up and down.**
4. **Transfer the required volume of AM-A (6 µl per sample + 54 µl dead volume per run) free of air bubbles to a 2 ml screw cap tube** (see chapter 4.5). Refreeze AM-A immediately after use.
5. **Transfer the required volume of AM-B (14 µl per sample + 98 µl dead volume per run) free of air bubbles to a 2 ml screw cap tube** (see chapter 4.5). Refreeze AM-A immediately after use.

<u>DNA extraction and detection area</u>

1. **Insert the PCR plate into the respective rack of the GenoXtract® 96** (see instructions for use of the **GXT96 X2 Extraction Kit**).
2. **Place the 2 ml screw cap tube containing AM-A into position A1 of the 24+4 rack in the GenoXtract® 96 and remove the cap.** Avoid formation of air bubbles.
3. **Place the 2 ml screw cap tube containing AM-B into position A2 of the 24+4 rack in the GenoXtract® 96 and remove the cap.** Avoid formation of air bubbles.
4. **Place an empty 5 ml screw cap tube** (see chapter 4.5) **into position A7 of the 24+4 rack and remove the cap.**
5. **Thaw C+ and equilibrate to room temperature** (15°C to 25°C)**, vortex for 5 s, and spin down briefly.**
6. **Place the original C+ tube into position A5 of the 24+4 rack in the GenoXtract® 96 and remove the cap.** Avoid formation of air bubbles.

   Note: Do not reuse the C+ tube after the **GenoXtract® 96** run. Discard the C+ tube (closed cap) after first use. Alternatively, transfer 40 µl C+ to a 0.5 ml screw cap tube (see chapter 4.5, Automated PCR setup) and place this in the **GenoXtract® 96** instead of the original C+ tube (refreeze original C+ tube immediately after use).

Please also refer to the instructions for use of the **GXT96 X2 Extraction Kit** and the operator's manual of the **GenoXtract® 96**.

7.1.4 Start of the GenoXtract® 96

Before starting the **GenoXtract® 96**, make sure that the instrument is correctly equipped. For details and options, please also refer to the instructions for use of the **GXT96 X2 Extraction Kit**, as well as the operator's manual of the **GenoXtract® 96** and the operator's manual of the **GenoXtract® 96 Software**.

1. **Switch on the GenoXtract® 96 and start the GenoXtract® 96 Software (version 1.0.0.1.0.12 or higher). Select method "X2_Extraction_PCRsetup_FT_MTBDR2" (version 1.2.0.4.14.63 or higher) to perform DNA extraction followed by PCR setup.**
   Alternatively, select method "X2_PCRsetup_FT_MTBDR2" (version 1.2.0.4.14.63 or higher) to perform only PCR setup without DNA extraction.
2. **Start the method and follow the instructions of the GenoXtract® 96 Software.**
   During PCR setup, the **GenoXtract® 96** prepares and aliquots the master mix and adds 20 µl DNA solution (isolate or C+) to each aliquot.
3. **After the PCR setup is finished, promptly seal the PCR plate using a suitable heat sealer** (see operator's manual of the **FluoroCycler® XT**). **Only use Clear Weld Seal Mark II sealing foils** (see chapter 4.5). Use the sealing foils according to the manufacturer's instructions. For additional information on sealing, please refer to the operator's manual of the **FluoroCycler® XT**. To avoid contaminations, the PCR plate must remain sealed.

Please proceed as described in chapter 8 without delay.

7.2 Automated DNA extraction with the GXT96 X2 Extraction Kit and manual PCR setup

For details and options, please also refer to the instructions for use of the **GXT96 X2 Extraction Kit**, the instructions for use of the **Inactivation Set**, the operator's manual of the **GenoXtract® 96**, and the operator's manual of the **GenoXtract® 96 Software**.

7.2.1 Preparation of samples

Make sure that the sample tubes are compatible to the **GenoXtract® 96** and respect the minimum and maximum filling levels (see instructions for use of the **GXT96 X2 Extraction Kit**).

1. **Mix sample material carefully but thoroughly by pipetting up and down using a disposable transfer pipette** (see chapter 4.5).
2. **Transfer 500 µl of sample material to a new sample tube** (see chapter 4.5), **add 167 µl Inactivation Reagent** (included in the **Inactivation Set**) **and close tube properly.** Mix thoroughly by vortexing (5 s to 10 s) and incubate the sample for 30 min at room temperature (15°C to 25°C).
3. **A negative control sample for detection of possible contamination events should be part of each run.**

   Negative control for NALC-NaOH-decontaminated sputum specimens:

   Transfer 500 µl phosphate buffer (pH 6.8; same phosphate buffer as used for decontamination of patient specimens) to an empty sample tube (see chapter 4.5) and add 167 µl **Inactivation Reagent**.

   Negative control for liquid culture samples:

   Transfer 500 µl water (molecular biology grade) or phosphate buffer (pH 6.8; same phosphate buffer as used for decontamination of patient specimens) to an empty sample tube (see chapter 4.5) and add 167 µl **Inactivation Reagent**.

   The negative control sample is processed like regular samples.
4. **Starting at position 1, place the sample tubes in the sample tube racks.** If applicable, make sure that the barcode on the sample tube is facing the right side of the instrument where the barcode reader is located (for details on barcode specifications, please refer to the **GenoXtract® 96** operator's manual).
5. **Remove caps of sample tubes.**

7.2.2 Preparation of the Internal Control DNA

The U-IC6 is added to each sample during DNA extraction.

1. **Determine the total number of samples** (number of samples to be analyzed plus control samples).
2. **Thaw U-IC6 and equilibrate to room temperature** (15°C to 25°C)**, vortex for 5 s and spin down briefly.**
3. **Prepare a mix of X2-B6** (included in the **GXT96 X2 Extraction Kit**) **and U-IC6:**

   Prepare a 30 ml container ("Container 06") as described in the instructions for use of the **GXT96 X2 Extraction Kit**. Label container and lid.

   Prepare a mix of X2-B6 and U-IC6 as described in the instructions for use of the **GXT96 X2 Extraction Kit**.

   The mix contains 50 µl X2-B6 and 1 µl U-IC6 for each sample.

   Consider an additional dead volume of 16 reactions per run of the **GenoXtract® 96** for both X2-B6 and the U-IC6.

Please proceed as described in the instructions for use of the **GXT96 X2 Extraction Kit** to equip the **GenoXtract® 96** with reservoirs, reagents, and labware.

7.2.3 Start of the GenoXtract® 96

Before starting the **GenoXtract® 96**, make sure that the instrument is correctly equipped. For details and options, please also refer to the instructions for use of the **GXT96 X2 Extraction Kit**, as well as the operator's manual of the **GenoXtract® 96** and the operator's manual of the **GenoXtract® 96 Software**.

1. **Switch on the GenoXtract® 96 and start the GenoXtract® 96 Software (version 1.0.0.1.0.12 or higher). Select method "X2_Extraction_FT_MTBDR2" (version 1.2.0.4.14.63 or higher) to perform DNA extraction.**
2. **Start the method and follow the instructions of the GenoXtract® 96 Software.**

7.2.4 Manual PCR setup

Please note that DNA extracted from sputum specimens and DNA extracted from cultivated samples may not be amplified in one PCR plate since different PCR protocols are required.

To avoid contamination, the following steps must be performed in separate working areas.

<u>Pre-PCR area (free from contaminating DNA)</u>

1. **Determine the total number of samples** (number of samples to be analyzed plus control samples).
2. **Prepare a PCR plate. Only use FrameStar® 96well PCR Plates Roche Style** (see chapter 4.5). Please note that due to the fluorescence measurement, the PCR plate may only be labeled on its frame.
3. **Thaw AM-A and AM-B shortly before preparing the master mix, spin down briefly, and mix carefully by pipetting up and down.**
4. **Prepare a master mix containing AM-A and AM-B and mix carefully but thoroughly (do not vortex). Refreeze AM-A and AM-B immediately after use. Please note that the master mix needs to be prepared freshly each time and needs to be processed quickly.**

   For each sample, the master mix contains:
   - 6 µl AM-A
   - 14 µl AM-B
5. **Aliquot 20 µl of the master mix into each of the wells needed on the prepared PCR plate.**

DNA extraction and detection area

1. **Thaw C+ and equilibrate to room temperature** (15°C to 25°C)**, vortex for 5 s, and spin down briefly.**
2. **Add 20 µl DNA solution (isolate or C+) to each aliquot of the master mix. Refreeze C+ immediately after use.**

   Each sample contains:
   – 20 µl master mix
   – 20 µl DNA solution
   Final volume: 40 µl
3. **Seal the PCR plate using a suitable heat sealer** (see operator's manual of the **FluoroCycler® XT**). **Only use Clear Weld Seal Mark II sealing foils** (see chapter 4.5). Use the sealing foils according to the manufacturer's instructions. For additional information on sealing, please refer to the operator's manual of the **FluoroCycler® XT**. To avoid contaminations, the PCR plate must remain sealed.

Please proceed as described in chapter 8 without delay.

7.3 Automated DNA extraction with the X2 Cartridge and manual PCR setup

For details and options, please also refer to the instructions for use of the **X2 Cartridge**, the instructions for use of the **Inactivation Set**, and the operator's manual of the **GenoXtract®**.

7.3.1 Preparation of samples and of the Internal Control DNA

1. **Determine the number of samples** (number of samples to be analyzed plus control samples).
2. **Prepare a mix of 10 µl Internal Control DNA** (U-IC6) **and 125 µl Inactivation Reagent** (included in the **Inactivation Set**) **per sample and close tube** (recommendation: see chapter 4.5) **properly.**
   Mix thoroughly by vortexing (5 s to 10 s) **and dispense 135 µl of the U-IC6/Inactivation Reagent mix into 2 ml screw cap tubes** (recommendation: see chapter 4.5).
3. **Mix decontaminated patient specimen or liquid culture sample carefully but thoroughly by pipetting up and down using a disposable transfer pipette.**
4. **Add 375 µl of sample material to the dispensed U-IC6/Inactivation Reagent mix and close tube properly. Mix thoroughly by vortexing (5 s to 10 s).**
5. **A negative control sample for detection of possible contamination events should be part of each run.**

   Negative control for NALC-NaOH-decontaminated sputum specimens:

   Transfer 375 µl phosphate buffer (pH 6.8; same phosphate buffer as used for decontamination of patient specimens) to the dispensed U-IC6/Inactivation Reagent mix and close tube properly. Mix thoroughly by vortexing (5 s to 10 s).

   Negative control for liquid culture samples:

   Transfer 375 µl water (molecular biology grade) or phosphate buffer (pH 6.8; same phosphate buffer as used for decontamination of patient specimens) to the dispensed U-IC6/Inactivation Reagent mix and close tube properly. Mix thoroughly by vortexing (5 s to 10 s).

   The negative control sample is processed like regular samples.
6. **Incubate closed sample tube for 30 min at room temperature** (15°C to 25°C) **and subsequently spin down briefly.**

7.3.2 Preparation and start of the GenoXtract®

For handling instructions of the **GenoXtract®** in combination with the **X2 Cartridge**, please refer to the instructions for use of the **X2 Cartridge** and the operator's manual of the **GenoXtract®**. Select the following parameters:

Parameter	Selection
Protocol	X2_V1
Sample volume	500 µl
Elution volume	100 µl

Please proceed as described in the instructions for use of the **X2 Cartridge**.
For details and options, please also refer to the instructions for use of the **X2 Cartridge** and the operator's manual of the **GenoXtract®**.

7.3.3 Manual PCR setup

Please note that DNA extracted from sputum specimens and DNA extracted from cultivated samples may not be amplified in one PCR plate since different PCR protocols are required.

To avoid contamination, the following steps must be performed in separate working areas.

Pre-PCR area (free from contaminating DNA)

1. **Determine the total number of samples** (number of samples to be analyzed plus control samples).
2. **Prepare a PCR plate. Only use FrameStar® 96well PCR Plates Roche Style** (see chapter 4.5). Please note that due to the fluorescence measurement, the PCR plate may only be labeled on its frame.
3. **Thaw AM-A and AM-B shortly before preparing the master mix, spin down briefly, and mix carefully by pipetting up and down.**
4. **Prepare a master mix containing AM-A and AM-B and mix carefully but thoroughly (do not vortex). Refreeze AM-A and AM-B immediately after use. Please note that the master mix needs to be prepared freshly each time and needs to be processed quickly.**

   For each sample, the master mix contains:
   - 6 µl AM-A
   - 14 µl AM-B
5. **Aliquot 20 µl of the master mix into each of the wells needed on the prepared PCR plate.**

DNA extraction and detection area

1. **Thaw C+ and equilibrate to room temperature** (15°C to 25°C)**, vortex for 5 s, and spin down briefly.**
2. **Add 20 µl DNA solution (isolate or C+) to each aliquot of the master mix. Refreeze C+ immediately after use.**

   Each sample contains:
   - 20 µl master mix
   - 20 µl DNA solution

   Final volume: 40 µl
3. **Seal the PCR plate using a suitable heat sealer** (see operator's manual of the **FluoroCycler® XT**). **Only use Clear Weld Seal Mark II sealing foils** (see chapter 4.5). Use the sealing foils according to the manufacturer's instructions. For additional information on sealing, please refer to the operator's manual of the **FluoroCycler® XT**. To avoid contaminations, the PCR plate must remain sealed.

Please proceed as described in chapter 8 without delay.

7.4 Manual DNA extraction with the FluoroLyse and manual PCR setup

For manual extraction, the **FluoroLyse** (see chapter 4.5) is used. For handling instructions, please refer to the respective instructions for use.

7.4.1 Manual extraction with the FluoroLyse

1. **Determine the number of samples** (number of samples to be analyzed plus negative control sample).
2. **Transfer 500 µl of decontaminated sample material to a 1.5 ml sample tube** (see chapter 4.5) **and close tube properly.**
3. **Centrifuge for 15 min at 10,000 x g in a standard table top centrifuge and discard supernatant.**
4. **Prepare a mix of 2 µl Internal Control DNA** (U-IC6) **and 100 µl Lysis Buffer (F-LYS;** included in the **FluoroLyse** kit**) per sample and close tube properly.**
   Mix thoroughly by vortexing (5 s to 10 s)**.**
5. **Resuspend the pellet in 100 µl F-LYS/U-IC6 mix by vortexing.**
6. **A negative control sample for detection of possible contamination events should be part of each run.**

   Negative control sample:

   Transfer 100 µl F-LYS/U-IC6 mix to an empty sample tube (see chapter 4.5).

   The negative control sample is processed like regular samples.
7. **Incubate closed sample tube for 5 min at 95°C in a water bath and subsequently spin down briefly.**
8. **Add 100 µl Neutralization Buffer** (**F-NB;** included in the **FluoroLyse** kit) **and vortex sample for 5 s.**
9. **Spin down for 5 min at full speed in a table top centrifuge and directly use 20 µl of the supernatant for PCR. It is important that the pipette tip does not touch the pellet.** In case the DNA solution is to be stored at least at –20°C for an extended period of time, transfer supernatant to a new tube.

7.4.2 Manual PCR setup

Please note that DNA extracted from sputum specimens and DNA extracted from cultivated samples may not be amplified in one PCR plate since different PCR protocols are required.

To avoid contamination, the following steps must be performed in separate working areas.

Pre-PCR area (free from contaminating DNA)

1. **Determine the total number of samples** (number of samples to be analyzed plus control samples).
2. **Prepare a PCR plate. Only use FrameStar® 96well PCR Plates Roche Style** (see chapter 4.5). Please note that due to the fluorescence measurement, the PCR plate may only be labeled on its frame.
3. **Thaw AM-A and AM-B shortly before preparing the master mix, spin down briefly, and mix carefully by pipetting up and down.**
4. **Prepare a master mix containing AM-A and AM-B and mix carefully but thoroughly (do not vortex). Refreeze AM-A and AM-B immediately after use. Please note that the master mix needs to be prepared freshly each time and needs to be processed quickly.**

   For each sample, the master mix contains:
   - 6 µl AM-A
   - 14 µl AM-B
5. **Aliquot 20 µl of the master mix into each of the wells needed on the prepared PCR plate.**

DNA extraction and detection area

1. **Thaw C+ and equilibrate to room temperature** (15°C to 25°C)**, vortex for 5 s, and spin down briefly.**
2. **Add 20 µl DNA solution (isolate or C+) to each aliquot of the master mix. Refreeze C+ immediately after use.**

   Each sample contains:
   – 20 µl master mix
   – 20 µl DNA solution
   Final volume: 40 µl
3. **Seal the PCR plate using suitable heat sealer** (see operator's manual of the **FluoroCycler® XT**). **Only use Clear Weld Seal Mark II sealing foils** (see chapter 4.5). Use the sealing foils according to the manufacturer's instructions. For additional information on sealing, please refer to the operator's manual of the **FluoroCycler® XT**. To avoid contaminations, the PCR plate must remain sealed.

Please proceed as described in chapter 8 without delay.

8 Test Procedure

If not done yet, register the kit lot of the test kit on hand in the **FluoroSoftware® XT-IVD** (see chapter 4.4). Please note that DNA extracted from sputum specimens and DNA extracted from cultivated samples may not be amplified in one PCR plate since different PCR protocols are required.

1. **Briefly centrifuge the sealed PCR plate to collect the liquid at the bottom of the wells.**
2. **Switch on the FluoroCycler® XT and start the FluoroSoftware® XT-IVD (version 1.0.1.1.1.17 or higher) on the connected computer.**
3. **Follow the instructions of the wizard in the FluoroSoftware® XT-IVD, start the run, and enter the sample information.** In order to obtain correct interpretations, it is crucial that the actual kit lot is allocated to the samples.

   For details and options, please refer to the operator's manual of the **FluoroCycler® XT** and the operator's manual of the **FluoroSoftware® XT-IVD**.

9 Test Evaluation

The interpretations of the FluoroSoftware® XT-IVD may only be evaluated in combination with additional laboratory and clinical data available to the responsible physician.
The user must have or acquire information about the local mutation distribution pattern of the genes investigated with this test.

The conducting laboratory is solely responsible for the output of the results.

When the protocol is finished, the **FluoroCycler® XT Report** is compiled automatically or can be generated by clicking the "PDF" button. The report includes the date, the run name, and all sample information as well as the respective fluorescence signatures and the interpretations as derived by the software.

9.1 Analysis by the FluoroSoftware® XT-IVD

rpoB

The *rpoB* gene is examined for detection of resistance to RMP and is used for detection of MTB complex DNA. The probes comprise the most important resistance-mediating regions of the *rpoB* gene. In case a resistance-mediating mutation was specified, it is displayed accordingly in the **FluoroCycler® XT Report**.

The following mutations in the rifampicin resistance-determining region (RRDR) of the *rpoB* gene (amino acid 510 to 533 (*Escherichia coli*-based annotation) or 429 to 452 (*M. tuberculosis* H37Rv-based annotation)) can be specified:

Escherichia coli-based annotation	*Mycobacterium tuberculosis* H37Rv-based annotation	*Escherichia coli*-based annotation	*Mycobacterium tuberculosis* H37Rv-based annotation
Substitutions		**Deletions**	
L511P	L430P	Del. at 514-516	Del. at 433-435
Q513K	Q432K	Del. at 517-518	Del. at 436-437
Q513L / D516V[4)]	Q432L / D435V[4)]	Del. at 531-533	Del. at 450-452
Q513P	Q432P		
D516F	D435F	**Insertions**	
D516G	D435G	Ins. at 514-515	Ins. at 433-434
D516Y	D435Y		
S522L	S441L	**Silent mutations**	
S522Q	S441Q	F514F	F433F
H526D / H526S[4)]	H445D / H445S[4)]	G523G	G442G
H526F	H445F	A532A	A451A
H526L	H445L		
H526P	H445P	**Combined mutations**	
H526N	H445N	Q513H + L533P	Q432H + L452P
H526R	H445R	M515I + D516Y	M434I + D435Y
H526Y / H526G[4)]	H445Y / H445G[4)]	D516G + L533P	D435G + L452P
R529K	R448K		
S531F	S450F		
S531L / S531W[4)]	S450L / S450W[4)]		
S531Q	S450Q		
L533P	L452P		

[4)] Mutations are not differentiated

katG

The *katG* gene is examined for detection of high-level resistance to INH.
The probes comprise the most important resistance-mediating region of the *katG* gene. In case a mutation was specified, it is displayed accordingly in the **FluoroCycler® XT Report**.

The following mutations in the *katG* gene (amino acid 307 to 316) can be specified:

S315T1 (ACC)	S315I
S315T2 (ACA)	S315N
	S315R

inhA

The *inhA* promoter region is examined for detection of a low-level INH resistance.
The probes comprise the most important resistance-mediating region of the *inhA* promoter region. In case a mutation was specified, it is displayed accordingly in the **FluoroCycler® XT Report**.

The following mutations in the *inhA* promoter region (nucleotide –20 to +9) can be specified:

G–17T	T–8A / T–8G
C–15T	T–8C

Internal Control (IC)

The **FluoroSoftware® XT-IVD** automatically checks for each sample whether U-IC6 detection is required or not and evaluates accordingly.

Please note:

Since the different mutations within the examined gene regions influence the melting properties of all probes, a multiplicity of fluorescence signatures is possible which cannot be evaluated visually but only by the **FluoroSoftware® XT-IVD**.

The detected mutations are based on the WHO catalogue [13].

The fluorescence signatures of MTB complex strains with identical target sequences may vary within a small tolerance range.

Differences in maximums and minimums of fluorescence signatures do not allow any conclusions about the number of target molecules in the sample and have no relevance for this qualitative test.

Theoretically, a resistance-mediating mutation can exist within the examined gene regions in spite of a detected wild type fluorescence signature. Possible reasons could be:

- The tested specimen contains a wild type and a resistant strain (e.g. due to mixed infection of the patient or contamination of the culture) or a heteroresistant strain, and at investigation, the ratio of the mutated sequence is too low for detection with the **FluoroSoftware® XT-IVD**.
- The tested specimen contains a wild type and a resistant strain (e.g. due to mixed infection of the patient or contamination of the culture) or a heteroresistant strain, and the resistance is caused by a mutation not covered by the mutation probes.

9.2 Interpretations in the FluoroCycler® XT Report

Interpretation	Explanation
MTB complex DNA detected	The *rpoB* locus was detected.
rpoB[5]	*rpoB* mutation status:
rpoB: WT	The wild type sequence is present.
rpoB: S531L / S531W	The respective resistance-mediating mutation is present.
rpoB: A532A	The respective silent mutation is present.
katG[5]	*katG* mutation status:
katG: WT	The wild type sequence is present.
katG: S315T1	The respective mutation is present.
katG: IND	The *katG* mutation status could not be determined (see below).
inhA[5]	*inhA* mutation status:
inhA: WT	The wild type sequence is present.
inhA: T–8A / T–8G	The respective mutation is present.
inhA: IND	The *inhA* mutation status could not be determined (see below).
Resistance indeterminate	The *rpoB* mutation status could not be determined (see below). In this case, the *katG* and *inhA* mutation status are not evaluated.
RMP[5]	RMP resistance of the MTB complex strain:
RMP: sensitive	No mutation or a silent mutation was detected in the *rpoB* locus (*rpoB*: WT / *rpoB*: A532A).
RMP: resistant	The *rpoB* locus carries a resistance-mediating mutation (e.g. *rpoB*: S531L / S531W).
INH[5]	INH resistance of the MTB complex strain:
INH: sensitive	No mutation was detected in the *katG* and in the *inhA* loci (*katG*: WT / *inhA*: WT).
INH: resistant	The *inhA* and/or the *katG* loci are mutated (e.g. *katG*: WT / *inhA*: C–15T).
INH: indeterminate	The *katG* and/or the *inhA* mutation status could not be determined (e.g. *katG*: WT / *inhA*: IND; see below).
No MTB complex DNA detected	The *rpoB* locus was not detected.
Negative Control valid	No MTB complex DNA was detected in the negative control.
Positive Control valid	The C+ was detected in the positive control.
Invalid	The fluorescence signature is invalid (see below).

[5] Exemplary mutation status are depicted

Resistance indeterminate

RMP resistance (i.e., the *rpoB* mutation status) could not be determined, e.g. because the quality of the fluorescence signature or the amount of the amplification product was too low for a safe identification. It is recommended to repeat the test with this isolate.

INH: indeterminate

INH resistance (i.e., the *katG* and/or *inhA* mutation status) could not be determined, e.g. because the quality of the fluorescence signature or the amount of the amplification product was too low for a safe identification. It is recommended to repeat the test with this isolate.

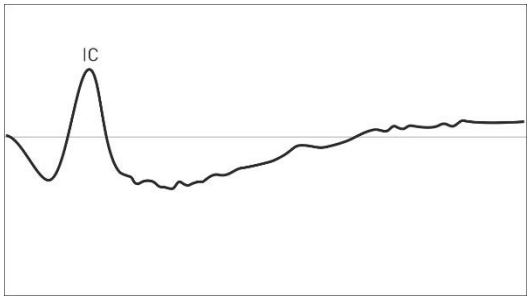
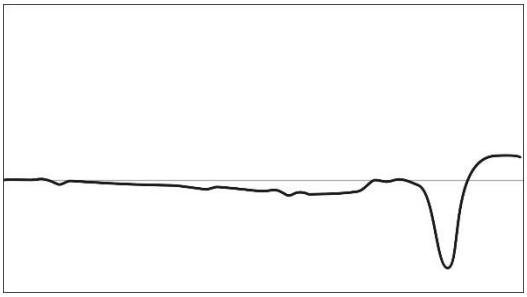
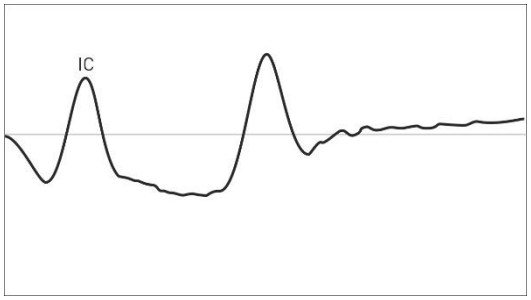
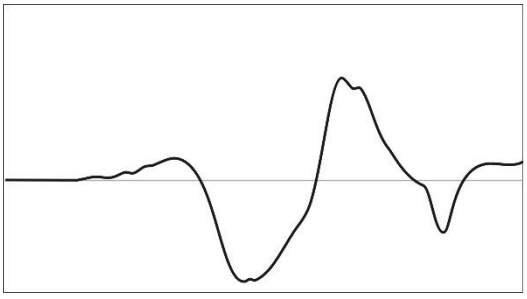
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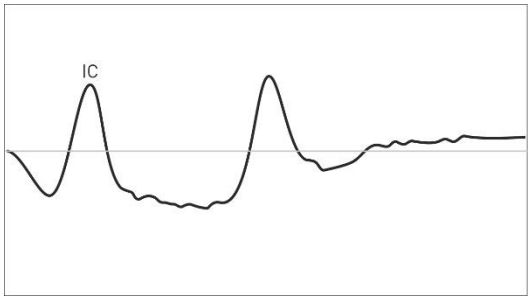
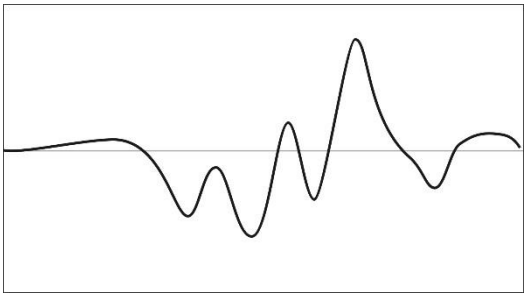
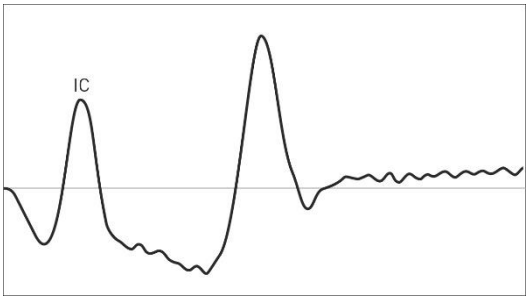
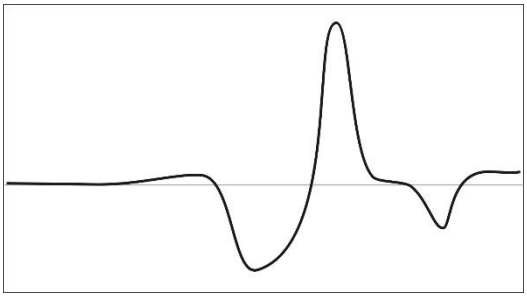
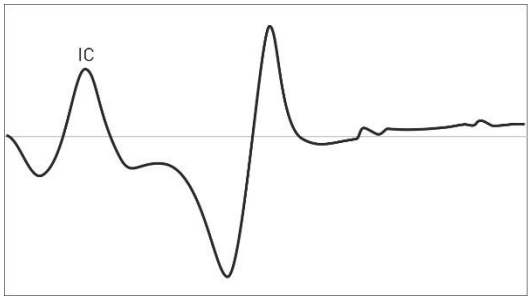
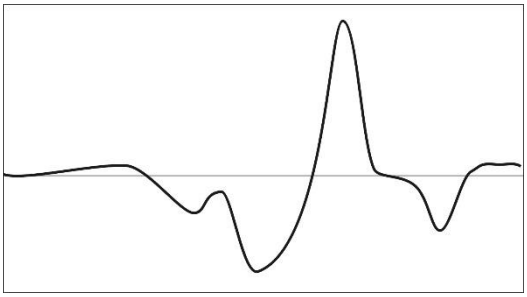
If a patient sample was interpreted as **Invalid**, the fluorescence signature could not be evaluated by the **FluoroSoftware® XT-IVD** or IC detection was required, but it was not detected. This indicates mistakes during DNA extraction or during setup and/or performance of the amplification reaction, presence of amplification inhibitors, or inhibition due to overload of the test system. It is recommended to repeat the test with this isolate (see chapter 11).

If a control was interpreted as **Invalid**, one of the following reasons applies:

- A contamination was detected in the negative control (see chapter 11).
- No IC was detected in the negative control.
- The C+ fluorescence signature was not as required (see chapter 11).

9.3 Examples for fluorescence signatures

IC - *katG*	***inhA - rpoB***
	
Interpretation No MTB complex DNA detected	
	
Interpretation MTB complex DNA detected *rpoB*: WT / *katG*: WT / *inhA*: WT RMP: sensitive / INH: sensitive	

IC - *katG*	*inhA - rpoB*
	
Interpretation MTB complex DNA detected *rpoB*: Q513L / D516V / *katG*: S315T2 / *inhA*: C-15T RMP: resistant / INH: resistant	
	
Interpretation MTB complex DNA detected *rpoB*: A532A / katG: WT / *inhA*: G-17T RMP: sensitive / INH: resistant	
	
Interpretation Positive Control valid	

10 Limitations

- Strictly adhere to the established protocols and procedures in order to obtain correct test results and to avoid contaminations.
- Use of this assay is limited to qualified personnel well trained in the test procedure and familiar with molecular biological methods.
- The interpretations of the **FluoroSoftware® XT-IVD** may only be evaluated in combination with additional laboratory and clinical data available to the responsible physician.
- The test reflects the current state of knowledge of Hain Lifescience.
- The test is indicated as an aid for diagnosis.
- The user must have or acquire information about the local mutation distribution pattern of the genes investigated with this test.
- In case of unexpected or indeterminate interpretations, further examination methods may be necessary.
- The very rare *rpoB:* H526C mutation causing RMP resistance is not detected by the **FluoroSoftware® XT-IVD**. If the clinical data of the patient indicate RMP resistance in spite of the interpretation "RMP: sensitive", the **GenoType MTBDR*plus*** VER 2.0 or phenotypic drug susceptibility testing is recommended for clarification.
- The conducting laboratory is solely responsible for the output of the results.
- The **FluoroType® MTBDR** VER 2.0 only detects those resistances that have their origins in the *rpoB*, *katG*, and *inhA* gene regions examined here. Resistances originating from mutations of other genes or gene regions as well as other RMP or INH resistance mechanisms will not be detected by this test.
- As any detection system based on hybridization, the test system on hand bears the possibility that sequence variations in the genomic regions the primers and probes were chosen from but the detection of which the test was not designed for may lead to false results. Due to the high variability of bacterial genomes, it is possible that certain subtypes might not be detected.
- As any DNA-based assay, this test only screens the nucleic acid sequence and not the amino acid sequence. Therefore, silent mutations in the probe region will only be interpreted as such if they are stored in the **FluoroSoftware® XT-IVD** database.
- As any DNA detection method, the test system on hand detects DNA from viable and nonviable bacteria. Therefore, this test may not be used for monitoring the progression or success of treatment of patients with antimicrobial therapy.
- The **FluoroType® MTBDR** VER 2.0 is a qualitative test. The fluorescence signature does not give information about the number of target molecules in the specimen.
- The members of the MTB complex cannot be differentiated.
- The presence of multiple bacterial species in the sample to be analyzed might hamper the interpretation of the test.
- Performance evaluation of this assay was carried out from NALC-NaOH-decontaminated sputum samples and from mycobacteria grown in liquid medium (MGIT; BD Diagnostics, Franklin Lakes, USA). DNA extraction was performed using the **FluoroLyse**, the **GXT96 X2 Extraction Kit** in combination with the **GenoXtract® 96**, and the **X2 Cartridge** in combination with the **GenoXtract®**. Prior to automated extraction with the **GXT96 X2 Extraction Kit** and the **X2 Cartridge**, samples were processed using the **Inactivation Set** VER 1.0. For correct evaluation it is mandatory that the validated DNA extraction methods are applied. The performance of the test has not been validated with other DNA extraction methods or sample materials.

11 Troubleshooting

11.1 Interpretation in FluoroCycler® XT Report: Invalid

Observation	Possible causes	Recommendation
The fluorescence signature of a sample cannot be evaluated by the **FluoroSoftware® XT-IVD**.	Improper sampling, storage, transport, or preparation of specimen.	Request a new specimen and repeat the test.
	DNA concentration too high for amplification or inhibition of amplification due to interfering substances.	Use only the intended sample material and the intended extraction method. Dilute the DNA solution and repeat the test. Please be aware that this could impair the sensitivity of the test.
	Error during DNA extraction.	Repeat the extraction with the intended extraction method. Strictly adhere to the respective instructions for use.
	Sample arrangement in the **FluoroCycler® XT** is not identical to sample allocation in the **FluoroSoftware® XT-IVD** and thus empty positions were measured as samples.	Correct the sample information in the Edit mode.
	FluoroCycler® device error.	Please contact your local Hain Lifescience representative.
A contamination was detected in the negative control.	Contamination of amplification reagents or carry-over contamination during sample preparation.	Retest the negative control. In case a contamination is detected again, exchange all amplification reagents and repeat the entire run. Perform all measures in accordance with institutional guidelines. Observe commonly accepted and proven measures when handling samples, kit constituents, and consumables. Perform cleaning measures and controls for detection of contaminations on a regular basis.
No C+ detected in the positive control or the detected C+ fluorescence signature is not within the specified range.	Mistakes in storage or handling of the C+ or amplification reagents.	Use a new aliquot or a new kit and repeat the entire run.

11.2 Unexpected result

Observation	Possible causes	Recommendation
Unexpected result	Improper sampling, storage, transport, or preparation of specimen.	Request a new specimen and repeat the test.
	Contamination of amplification reagents or carry-over contamination during sample preparation.	Exchange all amplification reagents and repeat the entire run. Observe commonly accepted and proven measures when handling samples, kit constituents, and consumables. Perform cleaning measures and controls for detection of contaminations on a regular basis.

In case of any further questions or problems, please contact your local Hain Lifescience representative.

12 Performance Characteristics

For performance evaluation of the **FluoroType® MTBDR** VER 2.0, the test was carried out according to the instructions on hand.

12.1 Diagnostic performance using the FluoroLyse and the GXT96 X2 Extraction Kit

12.1.1 Clinical specimens

Diagnostic performance characteristics of the **FluoroType® MTBDR** VER 2.0 were determined in a study with 610 sputum specimens (119 smear-positive, 491 smear-negative). The study was carried out in a high MDR-TB burden country. Patients with current or previous (within the last three months) anti-TB treatment were excluded from the study. All other patients were included irrespective of their age, gender, HIV status or concomitant diseases.

12.1.1.1 Detection of MTB complex

For detection of MTB complex, **FluoroType® MTBDR** VER 2.0 results were compared to results of MGIT culture (BD Diagnostics, Franklin Lakes, USA) and subsequent identification of the MTB complex with the **GenoType MTBDR*plus*** VER 2.0 from the cultured material. Additionally, all NALC-NaOH-decontaminated specimens and all culture samples were examined by microscopy. False-positive results were resolved by **FluoroType® MTB** VER 1.0 (reference method). For all samples, DNA was extracted firstly using the **FluoroLyse** and secondly using the **GXT96 X2 Extraction Kit** in combination with the **GenoXtract® 96**. NALC-NaOH-decontaminated specimens and culture samples were inactivated using the **Inactivation Set** VER 1.0 prior to DNA extraction using the **GXT96 X2 Extraction Kit**.

FluoroLyse
In total, 9 sputum specimens (5 smear-positive and 4 smear-negative) were invalid with the **FluoroType® MTBDR** VER 2.0 (9 out of 610, total rate of invalid samples 1.5%).

Table 1
Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-positive sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Methods of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	113	0	113
	Negative	0	1	1
	Total	113	1	114

Diagnostic sensitivity:	100%	95% CI: 96.8% to 100%
Diagnostic specificity:	100%	95% CI: n.d.
Positive predictive value:	100%	95% CI: 96.8% to 100%
Negative predictive value:	100%	95% CI: n.d.

Table 2

Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-negative sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Methods of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	178	7[1)]	185
	Negative	33	269	302
	Total	211	276	487

[1)] All specimens were tested MTB complex-positive with the reference method (**FluoroType® MTB** VER 1.0).

Diagnostic sensitivity:	84.4%	95% CI: 78.7% to 89.0%
Diagnostic specificity:	97.5%	95% CI: 94.8% to 99.0%
Positive predictive value:	96.2%	95% CI: 92.4% to 98.5%
Negative predictive value:	89.1%	95% CI: 85.0% to 92.4%

Table 3

Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-negative sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0 and reference method (**FluoroType® MTB** VER 1.0 for false-positive results)

		Methods of comparison and reference method		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	185	0	185
	Negative	33	269	302
	Total	218	269	487

Diagnostic sensitivity:	84.9%	95% CI: 79.4% to 89.3%
Diagnostic specificity:	100%	95% CI: 98.6% to 100%
Positive predictive value:	100%	95% CI: 98.0% to 100%
Negative predictive value:	89.1%	95% CI: 85.0% to 92.4%

GXT96 X2 Extraction Kit

All samples were tested MTB complex-positive or MTB complex-negative. The total rate of invalid samples was 0% (0 out of 610).

Table 4

Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-positive sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Methods of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	118	1[2)]	119
	Negative	0	0	0
	Total	118	1	119

[2)] Specimen was tested MTB complex-positive with the reference method (**FluoroType® MTB** VER 1.0).

Diagnostic sensitivity:	100%	95% CI: 96.9% to 100%
Diagnostic specificity:	0%	95% CI: n.d.
Positive predictive value:	99.2%	95% CI: 95.4% to 100%
Negative predictive value:	–	95% CI: –

Table 5

Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-positive sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0 and reference method (**FluoroType® MTB** VER 1.0 for false-positive results)

		Methods of comparison and reference method		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	119	0	119
	Negative	0	0	0
	Total	119	0	119

Diagnostic sensitivity:	100%	95% CI: 96.9% to 100%
Diagnostic specificity:	–	95% CI: –
Positive predictive value:	100%	95% CI: 96.9% to 100%
Negative predictive value:	–	95% CI: –

Table 6
Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-negative sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Methods of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	173	8[3)]	181
	Negative	41	269	310
	Total	214	277	491

[3)] All specimens were tested MTB complex-positive with the reference method (**FluoroType® MTB** VER 1.0).

Diagnostic sensitivity:	80.8%	95% CI: 74.9% to 85.9%
Diagnostic specificity:	97.1%	95% CI: 94.4% to 98.7%
Positive predictive value:	95.6%	95% CI: 91.5% to 98.1%
Negative predictive value:	86.8%	95% CI: 82.5% to 90.3%

Table 7
Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-negative sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0 and reference method (**FluoroType® MTB** VER 1.0 for false-positive results)

		Methods of comparison and reference method		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	181	0	181
	Negative	41	269	310
	Total	222	269	491

Diagnostic sensitivity:	81.5%	95% CI: 75.8% to 86.4%
Diagnostic specificity:	100%	95% CI: 98.6% to 100%
Positive predictive value:	100%	95% CI: 98.0% to 100%
Negative predictive value:	86.8%	95% CI: 82.5% to 90.3%

12.1.1.2 Detection of RMP resistance

For evaluation of RMP resistance detection, the 291 samples were used that were positive with both culture and **FluoroType® MTBDR** VER 2.0. A test result was assessed as true-resistant/sensitive when the RMP resistance status detected with the **FluoroType® MTBDR** VER 2.0 was confirmed by the results of the **GenoType MTBDR*plus*** VER 2.0 from cultured material. Discrepancies were resolved by sequencing or **GenoType MTBDR*plus*** VER 2.0 from the patient sample. In case a heteroresistance or a mixed infection was detected with the method of comparison or reference method, the sample was assessed as resistant.

FluoroLyse
In total, 3 MTB complex-positive sputum specimens (smear-negative) were tested RMP resistance indeterminate with the **FluoroType® MTBDR** VER 2.0 (3 out of 291, total indeterminate rate for RMP resistance detection 1.0%). All other MTB complex-positive samples were tested RMP-susceptible or RMP-resistant.

Table 8

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from smear-positive sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	24	2[4)]	26
	Sensitive	0	87	87
	Total	24	89	113

[4)] Sequencing of the culture samples revealed RMP resistance.		
Diagnostic sensitivity:	100%	95% CI: 85.8% to 100%
Diagnostic specificity:	97.8%	95% CI: 92.1% to 99.7%

Table 9

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from smear-positive sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0 and reference method (sequencing)

		Method of comparison and reference method		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	26	0	26
	Sensitive	0	87	87
	Total	26	87	113

Diagnostic sensitivity:	100%	95% CI: 86.8% to 100%
Diagnostic specificity:	100%	95% CI: 95.8% to 100%

Table 10

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from smear-negative sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	44	4[5)]	48
	Sensitive	0	127	127
	Total	44	131	175

[5)] For 3 samples, sequencing of the culture sample revealed RMP resistance.		
Diagnostic sensitivity:	100%	95% CI: 92.0% to 100%
Diagnostic specificity:	96.9%	95% CI: 92.4% to 99.2%

Table 11

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from smear-negative sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0 and reference method (sequencing)

		Method of comparison and reference method		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	47	1	48
	Sensitive	0	127	127
	Total	47	128	175

Diagnostic sensitivity:	100%	95% CI: 92.5% to 100%
Diagnostic specificity:	99.2%	95% CI: 95.7% to 100%

GXT96 X2 Extraction Kit

In total, 8 MTB complex-positive sputum specimens (smear-negative) were tested RMP resistance indeterminate with the **FluoroType® MTBDR** VER 2.0 (8 out of 291, total indeterminate rate for RMP resistance detection 2.7%). All other MTB complex-positive samples were tested RMP-susceptible or RMP-resistant.

Table 12

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from smear-positive sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	24	2[6)]	26
	Sensitive	0	92	92
	Total	24	94	118

[6)] Sequencing of the culture samples revealed RMP resistance.		
Diagnostic sensitivity:	100%	95% CI: 85.8% to 100%
Diagnostic specificity:	97.9%	95% CI: 92.5% to 99.7%

Table 13

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from smear-positive sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0 and reference method (sequencing)

		Method of comparison and reference method		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	26	0	26
	Sensitive	0	92	92
	Total	26	92	118

Diagnostic sensitivity:	100%	95% CI: 86.8% to 100%
Diagnostic specificity:	100%	95% CI: 96.1% to 100%

Table 14

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from smear-negative sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	40	1[7)]	41
	Sensitive	1	123	124
	Total	41	124	165

[7)] For this sample, sequencing of the culture sample revealed RMP resistance.

Diagnostic sensitivity:	97.6%	95% CI: 87.1% to 99.9%
Diagnostic specificity:	99.2%	95% CI: 95.6% to 100%

Table 15

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from smear-negative sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0 and reference method (sequencing)

		Method of comparison and reference method		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	41	0	41
	Sensitive	1	123	124
	Total	42	123	165

Diagnostic sensitivity:	97.6%	95% CI: 87.4% to 99.9%
Diagnostic specificity:	100%	95% CI: 97% to 100%

12.1.1.3 Detection of INH resistance

For evaluation of INH resistance detection, the 291 samples were used that were positive with both culture and **FluoroType® MTBDR** VER 2.0.
A test result was assessed as true-resistant/sensitive when the INH resistance status detected with the **FluoroType® MTBDR** VER 2.0 was confirmed by the results of the **GenoType MTBDR*plus*** VER 2.0 from cultured material. Discrepancies were resolved by sequencing or **GenoType MTBDR*plus*** VER 2.0 from the patient sample. In case a heteroresistance or a mixed infection was detected with the method of comparison or reference method, the sample was assessed as resistant.

FluoroLyse
In total, 14 MTB complex-positive sputum specimens (smear-negative) were tested INH resistance indeterminate with the **FluoroType® MTBDR** VER 2.0 (14 out of 291, total indeterminate rate for INH resistance detection 4.8%). All other MTB complex-positive samples were tested INH-susceptible or INH-resistant.

Table 16
Results of the **FluoroType® MTBDR** VER 2.0 for detection of INH resistance from smear-positive sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	INH	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	26	0	26
	Sensitive	0	87	87
	Total	26	87	113

Diagnostic sensitivity:	100%	95% CI: 86.8% to 100%
Diagnostic specificity:	100%	95% CI: 95.8% to 100%

Table 17
Results of the **FluoroType® MTBDR** VER 2.0 for detection of INH resistance from smear-negative sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	INH	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	45	3[8)]	48
	Sensitive	3[9)]	113	116
	Total	48	116	164[9)]

[8)] For 1 sample, **GenoType MTBDR*plus*** VER 2.0 from the sputum specimen revealed susceptibility to INH.
[9)] For two samples, **GenoType MTBDR*plus*** VER 2.0 from the sputum specimen revealed resistance to INH.

Diagnostic sensitivity:	93.8%	95% CI: 82.8% to 98.7%
Diagnostic specificity:	97.4%	95% CI: 92.6% to 99.5%

Table 18

Results of the **FluoroType® MTBDR** VER 2.0 for detection of INH resistance from smear-negative sputum specimens after DNA extraction with **FluoroLyse** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0 and reference method (sequencing and **GenoType MTBDR*plus*** VER 2.0 from sputum specimen)

		Method of comparison and reference method		
	INH	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	47	1	48
	Sensitive	2	114	116
	Total	49	115	164

Diagnostic sensitivity:	95.9%	95% CI: 86.0% to 99.5%
Diagnostic specificity:	99.1%	95% CI: 95.3% to 100%

GXT96 X2 Extraction Kit

In total, 19 MTB complex-positive sputum specimens (smear-negative) were tested INH resistance indeterminate with the **FluoroType® MTBDR** VER 2.0 (19 out of 291, total indeterminate rate for INH resistance detection 6.5%). All other MTB complex-positive samples were tested INH-susceptible or INH-resistant.

Table 19

Results of the **FluoroType® MTBDR** VER 2.0 for detection of INH resistance from smear-positive sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	INH	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	26	0	26
	Sensitive	0	92	92
	Total	26	92	118

Diagnostic sensitivity:	100%	95% CI: 86.8% to 100%
Diagnostic specificity:	100%	95% CI: 96.1% to 100%

Table 20

Results of the **FluoroType® MTBDR** VER 2.0 for detection of INH resistance from smear-negative sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	INH	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	40	1[10)]	41
	Sensitive	4[11)]	109	113
	Total	44	110	154

[10)] For 1 sample, **GenoType MTBDR*plus*** VER 2.0 from the sputum specimen revealed resistance to INH. [11)] For 1 sample, **GenoType MTBDR*plus*** VER 2.0 from the sputum specimen revealed susceptibility to INH.		
Diagnostic sensitivity:	90.9%	95% CI: 78.3% to 97.5%
Diagnostic specificity:	99.1%	95% CI: 95% to 100%

Table 21

Results of the **FluoroType® MTBDR** VER 2.0 for detection of INH resistance from smear-negative sputum specimens after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to culture/**GenoType MTBDR*plus*** VER 2.0 and reference method (sequencing and **GenoType MTBDR*plus*** VER 2.0 from sputum specimen)

		Method of comparison and reference method		
	INH	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	41	0	41
	Sensitive	3	110	113
	Total	44	110	154

Diagnostic sensitivity:	93.2%	95% CI: 81.3% to 98.6%
Diagnostic specificity:	100%	95% CI: 96.7% to 100%

12.1.2 Cultivated samples

Diagnostic performance characteristics of the **FluoroType® MTBDR** VER 2.0 were determined in a study with 323 cultured MTB complex-positive samples. The study was carried out in a high MDR-TB burden country. Untreated patients as well as patients with previous or current therapy and HIV-positive patients were included in the study.

12.1.2.1 Detection of MTB complex

Diagnostic performance characteristics of the **FluoroType® MTBDR** VER 2.0 from culture samples were determined in a study with 323 MGIT culture samples derived from decontaminated sputum specimens.

For detection of MTB complex, **FluoroType® MTBDR** VER 2.0 results were compared to results of **GenoType MTBDR*plus*** VER 2.0 from the same cultured material (MGIT culture (BD Diagnostics, Franklin Lakes, USA)). For all samples, DNA was extracted firstly using the **FluoroLyse** and secondly using the **GXT96 X2 Extraction Kit** in combination with the **GenoXtract® 96**. NALC-NaOH-decontaminated specimens and culture samples were inactivated using the **Inactivation Set** VER 1.0 prior to DNA extraction using the **GXT96 X2 Extraction Kit**.

FluoroLyse

Table 22
Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from cultured material after DNA extraction with **FluoroLyse** in comparison to the **GenoType MTBDR*plus*** VER 2.0 from the same culture sample

		Methods of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	315	0	315
	Negative	1	7	8
	Total	316	7	323

Diagnostic sensitivity:	99.7%	95% CI: 98.3% to 100%
Diagnostic specificity:	100%	95% CI: 59.0% to 100%
Positive predictive value:	100%	95% CI: 98.8% to 100%
Negative predictive value:	87.5%	95% CI: 47.3% to 100%

GXT96 X2 Extraction Kit

Table 23
Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from cultured material after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to the **GenoType MTBDR*plus*** VER 2.0 from the same culture sample

		Methods of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	316	0	316
	Negative	0	7	7
	Total	316	7	323

Diagnostic sensitivity:	100%	95% CI: 98.8% to 100%
Diagnostic specificity:	100%	95% CI: 59.0% to 100%
Positive predictive value:	100%	95% CI: 98.8% to 100%
Negative predictive value:	100%	95% CI: 59.0% to 100%

12.1.2.2 Detection of RMP resistance

A test result was assessed as true-resistant/sensitive when the RMP resistance status detected with the **FluoroType® MTBDR** VER 2.0 was confirmed by the results of the **GenoType MTBDR*plus*** VER 2.0. Discrepancies were resolved by sequencing. In case a heteroresistance or a mixed infection was detected with the method of comparison or reference method, the sample was assessed as resistant.

FluoroLyse
For evaluation of RMP resistance detection, the 315 samples were used that were positive with **GenoType MTBDR*plus*** VER 2.0 and **FluoroType® MTBDR** VER 2.0 from culture sample.
In total, 2 MTB complex-positive culture samples were tested RMP resistance indeterminate with the **FluoroType® MTBDR** VER 2.0 (2 out of 315, total indeterminate rate for RMP resistance detection 0.6%). All other MTB complex-positive samples were tested RMP-susceptible or RMP-resistant.

Table 24

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from cultured material after DNA extraction with **FluoroLyse** in comparison to the **GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	65	3[12)]	68
	Sensitive	0	245	245
	Total	65	248	313

[12)] Sequencing of the culture samples revealed RMP resistance in all 3 cases.		
Diagnostic sensitivity:	100%	95% CI: 94.5% to 100%
Diagnostic specificity:	98.8%	95% CI: 96.5% to 99.8%

Table 25

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from cultured material after DNA extraction with **FluoroLyse** in comparison to the **GenoType MTBDR*plus*** VER 2.0 and reference method (sequencing)

		Method of comparison and reference method		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	68	0	65
	Sensitive	0	245	245
	Total	68	245	313

Diagnostic sensitivity:	100%	95% CI: 94.7% to 100%
Diagnostic specificity:	100%	95% CI: 98.5% to 100%

GXT96 X2 Extraction Kit

For evaluation of RMP resistance detection, the 316 samples were used that were positive with **GenoType MTBDR*plus*** VER 2.0 and **FluoroType® MTBDR** VER 2.0 from culture sample.
In total, 1 MTB complex-positive culture sample was tested RMP resistance indeterminate with the **FluoroType® MTBDR** VER 2.0 (1 out of 316, total indeterminate rate for RMP resistance detection 0.32%). All other MTB complex-positive samples were tested RMP-susceptible or RMP-resistant.

Table 26

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from cultured material after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to the **GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	66	4[13)]	70
	Sensitive	0	245	245
	Total	66	249	315

[13)] Sequencing of the culture samples revealed RMP resistance in all 4 cases.		
Diagnostic sensitivity:	100%	95% CI: 94.6% to 100%
Diagnostic specificity:	98.4%	95% CI: 95.9% to 99.6%

Table 27

Results of the **FluoroType® MTBDR** VER 2.0 for detection of RMP resistance from cultured material after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to the **GenoType MTBDR*plus*** VER 2.0 and reference method (sequencing)

		Method of comparison and reference method		
	RMP	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	70	0	70
	Sensitive	0	245	245
	Total	70	245	315

Diagnostic sensitivity:	100%	95% CI: 94.9% to 100%
Diagnostic specificity:	100%	95% CI: 98.5% to 100%

12.1.2.3 Detection of INH resistance

A test result was assessed as true-resistant/sensitive when the INH resistance status detected with the **FluoroType® MTBDR** VER 2.0 was confirmed by the results of the **GenoType MTBDR*plus*** VER 2.0. Discrepancies were resolved by sequencing. In case a heteroresistance or a mixed infection was detected with the method of comparison or reference method, the sample was assessed as resistant.

FluoroLyse

For evaluation of INH resistance detection, the 315 samples were used that were positive with **GenoType MTBDR*plus*** VER 2.0 and **FluoroType® MTBDR** VER 2.0 from culture sample.
In total, 2 MTB complex-positive culture samples were tested INH resistance indeterminate with the **FluoroType® MTBDR** VER 2.0 (2 out of 315, total indeterminate rate for INH resistance detection 0.6%). All other MTB complex-positive samples were tested INH-susceptible or INH-resistant.

Table 28

Results of the **FluoroType® MTBDR** VER 2.0 for detection of INH resistance from cultured material after DNA extraction with **FluoroLyse** in comparison to the **GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	INH	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	74	0	74
	Sensitive	1[14)]	238	239
	Total	75	238	313

[14)] The sample was heteroresistant with the **GenoType MTBDR*plus*** VER 2.0 (C–15T/WT) and INH sensitive by sequencing.		
Diagnostic sensitivity:	98.7%	95% CI: 92.8% to 100%
Diagnostic specificity:	100%	95% CI: 98.5% to 100%

GXT96 X2 Extraction Kit

For evaluation of INH resistance detection, the 316 samples were used that were positive with **GenoType MTBDR*plus*** VER 2.0 and **FluoroType® MTBDR** VER 2.0 from culture sample.

In total, 2 MTB complex-positive culture samples were tested INH resistance indeterminate with the **FluoroType® MTBDR** VER 2.0 (2 out of 316, total indeterminate rate for INH resistance detection 0.64%). All other MTB complex-positive samples were tested INH-susceptible or INH-resistant.

Table 29

Results of the **FluoroType® MTBDR** VER 2.0 for detection of INH resistance from cultured material after DNA extraction with the **GXT96 X2 Extraction Kit** in comparison to the **GenoType MTBDR*plus*** VER 2.0

		Method of comparison		
	INH	Resistant	Sensitive	Total
FluoroType® MTBDR VER 2.0	Resistant	75	0	75
	Sensitive	1[15)]	238	239
	Total	76	238	314

[15)] The sample was heteroresistant with the **GenoType MTBDR*plus*** VER 2.0 (C–15T/WT) and INH sensitive by sequencing.		
Diagnostic sensitivity:	98.7%	95% CI: 92.9% to 100%
Diagnostic specificity:	100%	95% CI: 98.5% to 100%

12.2 Diagnostic performance using the X2 Cartridge

12.2.1 Clinical specimens

Diagnostic performance characteristics of the **FluoroType® MTBDR** VER 2.0 were determined in a study with 156 sputum specimens. 5 out of 156 samples were excluded from the sputum specimens due to insufficient sample volume, resulting in 151 samples (16 smear-positive, 135 smear-negative). Only patients without current anti-TB treatment were included in the study irrespective of their age and gender.

12.2.1.1 Detection of MTB complex

For detection of the MTB complex, **FluoroType® MTBDR** VER 2.0 results were compared to results of MGIT culture (BD Diagnostics, Franklin Lakes, USA) and subsequent identification of the MTB complex with the **GenoType MTBC** VER 1.X from the cultured material. Additionally, all NALC-NaOH-decontaminated specimens and all culture samples were examined by microscopy. For all samples, DNA was extracted using the **X2 Cartridge** in combination with the **GenoXtract®**. NALC-NaOH-decontaminated specimens and culture samples were inactivated using the **Inactivation Set** VER 1.0 prior to DNA extraction. For 1 false-positive sample, the presence of mycobacterial DNA was confirmed by the **FluoroType® MTB** VER 1.0.
In total, 2 sputum specimens (0 smear-positive and 2 smear-negative) were invalid with the **FluoroType® MTBDR** VER 2.0 (2 out of 151, total rate of invalid samples 1.3%). After repetition, 1 sputum sample showed a valid result and 1 sample was invalid again with the **FluoroType® MTBDR** VER 2.0 (1 out of 151, total rate of invalid samples 0.7%).

Table 30
Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from sputum specimens after DNA extraction with the **X2 Cartridge** in comparison to culture/**GenoType MTBC** VER 1.X

		Method of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	37	1[17]	38
	Negative	13[16]	98	111
	Total	50	99	149[18]

[16] After repetition, 3 samples were true-positive.
[17] 1 sample was confirmed true-positive by the **FluoroType® MTB** VER 1.0.
[18] 2 out of 151 samples were invalid.

Diagnostic sensitivity:	74.0%	95% CI: 59.7% to 85.4%
Diagnostic specificity:	99.0%	95% CI: 94.5% to 100%
Positive predictive value:	97.4%	95% CI: 86.2% to 99.9%
Negative predictive value:	88.3%	95% CI: 80.8% to 93.6%

Table 31

Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from sputum specimens after DNA extraction with the **X2 Cartridge** in comparison to culture/**GenoType MTBC** VER 1.X after repetition

		Method of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	40	1[19)]	41
	Negative	10	99	108
	Total	50	100	150[20)]

[19)] 1 sample was confirmed true-positive by the **FluoroType® MTB** VER 1.0.

[20)] 1 out of 151 samples remained invalid after repetition.

Diagnostic sensitivity:	80.0%	95% CI: 66.3% to 90.0%
Diagnostic specificity:	99.0%	95% CI: 94.6% to 100%
Positive predictive value:	97.6%	95% CI: 87.1% to 99.9%
Negative predictive value:	90.8%	95% CI: 83.8% to 95.5%

Table 32

Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-positive sputum specimens after DNA extraction with the **X2 Cartridge** in comparison to culture/**GenoType MTBC** VER 1.X

		Method of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	15	0	15
	Negative	1[21)]	0	1
	Total	16	0	16

[21)] After repetition, 1 sample was true-positive.

Diagnostic sensitivity:	93.8%	95% CI: 69.8% to 99.8%
Diagnostic specificity:	–	95% CI: –
Positive predictive value:	100%	95% CI: 78.2% to 100%
Negative predictive value:	–	95% CI: –

Table 33

Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-positive sputum specimens after DNA extraction with the **X2 Cartridge** in comparison to culture/**GenoType MTBC** VER 1.X after repetition

		Method of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	16	0	16
	Negative	0	0	0
	Total	16	0	16

Diagnostic sensitivity:	100%	95% CI: 79.4% to 100%
Diagnostic specificity:	–	95% CI: –
Positive predictive value:	100%	95% CI: 79.4% to 100%
Negative predictive value:	–	95% CI: –

Table 34

Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-negative sputum specimens after DNA extraction with the **X2 Cartridge** in comparison to culture/**GenoType MTBC** VER 1.X

		Method of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	22	1[23)]	23
	Negative	12[22)]	98	110
	Total	34	99	133[24)]

[22)] After repetition, 2 samples were true-positive.

[23)] 1 sample was confirmed true-positive by the **FluoroType® MTB** VER 1.0.

[24)] 2 out of 135 samples were invalid.

Diagnostic sensitivity:	64.7%	95% CI: 46.5% to 80.3%
Diagnostic specificity:	99.0%	95% CI: 94.5% to 100%
Positive predictive value:	95.7%	95% CI: 78.1% to 99.9%
Negative predictive value:	89.1%	95% CI: 81.7% to 94.2%

Table 35
Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from smear-negative sputum specimens after DNA extraction with the **X2 Cartridge** in comparison to culture/**GenoType MTBC** VER 1.X after repetition

		Method of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	24	1[25]	25
	Negative	10	99	109
	Total	34	100	134[26]

[25] 1 sample was confirmed true-positive by the **FluoroType® MTB** VER 1.0.
[26] 1 out of 135 samples remained invalid after repetition.

Diagnostic sensitivity:	70.6%	95% CI: 52.5% to 84.9%
Diagnostic specificity:	99.0%	95% CI: 94.6% to 100%
Positive predictive value:	96.0%	95% CI: 79.6% to 99.9%
Negative predictive value:	90.8%	95% CI: 83.8% to 95.5%

12.2.1.2 Detection of RMP resistance

A result was assessed as true-resistant/sensitive when the RMP-resistance status detected with the **FluoroType® MTBDR** VER 2.0 was confirmed by the results of the phenotypic drug susceptibility testing (DST). RMP resistance detection was assessed for all 40 samples tested MTB complex-positive with the **FluoroType® MTBDR** VER 2.0 and culture/**GenoType MTBC** VER 1.X. All 40 MTB complex-positive samples were tested RMP-susceptible with the **FluoroType® MTBDR** VER 2.0 and phenotypic DST (0 out of 40 samples RMP resistance indeterminate, total indeterminate rate of the **FluoroType® MTBDR** VER 2.0 for RMP resistance detection 0%).

12.2.1.3 Detection of INH resistance

A result was assessed as true-resistant/sensitive when the INH-resistance status detected with the **FluoroType® MTBDR** VER 2.0 was confirmed by the results of the phenotypic drug susceptibility testing (DST). INH resistance detection was assessed for all 40 samples tested MTB complex-positive with the **FluoroType® MTBDR** VER 2.0 and culture/**GenoType MTBC** VER 1.X. 37 MTB complex-positive samples were tested INH-susceptible, 2 MTB complex-positive samples were tested INH-resistant (S315T1 mutation in *katG*) with the **FluoroType® MTBDR** VER 2.0 and phenotypic DST. 1 MTB complex-positive sample was tested indeterminate with the **FluoroType® MTBDR** VER 2.0 and INH-susceptible with the phenotypic DST (1 out of 40 samples INH resistance indeterminate, total indeterminate rate of the **FluoroType® MTBDR** VER 2.0 for INH resistance detection 2.5%).

12.2.2 Cultivated samples

Diagnostic performance characteristics of the **FluoroType® MTBDR** VER 2.0 were determined in a study with 55 cultured MTB complex-positive samples derived from the same decontaminated sputum specimens. Only patients without current anti-TB treatment were included in the study irrespective of their age and gender.

12.2.2.1 Detection of MTB complex

For detection of the MTB complex, **FluoroType® MTBDR** VER 2.0 results were compared to results of **GenoType MTBC** VER 1.X from the same cultured material (MGIT culture (BD Diagnostics, Franklin Lakes, USA)). For all samples, DNA was extracted using the **X2 Cartridge** in combination with the **GenoXtract®**. NALC-NaOH-decontaminated specimens and culture samples were inactivated using the **Inactivation Set** VER 1.0 prior to DNA extraction using the **X2 Cartridge**.
In total, 2 culture samples were invalid with the **FluoroType® MTBDR** VER 2.0 (2 out of 55, total rate of invalid samples 3.6%). After repetition, all culture samples showed a valid result with the **FluoroType® MTBDR** VER 2.0 (0 out of 55, total rate of invalid samples 0%).

Table 36
Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from cultured material after DNA extraction with the **X2 Cartridge** in comparison to the **GenoType MTBC** VER 1.X from the same culture sample

		Method of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	53	0	53
	Negative	0	0	0
	Total	53	0	53[27)]

[27)] 2 out of 55 samples were invalid.

Diagnostic sensitivity:	100%	95% CI: 93.3% to 100%
Diagnostic specificity:	–	95% CI: –
Positive predictive value:	100%	95% CI: 93.3% to 100%
Negative predictive value:	–	95% CI: –

Table 37
Results of the **FluoroType® MTBDR** VER 2.0 for detection of the MTB complex from cultured material after DNA extraction with the **X2 Cartridge** in comparison to the **GenoType MTBC** VER 1.X from the same culture sample after repetition

		Method of comparison		
		Positive	Negative	Total
FluoroType® MTBDR VER 2.0	Positive	55	0	55
	Negative	0	0	0
	Total	55	0	55

Diagnostic sensitivity:	100%	95% CI: 93.3% to 100%
Diagnostic specificity:	–	95% CI: –
Positive predictive value:	100%	95% CI: 93.3% to 100%
Negative predictive value:	–	95% CI: –

12.2.2.2 Detection of RMP resistance

A result was assessed as true-resistant/sensitive when the RMP-resistance status detected with the **FluoroType® MTBDR** VER 2.0 was confirmed by the results of the phenotypic drug susceptibility testing (DST). RMP resistance detection was assessed for all 55 samples tested MTB complex-positive with the **FluoroType® MTBDR** VER 2.0 and **GenoType MTBC** VER 1.X. All 55 MTB complex-positive samples were tested RMP-susceptible with the **FluoroType® MTBDR** VER 2.0 and phenotypic DST (0 out of 55 samples RMP resistance indeterminate, total indeterminate rate of the **FluoroType® MTBDR** VER 2.0 for RMP resistance detection 0%).

12.2.2.3 Detection of INH resistance

A result was assessed as true-resistant/sensitive when the INH-resistance status detected with the **FluoroType® MTBDR** VER 2.0 was confirmed by the results of the phenotypic drug susceptibility testing (DST). INH resistance detection was assessed for all 55 samples tested MTB complex-positive with the **FluoroType® MTBDR** VER 2.0 and **GenoType MTBC** VER 1.X. 53 MTB complex-positive samples were tested INH-susceptible and 2 MTB complex-positive samples were tested INH-resistant (S315T1 mutation in *katG)* with the **FluoroType® MTBDR** VER 2.0 and phenotypic DST (0 out of 55 samples INH resistance indeterminate, total indeterminate rate of the **FluoroType® MTBDR** VER 2.0 for INH resistance detection 0%).

12.3 Analytical performance

12.3.1 Analytical specificity

The specificity of this test is ensured by the accurate design of specific primers and probes which considers, among others, homology comparisons of the sequences published in gene databases, and by stringent reaction conditions.
The analytical specificity of the **FluoroType® MTBDR** VER 2.0 was determined with all MTB complex strains as well as with strains of mycobacterial and non-mycobacterial species that are not detectable with this test system.
All eight MTB complex isolates were correctly identified. The additional 120 isolates displayed fluorescence signatures resulting in the interpretation "No MTB complex DNA detected". It was shown that with the selected primers and probes, the Analyzer specifically identifies the MTB complex amplicon. Hence, an analytical specificity of 100% was achieved for detection of MTB complex DNA.

Analyzed MTB complex strains:

Mycobacterium tuberculosis	*Mycobacterium caprae*
Mycobacterium africanum	*"Mycobacterium canettii"*
Mycobacterium bovis	*Mycobacterium microti*
Mycobacterium bovis BCG	*Mycobacterium pinnipedii*

Analyzed species which can be present in the same sample material or are phylogenetically related:

Bordetella bronchiseptica	*Mycobacterium conspicuum*	*Mycobacterium palustre*
Bordetella pertussis	*Mycobacterium cookii*	*Mycobacterium paraffinicum*
Corynebacterium coyleae	*Mycobacterium cosmeticum*	*Mycobacterium parafortuitum*
Corynebacterium jeikeium	*Mycobacterium crocinum*	*Mycobacterium parascrofulaceum*
Corynebacterium kutscheri	*Mycobacterium diernhoferi*	*Mycobacterium peregrinum*
Epstein-Barr virus	*Mycobacterium duvalii*	*Mycobacterium porcinum*
Escherichia coli	*Mycobacterium engbaekii*	*Mycobacterium poriferae*
Gordona rubropertinctus	*Mycobacterium farcinogenes*	*Mycobacterium pulveris*
Gordona terrae	*Mycobacterium flavescens*	*Mycobacterium rhodesiae*
Haemophilus influenzae	*Mycobacterium fortuitum*	*Mycobacterium rufum*
Herpes simplex virus 1	*Mycobacterium fortuitum* subsp. *acetamidolyticum*	*Mycobacterium rutilum*
Herpes simplex virus 2	*Mycobacterium fortuitum* subsp. *fortuitum*	*Mycobacterium saskatchewanense*
Legionella pneumophila	*Mycobacterium franklinii*	*Mycobacterium scrofulaceum*
Klebsiella oxytoca	*Mycobacterium gadium*	*Mycobacterium senegalense*
Klebsiella pneumoniae	*Mycobacterium gilvum*	*Mycobacterium septicum*
MRSA	*Mycobacterium gordonae*	*Mycobacterium sherrisii*
Mycobacterium abscessus subsp. *bolletii*	*Mycobacterium hackensackense*	*Mycobacterium shimoidei*
Mycobacterium abscessus subsp. *massiliense*	*Mycobacterium haemophilum*	*Mycobacterium sphagni*
Mycobacterium agri	*Mycobacterium heckeshornense*	*Mycobacterium simiae*
Mycobacterium aichiense	*Mycobacterium heidelbergense*	*Mycobacterium smegmatis*
Mycobacterium aromaticivorans	*Mycobacterium hiberniae*	*Mycobacterium terrae*
Mycobacterium arosiense	*Mycobacterium houstonense*	*Mycobacterium thermoresistibile*
Mycobacterium arupense	*Mycobacterium immunogenum*	*Mycobacterium tokaiense*

Mycobacterium asiaticum	*Mycobacterium interjectum*	*Mycobacterium triplex*
Mycobacterium aurum	*Mycobacterium intermedium*	*Mycobacterium triviale*
Mycobacterium austroafricanum	*Mycobacterium intracellulare*	*Mycobacterium vaccae*
Mycobacterium avium	*Mycobacterium kansasii*	*Mycobacterium xenopi*
Mycobacterium avium subsp. *avium*	*Mycobacterium kubicae*	*Nocardia brasiliensis*
Mycobacterium avium subsp. *paratuberculosis*	*Mycobacterium lacus*	*Nocardia brevicatena*
Mycobacterium avium subsp. *silvaticum*	*Mycobacterium lentiflavum*	*Nocardia carnea*
Mycobacterium bacteremicum	*Mycobacterium mageritense*	*Nocardia cyracigeorgica*
Mycobacterium boenickei	*Mycobacterium malmoense*	*Pseudomonas aeruginosa*
Mycobacterium branderi	*Mycobacterium marinum*	*Tsukamurella paurometabola*
Mycobacterium brisbanense	*Mycobacterium moriokaense*	*Tsukamurella pulmonis*
Mycobacterium brumae	*Mycobacterium mucogenicum*	Varicella zoster virus
Mycobacterium chelonae	*Mycobacterium nebraskense*	*Rhodococcus erythropolis*
Mycobacterium chimaera	*Mycobacterium neoaurum*	*Rhodococcus rhodochrous*
Mycobacterium chitae	*Mycobacterium neworleansense*	*Rhodococcus ruber*
Mycobacterium chlorophenolicum	*Mycobacterium nonchromogenicum*	
Mycobacterium chubuense	*Mycobacterium obuense*	
Mycobacterium confluentis	*Mycobacterium pallens*	

12.3.2 Analytical sensitivity (limit of detection, LOD)

Clinical specimens

In order to determine the LOD for clinical specimens, an *M. tuberculosis* strain was spiked into NALC-NaOH-decontaminated, MTB complex-negative sputum samples. DNA extraction was performed using the **FluoroLyse**, the **GXT96 X2 Extraction Kit**, and the **X2 Cartridge**. Testing was performed with 8 concentrations in 20 replicates each. Using probit analysis, the following LODs were determined:

Table 38

LOD for different targets and extraction methods (clinical specimens)

	LOD (p = 0.05)[28]		
Target	**FluoroLyse**	**GXT96 X2 Extraction Kit**	**X2 Cartridge**
MTB complex	9 CFU/ml	19 CFU/ml	29 CFU/ml
RMP/INH	14 CFU/ml	31 CFU/ml	108 CFU/ml

[28] Lowest concentration that generates a positive test result with a confidence of 95% (in 19 out of 20 replicates)

Cultivated samples

In order to determine the LOD for cultivated samples, *M. tuberculosis* culture dilutions were extracted using the **FluoroLyse**, the **GXT96 X2 Extraction Kit**, and the **X2 Cartridge**. Testing was performed with 8 concentrations in 20 replicates each. Using probit analysis, the following LODs were determined:

Table 39

LOD for different targets and extraction methods (cultivated samples)

	LOD (p = 0.05)[29)]		
Target	**FluoroLyse**	**GXT96 X2 Extraction Kit**	**X2 Cartridge**
MTB complex	7 CFU/ml	23 CFU/ml	65 CFU/ml
RMP/INH	8 CFU/ml	77 CFU/ml	92 CFU/ml

[29)] Lowest concentration that generates a positive test result with a confidence of 95% (in 19 out of 20 replicates)

12.3.3 Precision

Precision using the **FluoroLyse** and the **GXT96 X2 Extraction Kit**:

Clinical specimens

In order to determine repeatability and reproducibility, *M. tuberculosis* cultures (RMP- and INH-susceptible) were spiked into NALC-NaOH-decontaminated, MTB complex-negative sputum samples at concentrations above and close to the LOD. A *Mycobacterium avium* culture and a negative control were also included in the sample set. DNA extraction was performed using the **FluoroLyse** and the **GXT96 X2 Extraction Kit**.
In order to determine repeatability, samples were set up in triplicate and tested under identical conditions (identical kit lot, instrument, operator, point in time, etc.). All parallel samples showed identical correct results, no deviations were detected; hence, a repeatability of 100% was achieved.
In order to determine reproducibility, the sample panel was tested at three different points in time, while all other testing conditions (kit lot, instrument, operator, etc.) were identical. All parallel samples showed identical correct results, no deviations were detected; hence, a reproducibility of 100% was achieved.

Cultivated samples

In order to determine repeatability and reproducibility, *M. tuberculosis* cultures (RMP- and INH-susceptible) were prepared at two concentrations above and close to the LOD. A *Mycobacterium avium* culture and a negative control were also included in the sample set. DNA extraction was performed using the **FluoroLyse** and the **GXT96 X2 Extraction Kit**.
In order to determine repeatability, samples were set up in triplicate and tested under identical conditions (identical kit lot, instrument, operator, point in time, etc.). All parallel samples showed identical correct results, no deviations were detected; hence, a repeatability of 100% was achieved.
In order to determine reproducibility, the sample panel was tested at three different points in time, while all other testing conditions (kit lot, instrument, operator, etc.) were identical. All parallel samples showed identical correct results, no deviations were detected; hence, a reproducibility of 100% was achieved.

Precision using the **X2 Cartridge**:

Clinical specimens

In order to determine repeatability and reproducibility, *M. tuberculosis* isolates (RMP- and INH-susceptible) from NALC-NaOH-decontaminated sputum were used. The samples were spiked into NALC-NaOH-decontaminated, MTB complex-negative sputum samples at concentrations above and close to the LOD. DNA extraction was performed using the **X2 Cartridge**.

In order to determine repeatability, samples were tested together with a negative analyte that is not detected by the test and a negative control in three parallels under identical conditions (identical kit lot, instrument, operator, point in time, etc.). All parallel samples showed identical correct results, no deviations were detected; hence, a repeatability of 100% was achieved.

In order to determine reproducibility, the sample panel was tested at three different points in time, while all other testing conditions (kit lot, instrument, operator, etc.) were identical. All parallel samples showed identical correct results, no deviations were detected; hence, a reproducibility of 100% was achieved.

Cultivated samples

In order to determine repeatability and reproducibility, *M. tuberculosis* cultures (RMP- and INH-susceptible) were prepared at two concentrations above and close to the LOD. DNA extraction was performed using the **X2 Cartridge**.

In order to determine repeatability, samples were tested together with a negative analyte that is not detected by the test and a negative control in three parallels under identical conditions (identical kit lot, instrument, operator, point in time, etc.). All parallel samples showed identical correct results, no deviations were detected; hence, a repeatability of 100% was achieved.

In order to determine reproducibility, the sample panel was tested at three different points in time, while all other testing conditions (kit lot, instrument, operator, etc.) were identical. All parallel samples showed identical correct results, no deviations were detected; hence, a reproducibility of 100% was achieved.

12.3.4 Interfering substances

Clinical specimens

Various substances which may be present in sputum may interfere with this test. *M. tuberculosis* culture dilutions (RMP- and INH-susceptible) were spiked into MTB complex-negative sputum in concentrations above and close to the LOD. Culture dilutions as well as negative controls were mixed with the substances indicated in table 40, then decontaminated using NALC-NaOH, extracted and analyzed with the **FluoroType® MTBDR** VER 2.0.

Table 40

Tested potential interferents of the **FluoroType® MTBDR** VER 2.0

Substance/class	Description/active ingredient	Test concentration(s)
Allergy relief medicines	Tea tree oil	0.5% v/v
Anesthetics (endotracheal intubation)	Lidocaine HCl	51.2 µmol/l
Anesthetics (oral)	Benzocaine 20%	5% w/v
Antibiotics (nasal ointment)	Mupirocin	2.4 mg/ml
Antibiotics (systemic)	Amoxicillin	206 µmol/l
Anti-tuberculosis drugs	Isoniazid	292 µmol/l
Anti-tuberculosis drugs	Rifampicin	78.1 µmol/l
Anti-tuberculosis drugs	Pyrazinamide	100 µg/ml
Anti-tuberculosis drugs	Ethambutol	58.7 µmol/l
Anti-tuberculosis drugs	Streptomycin	25 µg/ml
Anti-viral drugs	Zanamivir	800 µg/ml
Blood	Hemoglobin	2 g/l

Substance/class	Description/active ingredient	Test concentration(s)
Blood	Whole blood	5% v/v
Bronchodilators	Theophylline	222 µmol/l
DNA (human)[3)]		5 µg/ml
Expectorants (oral)	Guaifenesin	15.2 mmol/l
Gastric acid	0.5% HCl, 0.1 M KCl, 0.1 M NaCl, pH 1-2	5%
Influenza vaccine	Live attenuated influenza vaccine	5% v/v
Inhaled bronchodilators	Albuterol sulfate	1.67 µmol/l
Mouthwash	Listerine	20%
Mucin: bovine submaxillary gland, type I-S	Purified mucin protein (2.5% w/v)	10% w/v
Nasal corticosteroids	Dexamethasone	1.53 µmol/l
Nasal gel (homeopathic)	Sulfur	5% w/v
Nasal sprays or drops	Phenylephrine 0.5%	10% v/v
Nebulizing solutions (hypertonic saline)	NaCl	5% w/v
Physiologic saline[30)]	NaCl (0.9%)	90% w/v
Pneumocystis jirovecii medication	Pentamidine	300 ng/ml
Pus	Buffy coat	5% v/v
Specimen processing reagents[30)]	NALC-NaOH (N-acetyl-L-cysteine-sodium hydroxide)	1% v/v
Specimen processing reagents[31)]	Isopropanol	1% v/v
Tobacco	Nicogel (40% tobacco extract)	0.5% w/v

30) Substance was spiked in the sample after NALC-NaOH-decontamination

31) Substance was spiked in the DNA isolate after DNA extraction

No inhibition of this test (leading to invalid or erroneous test results) was observed in the presence of any of the substances at the analyzed concentrations; all MTB complex-negative samples showed a valid negative test result, all MTB complex-positive samples were correctly identified.

Cultivated samples

There are substances that may inhibit PCR reactions or interfere with fluorescence measurement. Such inhibitors may, for example, originate from the culture medium. In order to assess if the medium affects the **FluoroType® MTBDR** VER 2.0, *M. tuberculosis* culture dilutions (RMP- and INH-susceptible), spiked into liquid medium in a concentration above the LOD as well as negative controls in order to assess potential interference.

Table 41

Tested potentially interfering medium of the **FluoroType® MTBDR** VER 2.0

Substance/class	Description/active ingredient	Test concentration(s)
Growth media	BD BBL MGIT + BD BACTEC MGIT growth supplement + BD BBL MGIT PANTA (Prepared according to manufacturer's instructions)	50 µl supernatant from culture

All MTB complex samples were correctly identified and displayed similar fluorescence signatures. Hence, an interference of substances from the tested medium with this test can be excluded.

12.3.5 Cross-contamination

Clinical specimens

For determination of the cross-contamination rate for NALC-NaOH decontaminated sputum samples, 240 MTB complex-positive samples (1E6 CFU/ml) and 240 MTB complex-negative samples were prepared in a checkerboard order for DNA extraction and PCR setup with the **GenoXtract® 96** in five consecutive runs. The cross-contamination rate of the **FluoroType® MTBDR** VER 2.0 with automated DNA extraction of 1E6 copies/ml and automated PCR setup with the **GenoXtract® 96** was 0.0%.

Cultivated samples

For determination of the cross-contamination rate for cultivated samples, 240 MTB complex-positive samples (1E6 CFU/ml, MGIT) and 240 MTB complex-negative samples were prepared in a checkerboard order for DNA extraction and PCR setup with the **GenoXtract® 96** in five consecutive runs. The cross-contamination rate of the **FluoroType® MTBDR** VER 2.0 with automated DNA extraction of 1E6 copies/ml and automated PCR setup with the **GenoXtract® 96** was 0.0%.

12.3.6 Stability

Shelf life of the kit when stored as recommended: see box label.
Stability is determined according to DIN EN ISO 23640.

13 References

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14 Table of Symbols

14.1 Non-harmonized symbols

Symbol	Meaning
	Analyzer version; see chapter 4.4
	Checksum

14.2 Harmonized symbols

Symbol	Meaning
	CE-mark
	In vitro diagnostic medical device
	Sufficient for <n> assays
	Manufacturer
	Consult instructions for use
	Caution

Symbol	Meaning
	Order number
	Use by
	Batch code
	Date of manufacture
	Temperature limitations

15 Important Changes

15.1 Important Changes in IFU-628-05

Chapter	Change
Materials required but not included in the kit	Further compatible sample tubes for use with the **FluoroType® MTBDR** VER 2.0 were added.
Performance characteristics	Completion of diagnostic performance characteristics.

15.2 Important Changes in IFU-628-06

Chapter	Change
Several (e.g. Principles of the Procedure, DNA Extraction and PCR Setup, Performance Characteristics)	The DNA extraction for the **FluoroType® MTBDR** VER 2.0 can also be performed with the new **X2 Cartridge**.
Kit contents	The **FluoroType® MTBDR** VER 2.0 with the catalog number 62896 contains two tubes of the Universal Internal Control DNA 6 (U-IC6).
Automated DNA extraction with the **GXT96 X2 Extraction Kit** and automated PCR setup, Automated DNA extraction with the **GXT96 X2 Extraction Kit** and manual PCR setup	**New:** "Mix sample material carefully but thoroughly by pipetting up and down with a disposable transfer pipette (see chapter 4.5)."
Manual extraction with the **FluoroLyse**	New chapter

15.3 Important Changes in IFU-628-07

Chapter	Change
Kit contents	The **FluoroType® MTBDR** VER 2.0 for analysis of 24 samples (order no.:62824) is available on request.
Internal Control	**New:** "A multiple addition of the U-IC6 can have a negative effect on the test evaluation and/or test performance."
Specimen Requirements	**New:** "Due to the potential inhomogeneity of the cultured sample, it must be mixed before removing the aliquot to be analyzed; otherwise the sensitivity of the test might be influenced."
DNA Extraction and PCR Setup	**New:** "Thaw C+ and equilibrate to room temperature (15°C to 25°C), vortex for 5 s, and spin down briefly."
Test Evaluation	**New:** "The following mutations in the rifampicin resistance determining region (RRDR) of the *rpoB* gene (amino acid 510 to 533 (*Escherichia coli*-based annotation) or 429 to 452 (*M. tuberculosis* H37Rv-based annotation)) can be specified:"
Test Evaluation	The detected mutations are based on the WHO catalogue [13].

CE IVD

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