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Name + Adresse der Firma:
Nom + adresse de l'entreprise:
Name + address of manufacturer:
Nome + indirizzo della ditta:
Nombre + dirección del fabricante:

Hain Lifescience GmbH
Hardwiesenstraße 1
72147 Nehren
Germany
Tel +49-7473-9451-0
Fax +49-7473-9451-99

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GenoType MTBDR*sl*
VER 2.0

317A (12)
31796A (96)

allen anwendbaren Anforderungen der Richtlinie 98/79/EG entspricht.
remplit toutes les exigences applicables de la Directive 98/79/CE.
meets all the applicable provisions of the Directive 98/79/EC.
adempie a tutte le applicabile esigenze della Direttiva 98/79/CE.
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May 26, 2022

Nehren, May 23, 2022

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GenoType MTBDR*sl*

VER 2.0

Instructions for Use

IFU-317A-06

CE

IVD for in vitro diagnostic use only

2023-06-22

HAIN LIFESCIENCE

Please read the instructions on hand completely and carefully before using the kit. Strictly adhere to the established procedure to obtain correct test results.

Intended Use

The **GenoType MTBDR*sl*** VER 2.0 is a qualitative in vitro diagnostic test for the manual detection of the *Mycobacterium tuberculosis* (MTB) complex and its mutations conferring resistances to fluoroquinolones (FLQ) and second-line injectable drugs (SLID) from smear-positive or -negative sputum specimens and cultivated samples, using compatible devices.
The tuberculosis-causing MTB complex is identified by detection of a locus that is specific to members of MTB complex, including: *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. caprae*, *M. bovis* BCG, *M. microti*, *M. canettii*, and *M. pinnipedii*.
The detection of FLQ resistance is enabled by the detection of the most significant resistance-associated mutations of the *gyrA* and *gyrB* genes. For detection of SLID resistance, the 16S rRNA gene *(rrs)* is examined, the promoter region of the *eis* gene is also examined.

The product is indicated as an aid for diagnosis of patients suspected of MTB complex infection and intended for use by laboratory professionals in clinical diagnostic laboratories.

Summary and Explanation

Tuberculosis (TB) is a bacterial infectious disease passed on by droplet infection. In 2021, there were an estimated 10.6 million incident cases of TB globally, and an estimated 1.6 million TB deaths [1]. TB treatment requires a therapy over several months. Emergence and spread of drug-resistant tuberculosis is a major medical and public health problem threatening global health. Multidrug-resistant (MDR-)TB is defined as TB that is resistant at least to the first-line drugs rifampicin and isoniazid. The other first-line anti-TB drugs are pyrazinamide, ethambutol, and streptomycin. All other anti-TB drugs are generally referred to as second-line drugs, and include fluoroquinolones, such as levofloxacin (LEV) and moxifloxacin (MFX), and second-line injectable drugs, such as amikacin (AMK), and formerly, capreomycin (CAP) and kanamycin (KAN) [2]. Due to its complex diagnosis and obstacles in treatment, MDR-TB that is also resistant to second-line drugs is a major challenge to TB control. Rapid diagnosis of TB resistant to second-line drugs is a prerequisite for appropriate treatment and effective containment of the infection.
Identification of FLQ resistance is enabled by the detection of mutations of the *gyrA* and *gyrB* genes (coding for the A and B subunits of the DNA gyrase, respectively). Identification of SLID resistances to AMK, CAP, and KAN is enabled by detection of mutations of the *rrs* gene (coding for the 16S rRNA) and the promoter region of the *eis* gene (coding for the acetyltransferase Eis). KAN and CAP use in TB treatment is no longer recommended by the WHO and resistances to these drugs are provided for information only.

Principles of the Procedure

The **GenoType MTBDR*sl*** VER 2.0 is based on the **DNA•STRIP** technology. The whole procedure is divided into three steps: (i) DNA extraction from NALC-NaOH-decontaminated sputum specimens or cultured material (solid/liquid medium) – the necessary reagents are not included in the kit, (ii) a multiplex amplification with biotinylated primers, and (iii) a reverse hybridization.
All reagents needed for amplification, such as polymerase and primers, are included in the Amplification Mixes A and B (AM-A and AM-B) and are optimized for this test. The membrane strips are coated with specific probes complementary to the amplified nucleic acids. After chemical denaturation, the single-stranded amplicons bind to the probes (hybridization). Highly specific binding of complementary DNA strands is ensured by stringent conditions which result from the combination of buffer composition and a certain temperature. Thus, the probes reliably discriminate several sequence variations in the gene regions examined. The streptavidin-conjugated alkaline phosphatase binds to the amplicons' biotin via the streptavidin moiety. Finally, the alkaline phosphatase transforms an added substrate into a dye which becomes visible on the membrane strips as a colored precipitate. A template ensures the easy and fast interpretation of the banding pattern obtained.

Reagents and Instruments

Kit contents

Order no. Tests	317A 12	31796A 96
Kit Component 1 of 2 (store at 2°C to 8°C)		
Membrane strips coated with specific probes (MTBDRsl VER 2.0 STRIPS)	12	2x 48
Denaturation Solution (DEN) contains <2% NaOH, dye	240 µl	2x 960 µl
Hybridization Buffer (HYB) contains <10% anionic tenside, dye	12 ml	96 ml
Stringent Wash Solution (STR) contains >25% of a quaternary ammonium compound, <1% anionic tenside, dye	12 ml	96 ml
Rinse Solution (RIN) contains buffer, <1% NaCl, <1% nonionic tenside	36 ml	3x 96 ml
Conjugate Concentrate (CON-C) contains streptavidin-conjugated alkaline phosphatase, dye	120 µl	960 µl
Conjugate Buffer (CON-D) contains buffer, 1% blocking reagent, <1% NaCl	12 ml	96 ml
Substrate Concentrate (SUB-C) contains <70% dimethyl sulfoxide, <10% 4-nitro blue tetrazolium chloride, <10% 5-bromo-4-chloro-3-indolyl phosphate	120 µl	960 µl
Substrate Buffer (SUB-D) contains buffer, <1% $MgCl_2$, <1% NaCl	12 ml	96 ml
Tray, evaluation sheet	1 of each	4 of each
Instructions for use, template	1 of each	1 of each
Lot label	3	3
Kit Component 2 of 2 (store at –20°C to –18°C)		
Amplification Mix A (AM-A GT MTBDRsl VER 2.0) contains buffer, nucleotides, Taq polymerase	120 µl	4x 240 µl
Amplification Mix B (AM-B GT MTBDRsl VER 2.0) contains salts, specific primers, dye	420 µl	4x 840 µl

Storage and disposal of kit constituents

1/2 Kit Component 1 of 2

2/2 Kit Component 2 of 2

Store all constituents from Kit Component 1 at 2°C to 8°C. Store all constituents from Kit Component 2 at –20°C to –18°C and keep strictly separated from contaminating DNA. Avoid repeated freezing and thawing (>4x) of AM-A and AM-B; when processing only small sample numbers per run, aliquot AM-A and AM-B using suitable screw cap tubes (recommendation: see chapter Ordering Information). Do not use the reagents beyond their expiry date. Dispose of unused reagents and waste in accordance with federal, state, and local regulations.

Precautions for handling kit constituents

Observe all federal, state, and local safety and environmental regulations. Always wear suitable protective clothing, protective gloves, and eye protection.

For information on the hazardous substances included in the kit, please refer to the safety data sheets which can be downloaded from:
www.hain-lifescience.com/products/msds.html

Conjugate Concentrate **(CON-C)** and Conjugate Buffer **(CON-D)** contain biological material. Hence, they must be considered as potentially infectious and must be handled accordingly (e.g. see [3] or [4]).

Materials required but not included in the kit
- 0.5 ml screw cap tubes or 1.5 ml screw cap tubes for aliquots (Sarstedt, Nümbrecht, Germany, see chapter Ordering Information)
- Absorbent paper
- Adjustable pipettes for 10, 20, 200, and 1000 µl
- Disposable gloves
- Disposable sterile pipette tips with filter
- DNA extraction kit (**GenoLyse**®, see chapter Ordering Information) and necessary equipment:
  - 1.5 ml screw cap tubes (Sarstedt, Nümbrecht, Germany, see chapter Ordering Information)
  - Inoculation loop (for transfer of solid cultures)
- Graduated cylinder
- PCR tubes, DNase- and RNase-free
- Reagents for cultivation of mycobacteria as well as necessary equipment (when cultivated samples are to be used)
- Sample decontamination reagents as well as necessary equipment
- Shaking water bath + shaking platform ***or*** **TwinCubator** (instrument for manual hybridization) ***or*** **GT-Blot 48** (instrument for automated hybridization)
- Thermal cycler (ramp rate adjustable to ≤2.2°C/s; thermal cyclers with higher ramp rate are not suitable for this test system)
- Timer
- Tweezers
- Water (distilled)
- Water (molecular biology grade; for negative controls)

Quality Control

In order to control the correct performance of the test and the proper functioning of kit constituents, each strip includes 6 control zones:
- a Conjugate Control zone (CC) to check the binding of the conjugate on the strip and a correct chromogenic reaction
- an Amplification Control zone (AC) to check for a successful amplification reaction
- four Locus Control zones (*gyrA*, *gyrB*, *rrs*, and *eis*) checking the optimal sensitivity of the reaction for each of the tested gene loci

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 Amplification Mixes or membrane strips from different kits unless the lots are identical. You can find the kit lot and the corresponding lots of the kit constituents on the lot labels included in the kit.

A positive sample (not provided with the kit) must be included in each run.
A negative control for detection of possible contamination events should be part of each DNA extraction. Alternatively, a negative control can be included during PCR setup (see chapter Amplification). The test strip of the negative control should show the bands CC and AC only.

Specimen Requirements

NALC-NaOH-decontaminated smear-positive or -negative sputum samples as well as cultivated samples (solid/liquid medium) can be used as starting material for DNA extraction. The performance of the test has not been validated with other sample materials.

Precautions for handling specimens
Patient specimens must always be considered as potentially infectious and must be handled accordingly (e.g. see [3] or [4]). 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)) 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 [3]). 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.

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" [5], the "Clinical Microbiology Procedures Handbook" [6], 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 2°C to 8°C must be carried out as soon as possible and should be done within 1 to 2 days [7,8]. Specimens used for decontamination must not be older than 3 days [9].
After decontamination and subsequent resuspension of the bacteria pellet with phosphate buffer, samples can be stored at 2°C to 8°C for a maximum of 7 days until performing DNA extraction.
Cultivated samples must be processed without delay.

Preparation
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" [5].
After decontamination, the cell pellet should be resuspended in a maximum of 1 to 1.5 ml of phosphate buffer. When testing patient specimens, higher volumes might hamper the sensitivity of the test. 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.
When the sample is to be cultivated, cultivation can be performed either on solid medium (e.g. Loewenstein-Jensen, Middlebrook) or in liquid medium (e.g. MGIT (BD Diagnostics, Franklin Lakes, USA)).

DNA Extraction

For DNA extraction from NALC-NaOH-decontaminated clinical specimens or cultured material, the **GenoLyse**® (see chapter Ordering Information) is used.
The method mentioned here was used for performance evaluation of the **GenoType MTBDR*sl*** VER 2.0.
Each DNA extracted from sputum or a cultivated sample using the **GenoLyse**® can be used for amplification with the **GenoType MTBDR*sl*** VER 2.0 (e.g. subsequent to the **GenoType MTBDR*plus*** VER 2.0).

Manual DNA extraction with GenoLyse®
For additional information, please refer to the current version of the instructions for use of the **GenoLyse®**.

Sample preparation for patient specimens
Transfer 500 µl of decontaminated sample material into a labeled 1.5 ml screw cap tube.

Sample preparation for bacteria grown in liquid medium
Transfer 1 ml into a labeled 1.5 ml screw cap tube.

Sample preparation for bacteria grown on solid medium
Collect bacteria with an inoculation loop and suspend in 100 µl of Lysis Buffer (A-LYS) in a labeled 1.5 ml screw cap tube, vortex, and continue with step 4 (see instructions for use of the **GenoLyse®**).

Sample preparation for negative controls
Please refer to the current version of the instructions for use of the **GenoLyse®**.

Please proceed as described in the current version of the instructions for use of the **GenoLyse®** (procedure without Internal Control).

Amplification

All reagents needed for amplification, such as polymerase and primers, are included in the Amplification Mixes A and B (AM-A and AM-B) and are optimized for this test. Thaw AM-A and AM-B shortly before preparing the master mix, spin down briefly, and mix carefully by pipetting up and down. Pipette AM-A and AM-B only in a room free from contaminating DNA. To avoid contamination, the DNA solution must be added in a separate working area.

Prepare for each sample:
- 10 µl AM-A (see Kit Component 2)
- 35 µl AM-B (see Kit Component 2)
- 5 µl DNA solution

Final volume: 50 µl

Determine the total number of samples (number of samples to be analyzed plus control samples). Prepare the number of PCR tubes needed. Prepare a master mix containing AM-A and AM-B and mix carefully but thoroughly (do not vortex). Alternatively, the content of an AM-A reaction tube may completely be transferred to an AM-B reaction tube. This will lead to master mix sufficient for 12 amplification reactions (12 tests kit) or for 4x 24 amplification reactions (96 tests kit). Please note that the master mix needs to be prepared freshly each time and needs to be processed quickly.
Avoid any delay in processing. DNA addition and start of the thermocycler should be done within one hour after preparation of the master mix.
Aliquot 45 µl of the master mix into each of the prepared PCR tubes.
If a negative control sample is included during PCR setup, add 5 µl water (molecular biology grade) to one aliquot. A negative control that has been included during DNA extraction (see chapter DNA Extraction) is processed like a regular sample (see below).
In a separate working area, add 5 µl DNA solution to each aliquot of the master mix (except for a negative control which has been included during PCR setup).

Amplification profile:
When using a thermal cycler from Hain Lifescience with the respective preinstallation, select protocol "MDR DIR" for clinical specimens or protocol "MDR CUL" for cultivated samples.
When using a different thermal cycler, use the following amplification profile. The heating rate must not be higher than 2.2°C/s. Higher heating rates can lead to invalid, not interpretable, or wrong results [10].

		Clinical specimens	Cultivated samples
15 min	95°C	1 cycle	1 cycle
30 s 2 min	95°C 65°C	20 cycles	10 cycles
25 s 40 s 40 s	95°C 50°C 70°C	30 cycles	20 cycles
8 min	70°C	1 cycle	1 cycle
Heating rate		≤2.2°C/s	≤2.2°C/s

Amplification products can be stored at –20°C to +8°C.

Hybridization

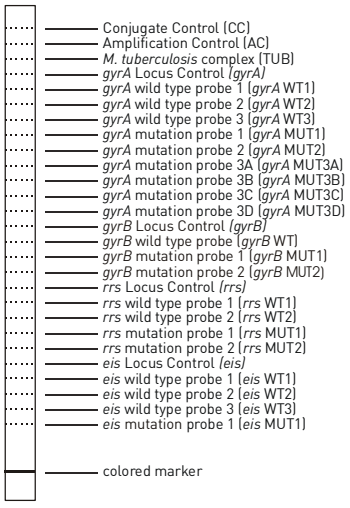
When using a hybridization instrument from Hain Lifescience (**GT-Blot 48** or **TwinCubator**), please refer to the document "Overview equipment programs" available on www.hain-lifescience.com for the name of the hybridization protocol to be used.
The following protocol describes the manual hybridization using a water bath or a **TwinCubator**.

Preparation
Prewarm shaking water bath to **45°C** (the maximum tolerated deviation from the target temperature is +/–1°C) or switch on **TwinCubator**. Prewarm solutions HYB and STR to 37°C to 45°C before use. The reagents must be free from precipitates (note, however, that solution CON-D is opaque). Mix if necessary. Warm the remaining reagents with the exception of CON-C and SUB-C to room temperature. Using a suitable tube, dilute Conjugate Concentrate (CON-C, orange) and Substrate Concentrate (SUB-C, yellow) 1:100 with the respective buffer **(CON-C with CON-D, SUB-C with SUB-D)** in the amounts needed. Mix well and bring to room temperature. For each strip, add 10 µl concentrate to 1 ml of the respective buffer. Dilute CON-C before each use. Diluted SUB-C is stable for 4 weeks if stored at room temperature and protected from light.

1. **Dispense 20 µl of Denaturation Solution (DEN, blue) in a corner of each of the wells used.**
2. **Add to the solution 20 µl of amplified sample, pipette up and down to mix well and incubate at room temperature for 5 minutes.**
   Meanwhile, take strips out of the tube using tweezers and mark them with a pencil underneath the colored marker. Always wear gloves when handling strips.
3. **Carefully add to each well 1 ml of prewarmed Hybridization Buffer (HYB, green). Gently shake the tray until the solution has a homogenous color.**
   Take care not to spill solution into the neighboring wells.
4. **Place a strip in each well.**
   The strips must be completely covered by the solution and the coated side (identifiable by the colored marker near the lower end) must face upward. Using tweezers, turn over strips which might have turned when immersed in the solution. Carefully clean tweezers after each use to avoid contamination. This also applies to all following steps.
5. **Place tray in shaking water bath/TwinCubator and incubate for 30 minutes at 45°C.**
   Adjust the shaking frequency of the water bath to achieve a constant and thorough mixing of the solution. To allow adequate heat transfer, the tray must be dipped into the water to at least 1/3 of its height.
6. **Completely aspirate Hybridization Buffer.**
   For example, use a Pasteur pipette connected to a vacuum pump.
7. **Add 1 ml of Stringent Wash Solution (STR, red) to each strip and incubate for 15 minutes at 45°C in shaking water bath/TwinCubator.**
8. **Work at room temperature from this step forward. Completely remove Stringent Wash Solution.**
   Pour out Wash Solution in a waste container and remove all remaining fluid by turning the tray upside down and gently striking it on an absorbent paper. This also applies to all other wash steps.
9. **Wash each strip once with 1 ml of Rinse Solution (RIN) for 1 minute on shaking platform/TwinCubator (pour out RIN after incubation).**
10. **Add 1 ml of diluted Conjugate (see above) to each strip and incubate for 30 minutes on shaking platform/TwinCubator.**
11. **Remove solution and wash each strip twice for 1 minute with 1 ml of Rinse Solution (RIN) and once for 1 minute with approx. 1 ml of distilled water (e.g. use wash bottle) on shaking platform/TwinCubator (pour out solution each time).**
    Make sure to remove any trace of water after the last wash.
12. **Add 1 ml of diluted substrate (see above) to each strip and incubate protected from light without shaking.**
    Depending on the test conditions (e.g. room temperature), the substrate incubation time, i.e. the time until the bands are clearly visible, can vary between 3 and 20 minutes. Extended substrate incubation times can lead to increased background staining and might impair interpretation of the results.
13. **Stop reaction as soon as bands are clearly visible by briefly rinsing twice with distilled water.**
14. **Using tweezers, remove strips from the tray and dry them between two layers of absorbent paper.**

Evaluation and Interpretation of Results

Paste strips and store protected from light. An evaluation sheet is included in the kit. When using this evaluation sheet, paste the developed strips in the designated fields by aligning the bands CC and AC with the respective lines on the sheet. For technical reasons the distances between single probes on the strips may vary slightly. **For an accurate evaluation therefore please use the provided template and align it – separately for each locus – with the respective Locus Control band.** Determine the resistance status and note down in the respective column.
As a help for interpretation, evaluation examples are given below.
Each strip has a total of 27 reaction zones (see figure).



Note: The strip is not displayed in original size.

General notes for evaluation
Not all bands of a strip have to show the same signal strength.
Only those bands whose intensities are about as strong as or stronger than that of the Amplification Control zone (AC) are to be considered.

Identification of FLQ resistance is enabled by the detection of mutations of the *gyrA* and *gyrB* genes. Identification of SLID resistances to AMK, CAP and KAN are enabled by detection of mutations of the *rrs* gene. In addition, the promoter region of the *eis* gene is examined to identify resistances to AMK and KAN. The wild type and mutation probes cover the most important resistance-associated regions of the examined genes. In case a mutation band is present, resistance to the respective antibiotic is detected (D). In case a wild type band is absent and no mutation band is present for the respective locus, the exact mutation is unknown, but resistance to the respective antibiotic is very probable and therefore inferred (I). In case all wild type bands, but no mutation band is present for one locus, resistance to the respective antibiotic is not detected (N).
If there are different evaluations (D/I/N) for one antibiotic by different loci, the resistance status with the highest rank counts (D overrules I and I overrules N). If a high-level resistance to an antibiotic is detected, the identification of a low-level resistance is not relevant.

When a complete gene locus (all bands including the Locus Control band) is missing, the result of this strip is not interpretable and the test must be repeated (see special cases section for exception concerning *eis* locus).

Conjugate Control (CC)
A line must develop in this zone, documenting the efficiency of conjugate binding and substrate reaction.

Amplification Control (AC)
When the test is performed correctly, a control amplicon will bind to the Amplification Control zone. Only those bands whose intensities are about as strong as or stronger than that of the Amplification Control zone (AC) are to be considered.
In case of a positive test result, the signal of the Amplification Control zone can be weak or even vanish totally. This might be due to competition of the single reactions during amplification. In this case the test was performed correctly and does not have to be repeated.
When only the CC and AC bands are developed, this represents a valid negative result. A missing AC band in case of a negative test result indicates mistakes during setup and/or performance of the amplification reaction, or presence of amplification inhibitors. In this case, the test result is invalid and the test must be repeated with the respective sample.

***M. tuberculosis* complex (TUB)**
This zone hybridizes with amplicons generated from all members of the *Mycobacterium tuberculosis* complex. If the TUB zone is negative while no evaluable resistance pattern is developed, the tested specimen does not contain bacteria belonging to the *M. tuberculosis* complex and cannot be evaluated by this test system.

Locus Controls *(gyrA, gyrB, rrs, eis)*
The Locus Control zones detect gene regions specific for the respective locus. In case of a positive test result (evaluable wild type and mutation banding pattern), the signals of the Locus Control bands may be weak.

gyrA
Both the *gyrA* and *gyrB* genes are examined for detection of resistance to the fluoroquinolones levofloxacin (LEV) and moxifloxacin (MFX; low-level resistance/high-level resistance).
If a high-level resistance to moxifloxacin is detected, the identification of a low-level resistance is not relevant.
The wild type probes comprise the most important resistance regions of the *gyrA* gene (see table 5).

Table 1: Evaluation of *gyrA* wild type and mutation bands (according to [11]); LEV, levofloxacin; MFX, moxifloxacin; D, resistance detected; I, resistance inferred; LL, low-level; HL, high-level

Negative wild type band	Positive mutation band	LEV resistance	MFX resistance
gyrA WT1	-	I	I (LL)
gyrA WT2	-	I	I (LL)
	gyrA MUT1	D	D (LL)
	gyrA MUT2	D	D (LL)
gyrA WT3	-	I	I (LL)
	gyrA MUT3A	D	D (LL)
	gyrA MUT3B	D	D (HL)
	gyrA MUT3C	D	D (HL)
	gyrA MUT3D	D	D (HL)

gyrB
The *gyrB* gene is examined for detection of resistance to the fluoroquinolones levofloxacin (LEV) and moxifloxacin (MFX; low-level resistance).
If a high-level resistance to moxifloxacin is detected through the *gyrA* gene, the identification of a low-level resistance through *gyrB* is not relevant.
The wild type probe comprises the most important resistance region of the *gyrB* gene (see table 5).

Table 2: Evaluation of *gyrB* wild type and mutation bands (according to [11]); LEV, levofloxacin; MFX, moxifloxacin; D, resistance detected; I, resistance inferred; LL, low-level)

Negative wild type band	Positive mutation band	LEV resistance	MFX resistance
gyrB WT	-	I	I (LL)
	gyrB MUT1	D	D (LL)
	gyrB MUT2	D	D (LL)

rrs
The *rrs* gene is examined for detection of cross-resistance to SLID such as amikacin (AMK), capreomycin (CAP), or kanamycin (KAN).
The wild type probes comprise the most important resistance regions of the *rrs* gene (see table 5).

Table 3: Evaluation of *rrs* wild type and mutation bands (according to [11]); AMK, amikacin; CAP, capreomycin; KAN, kanamycin; D, resistance detected; I, resistance inferred

Negative wild type band	Positive mutation band	AMK/CAP/KAN resistance
rrs WT1	-	I
	rrs MUT1	D
rrs WT2	-	I
	rrs MUT2	D

eis

The promoter region of the *eis* gene is examined for detection of an amikacin (AMK) resistance and a kanamycin (KAN) resistance.
The wild type probes comprise the most important resistance regions of the promoter region of the *eis* gene (see table 5).

Table 4: Evaluation of *eis* wild type and mutation bands (according to [11]); KAN, kanamycin; AMK, amikacin; D, resistance detected; I, resistance inferred

Negative wild type band	Positive mutation band	KAN resistance	AMK resistance[2]
eis WT1	-	I	-
eis WT2	-	I	-
	eis MUT1	D	D
eis WT3	-	I[1]	-

[1] If the *eis* WT3 band is negative, this might be due to an unknown resistance-conferring mutation, although no such mutation has been described to date [11,12].
[2] In the *eis* locus, only the c–14t mutation (*eis* MUT1 band) is associated with an AMK resistance [11].

Table 5: Mutations and the corresponding wild type and mutation bands

Negative wild type band	Region covered	Positive mutation band	Known resistance-conferring mutations
gyrA WT1	Codons 85–90[3]	-	G88A
			G88C
gyrA WT2	Codons 89–93[3]	*gyrA* MUT1	A90V
		gyrA MUT2	S91P
gyrA WT3	Codons 92–96[3]	*gyrA* MUT3A	D94A
		gyrA MUT3B	D94N
			D94Y
		gyrA MUT3C	D94G
		gyrA MUT3D	D94H[4]
gyrB WT	Codons[3] 497–502[5]/ 536–541[6]	*gyrB* MUT1	N499D[5]/N538D[6]
		gyrB MUT2	E501V[5]/E540V[6]
rrs WT1	1400 region	*rrs* MUT1	a1401g
		-	c1402t
rrs WT2	1484 region	*rrs* MUT2	g1484t
eis WT1	-37 region	-	g–37t
eis WT2	-12 region	*eis* MUT1	c–14t
		-	c–12t
		-	g–10a
eis WT3	-2 region	-	unknown[7]

[3] Not all mutations in these codons, particularly if they occur at the edges of the probes, lead to a negative band.
[4] This rare mutation has only been detected theoretically (in silico).
[5] Numbering system 2002 according to [13]
[6] Numbering system 1998 according to [13]
[7] If the *eis* WT3 band is negative, this might be due to an unknown resistance-conferring mutation, although no such mutation has been described to date [11,12].

Special cases

When both a mutation probe and the corresponding wild type probe of a strip are developed, this represents a valid result. Possible reasons could be:
- The tested specimen contains a heteroresistant strain.
- The tested specimen contains more than one *M. tuberculosis* complex strain (e.g. due to mixed infection of the patient).

Theoretically, a resistance can exist in spite of a wild type pattern. Possible reasons could be:
- The tested specimen contains a strain that has developed a heteroresistance and the resistance is caused by a mutation not covered by the mutation probes.
- The tested specimen contains a wild type and a resistant strain (e.g. due to mixed infection of the patient) and the resistance is caused by a mutation not covered by the mutation probes.

In rare cases, all bands of a gene locus (including the Locus Control band) may be missing completely on a test strip. If this result is generated from a clinical specimen, possible reasons could be, but are not limited to, a DNA concentration below the limit of detection or the presence of interfering substances in the sample. Such a banding pattern is not interpretable and the test must be repeated. If a cultivated sample generates a result with the complete *eis* locus missing whilst the bands of all other loci are developed, this is most likely due to a deletion that does not confer resistance to kanamycin or amikacin. The remaining loci can be evaluated in this case [14].

Evaluation examples

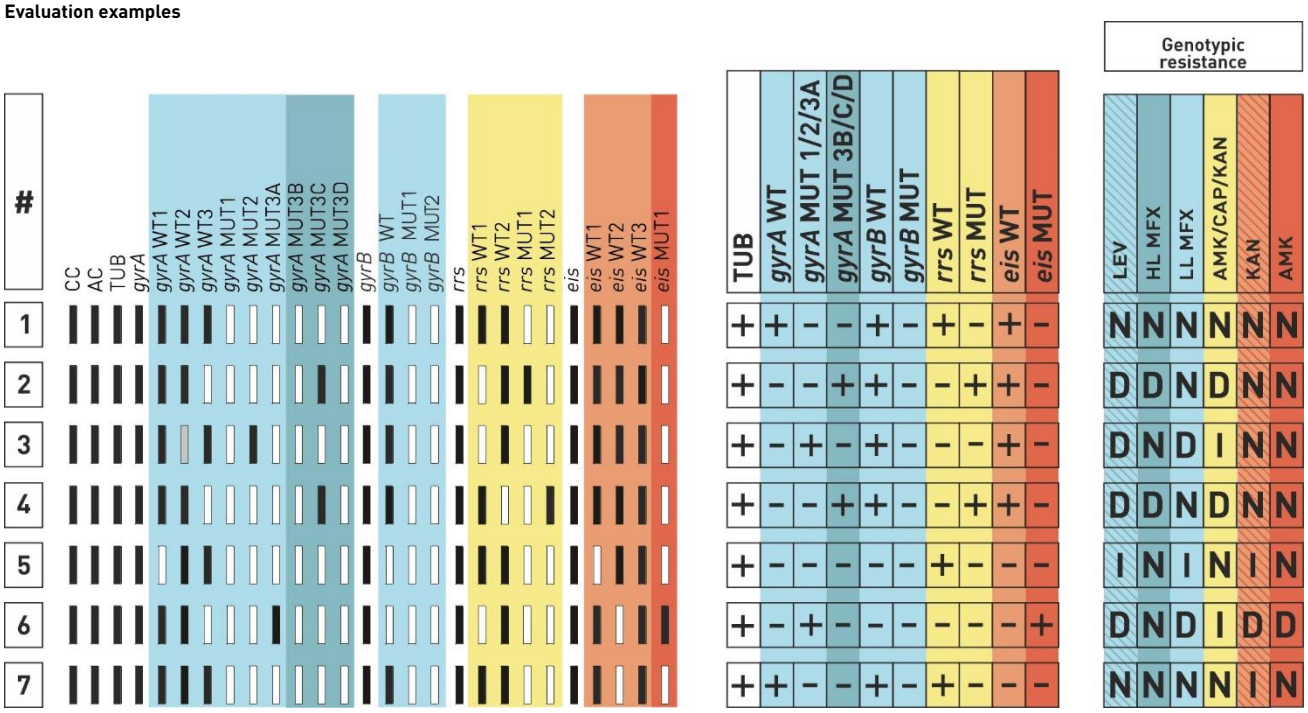


Figure 1: Examples for banding patterns and their evaluation according to [11] with respect to levofloxacin (LEV) resistance, high-level moxifloxacin (HL MFX) resistance, low-level moxifloxacin (LL MFX) resistance, amikacin/capreomycin/kanamycin (AMK/CAP/KAN) resistance, kanamycin (KAN) resistance, and amikacin (AMK) resistance. D, resistance detected; N, resistance not detected; I, resistance inferred.

General notes for using the evaluation sheet

If all wild type bands of a locus are positive, enter "+" in the respective WT column. If at least one of the wild type bands is absent, enter "–".
If none of the mutation bands of a locus are positive, enter "–" in the respective MUT column (for *gyrA*, the mutation bands MUT 1, 2, and 3A are evaluated separately from the mutation bands MUT 3B, C, and D). If at least one of the mutation bands is positive, enter "+".

In case a mutation band is positive, enter "D" (resistance detected) in the respective resistance column (see tables 1-4).
In case a wild type band is absent and no mutation band is present for the respective locus, enter "I" (resistance inferred) in the respective resistance column (see tables 1-4).
In case all wild type bands are positive and no mutation bands are present, enter "N" (resistance not detected).

Table 6: Evaluation of the examples (figure 1) according to [11]; LEV, levofloxacin; MFX, moxifloxacin; LL, low-level; HL, high-level; AMK, amikacin; CAP, capreomycin; KAN, kanamycin

Example #	Banding pattern (deviation from wild type)	WT/MUT columns on evaluation sheet	"Genotypic resistance" on evaluation sheet (see tables 1-4)
1	**Wild type banding pattern:**		
	gyrA/*gyrB*: All WT bands positive All MUT bands negative	*gyrA*/*gyrB*: *gyrA* WT: "+" *gyrA* MUT 1/2/3A: "–" *gyrA* MUT 3B/C/D: "–" *gyrB* WT: "+" *gyrB* MUT: "–"	LEV: "N" (resistance not detected) HL MFX: "N" (resistance not detected) LL MFX: "N" (resistance not detected)
	rrs: All WT bands positive All MUT bands negative	*rrs*: *rrs* WT: "+" *rrs* MUT: "–"	AMK/CAP/KAN: "N" (resistance not detected)
	eis: All WT bands positive MUT band negative	*eis*: *eis* WT: "+" *eis* MUT: "–"	KAN: "N" (resistance not detected) AMK: "N" (resistance not detected)
	Example 1 shows the wild type banding pattern. All wild type probes but none of the mutation probes display a signal; hence, the evaluation chart shows "+" in the four wild type columns and "–" in the five mutation columns. Accordingly, "N" (resistance not detected) is entered in the resistance columns.		
	Final evaluation: no resistance detected		

Example #	Banding pattern (deviation from wild type)	WT/MUT columns on evaluation sheet	"Genotypic resistance" on evaluation sheet (see tables 1-4)
2	*gyrA*/*gyrB*: *gyrA* WT3 band negative *gyrA* MUT3C band positive	*gyrA*/*gyrB*: *gyrA* WT: "–" *gyrA* MUT 1/2/3A: "–" *gyrA* MUT 3B/C/D: "+" *gyrB* WT: "+" *gyrB* MUT: "–"	LEV: "D" (resistance detected) HL MFX: "D" (resistance detected) LL MFX: "N" (not relevant because HL MFX resistance is detected)
	rrs: *rrs* WT1 band negative *rrs* MUT1 band positive	*rrs*: *rrs* WT: "–" *rrs* MUT: "+"	AMK/CAP/KAN: "D" (resistance detected)
	eis: Wild type banding pattern	*eis*: *eis* WT: "+" *eis* MUT: "–"	KAN: "N" (resistance not detected) AMK: "N" (resistance not detected)
	The *gyrA* WT3 band is missing and the *gyrA* MUT3C band is developed. Hence, the evaluation chart shows a "–" in the "*gyrA* WT" and "*gyrA* MUT 1/2/3A" columns and a "+" in the "*gyrA* MUT 3B/C/D" column. The *gyrB* locus displays the wild type banding pattern resulting in a wild type entry as in example 1. Due to the *gyrA* banding pattern, the strain is evaluated as LEV-resistant and HL MFX-resistant (see table 1). LL MFX resistance is not detected; however, since HL MFX resistance is detected, the LL MFX resistance status is not relevant. The *rrs* WT1 band is missing, and the *rrs* MUT1 band is developed; hence, the "*rrs* WT" column is marked with a "–", the "*rrs* MUT" column is marked with a "+", and the strain is evaluated as resistant to AMK/CAP/KAN. The *eis* locus displays the wild type banding pattern; hence, the columns are marked according to example 1. **Final evaluation: resistance detected for LEV, HL MFX, AMK, CAP, and KAN**		
3	*gyrA*/*gyrB*: *gyrA* WT2 band negative (signal intensity lower than that of the AC band) *gyrA* MUT2 band positive	*gyrA*/*gyrB*: *gyrA* WT: "–" *gyrA* MUT 1/2/3A: "+" *gyrA* MUT 3B/C/D: "–" *gyrB* WT: "+" *gyrB* MUT: "–"	LEV: "D" (resistance detected) HL MFX: "N" (resistance not detected) LL MFX: "D" (resistance detected)
	rrs: *rrs* WT1 band negative	*rrs*: *rrs* WT: "–" *rrs* MUT: "–"	AMK/CAP/KAN: "I" (resistance inferred)
	eis: Wild type banding pattern	*eis*: *eis* WT: "+" *eis* MUT: "–"	KAN: "N" (resistance not detected) AMK: "N" (resistance not detected)
	The *gyrA* WT2 band is missing (signal intensity is lower than that of the AC band) and the *gyrA* MUT2 band is developed. Hence, the evaluation chart shows a "–" in the "*gyrA* WT" and "*gyrA* MUT 3B/C/D" columns and a "+" in the "*gyrA* MUT 1/2/3A" column. The *gyrB* locus displays the wild type banding pattern resulting in a wild type entry as in example 1. Due to the *gyrA* banding pattern, the strain is evaluated as LEV-resistant and at least LL MFX-resistant (see table 1). HL MFX resistance is not detected. The *rrs* WT1 band is missing and none of the *rrs* mutation bands is developed; hence, the "*rrs* WT" and "*rrs* MUT" columns are marked with a "–" and resistance to AMK, CAP, and KAN is inferred. The *eis* locus displays the wild type banding pattern; hence, the columns are marked according to example 1. **Final evaluation: resistance detected for LEV and at least LL MFX - resistance inferred for AMK, CAP, and KAN**		
4	*gyrA*/*gyrB*: *gyrA* WT3 band negative *gyrA* MUT3C band positive	*gyrA*/*gyrB*: *gyrA* WT: "–" *gyrA* MUT 1/2/3A: "–" *gyrA* MUT 3B/C/D: "+" *gyrB* WT: "+" *gyrB* MUT: "–"	LEV: "D" (resistance detected) HL MFX: "D" (resistance detected) LL MFX: "N" (not relevant because HL MFX resistance is detected)
	rrs: *rrs* WT2 band negative *rrs* MUT2 band positive	*rrs*: *rrs* WT: "–" *rrs* MUT: "+"	AMK/CAP/KAN: "D" (resistance detected)
	eis: Wild type banding pattern	*eis*: *eis* WT: "+" *eis* MUT: "–"	KAN: "N" (resistance not detected) AMK: "N" (resistance not detected)
	The *gyrA* WT3 band is missing and the *gyrA* MUT3C band is developed. Hence, the evaluation chart shows a "–" in the "*gyrA* WT" and "*gyrA* MUT 1/2/3A" columns and a "+" in the "*gyrA* MUT 3B/C/D" column. The *gyrB* locus displays the wild type banding pattern resulting in a wild type entry as in example 1. Due to the *gyrA* banding pattern, the strain is evaluated as LEV-resistant and HL MFX-resistant (see table 1). LL MFX resistance is not detected; however, since HL MFX resistance is detected, the LL MFX resistance status is not relevant. The *rrs* WT2 band is missing and the *rrs* MUT2 band is developed; hence, the "*rrs* WT" column is marked with a "–", the "*rrs* MUT" column is marked with a "+", and the strain is evaluated as resistant to AMK/CAP/KAN. The *eis* locus displays the wild type banding pattern; hence, the columns are marked according to example 1. **Final evaluation: resistance detected for LEV, HL MFX, AMK, CAP, and KAN**		

Example #	Banding pattern (deviation from wild type)	WT/MUT columns on evaluation sheet	"Genotypic resistance" on evaluation sheet (see tables 1-4)
5	*gyrA*/*gyrB*: *gyrA* WT1 band negative *gyrB* WT band negative	*gyrA*/*gyrB*: *gyrA* WT: "–" *gyrA* MUT 1/2/3A: "–" *gyrA* MUT 3B/C/D: "–" *gyrB* WT: "–" *gyrB* MUT: "–"	LEV: "I" (resistance inferred) HL MFX: "N" (resistance not detected) LL MFX: "I" (resistance inferred)
	rrs: Wild type banding pattern	*rrs*: *rrs* WT: "+" *rrs* MUT: "–"	AMK/CAP/KAN: "N" (resistance not detected)
	eis: *eis* WT1 band negative	*eis*: *eis* WT: "–" *eis* MUT: "–"	KAN: "I" (resistance inferred) AMK: "N" (resistance not detected)
	From both the *gyrA* and the *gyrB* locus one wild type band is missing and none of the mutation bands are developed. Therefore, all *gyrA* and *gyrB* columns are marked with a "–" and LEV resistance and at least LL MFX resistance are inferred (see table 1). HL MFX resistance is not detected. The *rrs* locus displays the wild type banding pattern; hence, the columns are marked according to example 1. The *eis* WT1 band is missing and the mutation band is not developed; hence, the "*eis* WT" and "*eis* MUT" columns are marked with a "–" and KAN resistance is inferred (see table 4). **Final evaluation: resistance inferred for LEV, at least LL MFX, and KAN**		
6	*gyrA*/*gyrB*: *gyrA* WT3 band negative *gyrA* MUT3A band positive *gyrB* WT band negative	*gyrA*/*gyrB*: *gyrA* WT: "–" *gyrA* MUT 1/2/3A: "+" *gyrA* MUT 3B/C/D: "–" *gyrB* WT: "–" *gyrB* MUT: "–"	LEV: "D" (resistance detected) HL MFX: "N" (resistance not detected) LL MFX: "D" (resistance detected)
	rrs: *rrs* WT1 band negative	*rrs*: *rrs* WT: "–" *rrs* MUT: "–"	AMK/CAP/KAN: "I" (resistance inferred)
	eis: *eis* WT2 band negative *eis* MUT1 band positive	*eis*: *eis* WT: "–" *eis* MUT: "+"	KAN: "D" (resistance detected) AMK: "D" (resistance detected)
	The *gyrA* WT3 band is missing and the *gyrA* MUT3A band is developed. Hence, the evaluation chart shows a "–" in the "*gyrA* WT" and "*gyrA* MUT 3B/C/D" columns and a "+" in the "*gyrA* MUT 1/2/3A" column. The *gyrB* WT band is missing and none of the mutation bands are developed; hence, the "*gyrB* WT" and "*gyrB* MUT" columns are marked with a "–". Due to the *gyrA* banding pattern, the strain is evaluated as LEV-resistant and at least LL MFX-resistant (see table 1). Due to the *gyrB* banding pattern, LEV resistance and at least LL MFX resistance would be inferred (see table 2), but this is overruled since resistance is detected through *gyrA*. The *rrs* WT1 band is missing and no mutation band is developed; hence, the "*rrs* WT" and "*rrs* MUT" columns are marked with a "–", and resistance to AMK/CAP/KAN is inferred. However, the evaluation for KAN and AMK is overruled by the *eis* banding pattern. The *eis* WT2 band is missing and the *eis* MUT1 band is developed; hence, the "*eis* WT" column is marked with a "–", the "*eis* MUT" column is marked with a "+", and the strain is evaluated as resistant to KAN and AMK (see table 4). **Final evaluation: resistance detected for LEV, at least LL MFX, AMK, and KAN - resistance inferred for CAP**		
7	*gyrA*/*gyrB*: Wild type banding pattern	*gyrA*/*gyrB*: *gyrA* WT: "+" *gyrA* MUT 1/2/3A: "–" *gyrA* MUT 3B/C/D: "–" *gyrB* WT: "+" *gyrB* MUT: "–"	LEV: "N" (resistance not detected) HL MFX: "N" (resistance not detected) LL MFX: "N" (resistance not detected)
	rrs: Wild type banding pattern	*rrs*: *rrs* WT: "+" *rrs* MUT: "–"	AMK/CAP/KAN: "N" (resistance not detected)
	eis: *eis* WT2 band negative	*eis*: *eis* WT: "–" *eis* MUT: "–"	KAN: "I" (resistance inferred) AMK: "N" (resistance not detected)
	The *gyrA* locus, the *gyrB* locus, and the *rrs* locus display the wild type banding pattern; hence, the columns are marked according to example 1. The *eis* WT2 band is missing and the mutation band is not developed; hence, the "*eis* WT" and "*eis* MUT" columns are marked with a "–" and KAN resistance is inferred (see table 4). **Final evaluation: resistance inferred for KAN**		

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 test reflects the current state of knowledge of Hain Lifescience.

The results of this test may only be interpreted in combination with additional laboratory and clinical data available to the responsible physician. In addition, results of phenotypic drug susceptibility testing have to be considered in certain cases.
The user must have or acquire information about the local mutation distribution pattern of the genes investigated with this test. Confirmation of the test results by phenotypic drug susceptibility testing may be necessary.

As any DNA-based assay, this test only screens the nucleic acid sequence and not the amino acid sequence. Therefore, it is possible that mutations in the probe region that do not cause an amino acid exchange (silent mutations) will still produce the absence of one of the wild type bands.
The **GenoType MTBDR*sl*** VER 2.0 only detects those resistances that have their origins in the *gyrA*, *gyrB*, *rrs*, and *eis* gene regions examined here. Resistances originating from mutations of other genes or gene regions as well as other FLQ or SLID resistance mechanisms will not be detected by this test.
If a low-level resistance was detected for moxifloxacin, this should be reported as "at least low-level resistance detected", as a high-level resistance cannot be entirely excluded (e.g. conferred by a mutation not covered by the assay or by a combination of two low-level resistance mutations).
The mutation eis_-7_del_1_cg_c which is associated with kanamycin resistance does not lead to the absence of an *eis* WT band [15].
In rare cases when the Eis enzyme is inactive (e.g. due to loss-of-function mutations in the *eis* coding region, which are not interrogated by this assay), *eis* promoter mutations cannot confer resistance [16].
The test only works within the limits of the genomic regions the primers and probes were chosen from.

Please note that effects due to multiple mutations outside the investigated sequences cannot 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.

The members of the *M. tuberculosis* complex cannot be differentiated.
The presence of multiple bacterial species in the sample to be analyzed might hamper the interpretation of the test.

As any DNA detection method, the test system on hand detects DNA from viable and nonviable bacteria. Therefore, the **GenoType MTBDR*sl*** VER 2.0 may not be used for monitoring the progression or success of treatment of patients with antimicrobial therapy.
The **GenoType MTBDR*sl*** VER 2.0 generates qualitative results. The intensities of the bands on a strip do not give information about the number of cells in a positive sample.

Performance evaluation of this assay was carried out using the **GenoLyse®** for DNA extraction from NALC-NaOH-decontaminated smear-positive and smear-negative sputum samples as well as from cultivated samples. The performance of the test has not been validated with other DNA extraction methods or sample materials.

Troubleshooting

Overall weak or no signals (including Conjugate Control zone)
- Room temperature too low or reagents not equilibrated to room temperature.
- No or too little amount of CON-C and/or SUB-C used.
  Repeat reverse hybridization.

Weak or no signals except for Conjugate Control zone
- Quality of extracted DNA does not allow an efficient amplification. Repeat extraction.
- Amplification Mixes (AM-A and AM-B) were not mixed properly, interchanged, or added in wrong amounts. Prepare a new master mix and repeat amplification.
- Incubation temperature too high. Repeat reverse hybridization.

No homogeneous staining
- Strips were not completely immersed during incubation steps.
- Tray was not shaken properly.
  Repeat reverse hybridization.

High background color
- CON-C and/or SUB-C used too concentrated.
- Washing steps were not performed with the necessary care.
- Wash solutions too cold.
  Repeat reverse hybridization.

Unexpected result
- Wrong incubation temperature.
- Hybridization Buffer and/or Stringent Wash Solution were not properly prewarmed or mixed.
- Contamination of neighboring wells by spillage during addition of Hybridization Buffer.
  Repeat reverse hybridization.
- Contamination of extracted DNA with previously extracted or amplified DNA. Repeat extraction.
- Contamination of amplification reagents. In this case, a negative control sample shows additional bands besides CC and AC. Repeat amplification using fresh reagents.
- Depending on the amount of amplified DNA used and on the specific reaction conditions, a strong and fast color development may occur. In such cases, discontinue the substrate incubation as soon as the signals are clearly visible in order to prevent the development of cross-hybridizing bands.
- No pure culture as starting material. Re-culture in order to exclude contamination.
- Improper sampling, storage, transport, or preparation of specimen. Request new specimen and repeat test.
- Error during DNA extraction. Repeat extraction.

Ordering Information

Hain Lifescience	Order no.
GenoType MTBDR*sl* VER 2.0 (kit for analysis of 12 samples)	317A
GenoType MTBDR*sl* VER 2.0 (kit for analysis of 96 samples)	31796A
GenoLyse® (kit for manual DNA extraction of 12 samples)	51612
GenoLyse® (kit for manual DNA extraction of 96 samples)	51610
TwinCubator (instrument for manual hybridization of up to 12 samples per run)	7025009
GT-Blot 48 (instrument for automated hybridization of up to 48 samples per run)	1003/1

Sarstedt, Nümbrecht, Germany	Order no.
0.5 ml screw cap tubes	72.730.105
1.5 ml screw cap tubes	72.692.005

Performance Characteristics

Diagnostic performance

1. Clinical specimens

Diagnostic performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 were determined in a study with 352 sputum specimens. The study specimens were collected in a high MDR-TB burden country. Both untreated patients as well as patients with previous or current anti-TB-treatment were included in the study. The **GenoType MTBDR*sl*** VER 2.0 was compared to culture (successful cultivation on Loewenstein-Jensen solid medium or in MGIT (BD Diagnostics, Franklin Lakes, USA) and subsequent *M. tuberculosis* complex (MTBC) identification using the **GenoType Mycobacterium CM** VER 1.0). For discrepant culture-negative specimens, the result of the **GenoType MTBDR*plus*** VER 2.0 was used as reference method.
Furthermore, the **GenoType MTBDR*sl*** VER 2.0 was compared to conventional drug susceptibility testing (DST) using BACTEC MGIT 960 (BD Diagnostics, Franklin Lakes, USA) and Loewenstein-Jensen proportion method. Specimens with discrepant results were examined by sequencing the **GenoType MTBDR*sl*** VER 2.0 amplification region.
Additionally, all samples were examined by microscopy.
DNA extraction from NALC-NaOH-decontaminated sputum specimens was performed with the **GenoLyse®** according to the instructions for use.
21 specimens were excluded due to ambiguous DST results or contaminated cultures.

For the detection of MTBC, test results were rated true-positive if the result of **GenoType MTBDR*sl*** VER 2.0 was consistent with an MTBC-positive result of culture/**GenoType Mycobacterium CM** VER 1.0 or, in case of a culture-negative specimen, if a TB infection was indicated by an MTBC-positive **GenoType MTBDR*plus*** VER 2.0 result from the respective clinical specimen.

Table 1: Performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 for detection of MTBC from sputum specimens compared to culture/**GenoType Mycobacterium CM** VER 1.0 (GT Myco CM) or to culture/GT Myco CM and **GenoType MTBDR*plus*** VER 2.0 (GT MTBDR*plus* V2) result from the respective clinical specimens

			Culture/GT Myco CM				Culture/GT Myco CM + GT MTBDR*plus* V2		
			positive	negative	Sens: 98.8% Spec: 89.6%		positive	negative	Sens: 98.9% Spec: 100%
total	**GenoType MTBDR*sl*** VER 2.0	positive	251	8	PPV: 96.9%	positive	259	0	PPV: 100%
		negative	3	69	NPV: 95.8%	negative	3	69	NPV: 95.8%
			Culture/GT Myco CM				Culture/GT Myco CM + GT MTBDR*plus* V2		
			positive	negative	Sens: 99.6% Spec: /*		positive	negative	Sens: 99.6% Spec: /*
smear-positive	**GenoType MTBDR*sl*** VER 2.0	positive	232	8	PPV: 96.7%	positive	240	0	PPV: 100%
		negative	1	2	NPV: /*	negative	1	2	NPV: /*
			Culture/GT Myco CM		Sens: 90.5%				
			positive	negative	Spec: 100%				
smear-negative	**GenoType MTBDR*sl*** VER 2.0	positive	19	0	PPV: 100%				
		negative	2	67	NPV: 97.1%				

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value
* no value due to low sample number

For evaluation of resistance detection, the 251 MTBC-positive samples (positive both in culture and with **GenoType MTBDR*sl*** VER 2.0) were used. Test results were rated true-positive if the result of **GenoType MTBDR*sl*** VER 2.0 was consistent with the DST result or, in case of divergent results, if the result of **GenoType MTBDR*sl*** VER 2.0 was confirmed by sequencing from culture material of the respective sample.

Table 2: Performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 for detection of fluoroquinolone (FLQ) resistance from sputum specimens compared to culture/DST (tested with ofloxacin) or to culture/DST and sequencing

		Culture/DST		Sens: 93.1% Spec: 100%		Culture/DST + sequencing		Sens: 96.4% Spec: 100%
		FLQ-R	FLQ-S			FLQ-R	FLQ-S	
GenoType MTBDR*sl* VER 2.0	FLQ-R	54	0	PPV: 100%	FLQ-R	54	0	PPV: 100%
	FLQ-S	4	193	NPV: 98.0%	FLQ-S	2	195	NPV: 99.0%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value;
FLQ-R, fluoroquinolone-resistant; FLQ-S, fluoroquinolone-sensitive

Table 3: Performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 for detection of amikacin (AMK) resistance from sputum specimens compared to culture/DST or to culture/DST and sequencing

		Culture/DST		Sens: 97.4% Spec: 97.7%		Culture/DST + sequencing		Sens: 100% Spec: 97.7%
		AMK-R	AMK-S			AMK-R	AMK-S	
GenoType MTBDR*sl* VER 2.0	AMK-R	37	5	PPV: 88.1%	AMK-R	37	5	PPV: 88.1%
	AMK-S	1	208	NPV: 99.5%	AMK-S	0	209	NPV: 100%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value;
AMK-R, amikacin-resistant; AM-S, amikacin-sensitive

Table 4: Performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 for detection of capreomycin (CAP) resistance from sputum specimens compared to culture/DST or to culture/DST and sequencing

		Culture/DST			Culture/DST + sequencing		
		CAP-R	CAP-S	Sens: 90.0% Spec: 98.1%	CAP-R	CAP-S	Sens: 97.3% Spec: 98.1%
GenoType MTBDR*sl* VER 2.0	CAP-R	36	4	PPV: 90.0%	36	4	PPV: 90.0%
	CAP-S	4	207	NPV: 98.1%	1	210	NPV: 99.5%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value; CAP-R, capreomycin-resistant; CAP-S, capreomycin-sensitive

Table 5: Performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 for detection of kanamycin (KAN) resistance from sputum specimens compared to culture/DST or to culture/DST and sequencing

		Culture/DST		Sens: 94.8% Spec: 96.6%	Culture/DST + sequencing		Sens: 98.6% Spec: 96.6%
		KAN-R	KAN-S		KAN-R	KAN-S	
GenoType MTBDR*sl* VER 2.0	KAN-R	73	6	PPV: 92.4%	73	6	PPV: 92.4%
	KAN-S	4	168	NPV: 97.7%	1	171	NPV: 99.4%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value; KAN-R, kanamycin-resistant; KAN-S, kanamycin-sensitive

2. Culture samples

The diagnostic performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 were determined in a study with 100 MTBC-positive culture samples.
The study samples obtained from a culture collection comprised MTBC strains originating from high MDR-TB burden countries as well as from low MDR-TB burden countries.
The **GenoType MTBDR*sl*** VER 2.0 was compared to conventional drug susceptibility testing (DST) using BACTEC MGIT 960 (BD Diagnostics, Franklin Lakes, USA) and Loewenstein-Jensen proportion method. Specimens with discrepant results were examined by sequencing the **GenoType MTBDR*sl*** VER 2.0 amplification region.
DNA extraction was performed using the **GenoLyse**® according to the instructions for use.
For kanamycin (KAN), DST was performed retrospectively using frozen culture aliquots that were recultivated. 89/100 samples yielded results; 11 samples could not be recultivated and were therefore excluded from the evaluation of KAN resistance.

Table 6: Performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 for detection of fluoroquinolone (FLQ) resistance from culture samples compared to culture/DST (tested with ofloxacin) or to culture/DST and sequencing

		Culture/DST		Sens: 93.9% Spec: 98.5%	Culture/DST + sequencing		Sens: 100% Spec: 100%
		FLQ-R	FLQ-S		FLQ-R	FLQ-S	
GenoType MTBDR*sl* VER 2.0	FLQ-R	31	1	PPV: 96.9%	32	0	PPV: 100%
	FLQ-S	2	66	NPV: 97.1%	0	68	NPV: 100%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value; FLQ-R, fluoroquinolone-resistant; FLQ-S, fluoroquinolone-sensitive

Table 7: Performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 for detection of amikacin (AMK) resistance from culture samples compared to culture/DST or to culture/DST and sequencing

		Culture/DST		Sens: 100% Spec: 98.5%	Culture/DST + sequencing		Sens: 100% Spec: 100%
		AMK-R	AMK-S		AMK-R	AMK-S	
GenoType MTBDR*sl* VER 2.0	AMK-R	35	1	PPV: 97.2%	36	0	PPV: 100%
	AMK-S	0	64	NPV: 100%	0	64	NPV: 100%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value; AMK-R, amikacin-resistant; AM-S, amikacin-sensitive

Table 8: Performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 for detection of capreomycin (CAP) resistance from culture samples compared to culture/DST or to culture/DST and sequencing

		Culture/DST		Sens: 84.6% Spec: 100%	Culture/DST + sequencing		Sens: 100% Spec: 100%
		CAP-R	CAP-S		CAP-R	CAP-S	
GenoType MTBDR*sl* VER 2.0	CAP-R	33	0	PPV: 100%	33	0	PPV: 100%
	CAP-S	6	61	NPV: 91.0%	0	67	NPV: 100%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value; CAP-R, capreomycin-resistant; CAP-S, capreomycin-sensitive

Table 9: Performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 for detection of kanamycin (KAN) resistance from culture samples compared to culture/DST or to culture/DST and sequencing.

		Culture/DST		Sens: 94.4% Spec: 98.1%	Culture/DST + sequencing		Sens: 100% Spec:100%
		KAN-R	KAN-S		KAN-R	KAN-S	
GenoType MTBDR*sl* VER 2.0	KAN-R	34	1	PPV: 97.1%	35	0	PPV: 100%
	KAN-S	2	52	NPV: 96.3%	0	54	NPV: 100%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value; KAN-R, kanamycin-resistant; KAN-S, kanamycin-sensitive

Further diagnostic performance characteristics of the **GenoType MTBDR*sl*** VER 2.0 have been published within the scope of an international multicenter study [17].

Analytical performance

Analytical specificity

The specificity of the **GenoType MTBDR*sl*** VER 2.0 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 was determined with 8 *M. tuberculosis* complex strains: *M. tuberculosis*, *M. africanum*, *M. bovis* BCG, *M. bovis*, *M. caprae*, *M. canettii, M. microti*, and *M. pinnipedii* (all sensitive to FLQ and to SLID). The following 40 strains not detectable with the test system were also analyzed: *Bordetella pertussis, Corynebacterium* spec., *Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Mycobacterium abscessus, M. alvei, M. asiaticum, M. avium, M. celatum, M. chelonae, M. fortuitum, M. gastri, M. genavense, M. goodii, M. gordonae, M. haemophilum, M. immunogenum, M. interjectum, M. intermedium, M. intracellulare, M. kansasii, M. lentiflavum, M. mageritense, M. malmoense, M. marinum, M. mucogenicum, M. peregrinum, M. scrofulaceum, M. shimoidei, M. simiae, M. smegmatis, M. szulgai, M. triplex, M. ulcerans, M. xenopi, Nocardia* spec., *Pseudomonas aeruginosa, Staphylococcus aureus,* and *S. pneumoniae.*

The 8 *M. tuberculosis* complex isolates were correctly identified as FLQ- and SLID-sensitive MTBC strains. All other 40 isolates displayed invalid or not interpretable band patterns. Hence, an analytical specificity of 100% was achieved.

Analytical sensitivity (limit of detection, LOD)

To determine the LOD of the **GenoType MTBDR*sl*** VER 2.0 for clinical specimens, three BCG culture dilutions (FLQ- and SLID-sensitive, 1500, 150, and 15 CFU/ml) were prepared in triplicate. Including a negative control, DNA was extracted using the **GenoLyse®** and analyzed with the **GenoType MTBDR*sl*** VER 2.0 applying the "MDR DIR" PCR protocol. An LOD of 150 CFU/ml was determined.

To determine the LOD of the **GenoType MTBDR*sl*** VER 2.0 for culture samples, three BCG culture dilutions (FLQ- and SLID-sensitive, 1.65×10^6, 1.65×10^5, and 1.65×10^4 CFU/ml) were set up in triplicate. Including a negative control, DNA was extracted using the **GenoLyse®** and analyzed with the **GenoType MTBDR*sl*** VER 2.0 applying the "MDR CUL" PCR protocol. An LOD of 1.65×10^5 CFU/ml was determined.

Reproducibility

In order to determine the intra-assay precision of the **GenoType MTBDR*sl*** VER 2.0, three BCG culture dilutions (FLQ- and SLID-sensitive; one above, one at, and one below the LOD) and one negative control were set up in four parallels and tested under identical conditions applying the "MDR DIR" PCR protocol. DNA extraction was performed using the **GenoLyse®**. All parallels showed identical and correct banding patterns and comparable signal strengths. Additionally, signal strengths between different sample dilutions were comparable. Hence, an intra-assay precision of 100% was achieved.

In order to determine the inter-assay precision of the **GenoType MTBDR*sl*** VER 2.0, three BCG culture dilutions (one above, one at, and one below the LOD) and a negative control were tested at three different points in time. Apart from the varied parameter, all other testing conditions were identical. DNA extraction was performed using the **GenoLyse®** and the isolates were analyzed with the **GenoType MTBDR*sl*** VER 2.0 applying the "MDR DIR" PCR protocol. No deviations were detected between parallel samples, that is between runs banding patterns were identical and correct, and signal strengths were comparable. Hence, the inter-assay precision was 100%.

Interfering substances

There are substances that may inhibit PCR reactions. Such inhibitors may, for example, originate from the culture medium. In order to assess if the medium influences the **GenoType MTBDR*sl*** VER 2.0, 6 different *M. tuberculosis* complex samples (3x FLQ- and SLID-sensitive, 2x FLQ-sensitive and AMK/CAP/KAN-resistant, 1x FLQ- and AMK/CAP/KAN-resistant) were cultured in 4 different media (solid media: Loewenstein-Jensen, Stonebrink, and Middlebrook-7H10; liquid medium: MGIT (BD Diagnostics, Franklin Lakes, USA)). DNA extraction was performed using the **GenoLyse®**. Subsequently, the culture samples were tested with the **GenoType MTBDR*sl*** VER 2.0 applying the "MDR CUL" PCR protocol.

All *M. tuberculosis* complex samples showed the same correct results. Hence, it can be excluded that the tested media import inhibitors into the **GenoType MTBDR*sl*** VER 2.0.

Interfering substances may also be carried over from the sample material. Hence, the substances indicated in table 10 were tested in order to assess a potential interference with the **GenoType MTBDR*sl*** VER 2.0. Defined BCG culture dilutions above, at, and below the detection limit of clinical specimens were spiked with various amounts of the potential inhibitors. From all samples, DNA extraction was performed using the **GenoLyse®**. Then the culture dilutions were tested with the **GenoType MTBDR*sl*** VER 2.0 applying the "MDR DIR" protocol for PCR.

Table 10: Tested potential interferents of the **GenoType MTBDR*sl*** VER 2.0

Substance/class	Description/active ingredient	Substance concentrations
Blood	Whole blood	2.5% v/v to 90% v/v
Blood	Hemoglobin	0.05% v/v to 13.5% v/v
Pus		0.5% v/v to 90% v/v

Interference of the **GenoType MTBDR*sl*** VER 2.0 (invalid or not interpretable test result) was observed in samples containing concentrations greater than 10% whole blood, 1% hemoglobin, and 2.5% pus.

Stability

Shelf life of the test kit when stored as recommended: see box label.

Stability is determined according to DIN EN ISO 23640.

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Important Changes in IFU-317A-05

IFU	Note
IFU-317A-05	Pre-release changes.

Important Changes in IFU-317A-06

Chapter	Change
Intended Use, Summary and Explanation, Evaluation and Interpretation of Results, Performance Characteristics	The evaluation criteria and listed antibiotics were adapted according to [11].
Precautions for handling kit constituents	For additional information on the hazardous substances included in the kit, please refer to the safety data sheets which can be downloaded from: www.hain-lifescience.com/products/msds.html
Quality Control	**New:** "A positive sample (not provided with the kit) must be included in each run."
	A negative control for detection of possible contamination events should be part of each DNA extraction. Alternatively, a negative control containing water (molecular biology grade) instead of DNA can be included during PCR setup (see chapter Amplification).
Specimen Requirements	**New:** "All culture samples that may contain mycobacteria should be handled applying Biosafety Level 2 practices or, when indicated, Biosafety Level 3 practices (e.g. see [3]). Observe all federal, state, and local safety regulations."
	Specimens must no longer be transported at room temperature. Instead, the specimens must be transported at 2°C to 8°C within 1 to 2 days.
	New: "Specimens used for decontamination must not be older than 3 days."
	New: "After decontamination and subsequent resuspension of the bacteria pellet with phosphate buffer, samples can be stored at 2°C to 8°C for a maximum of 7 days until performing DNA extraction."
	New: "Cultivated samples must be processed without delay."
Manual DNA extraction with **GenoLyse®**	New chapter
Amplification	**New:** "Please note that the master mix needs to be prepared freshly each time and needs to be processed quickly. Avoid any delay in processing. DNA addition and start of the thermocycler should be done within one hour after preparation of the master mix."
	New: "When using a different thermal cycler, use the following amplification profile. The heating rate must not be higher than 2.2°C/s. Higher heating rates can lead to invalid, not interpretable, or wrong results."
	New: "If a negative control sample is included during PCR setup, add 5 µl water (molecular biology grade) to one aliquot. A negative control that has been included during DNA extraction (see chapter DNA Extraction) is processed like a regular sample (see below)."
Limitations	**New:** "The mutation eis_-7_del_1_cg_c which is associated with kanamycin resistance does not lead to the absence of an *eis* WT band [15]."
	New: "In rare cases, the *eis* c-14t mutation coincides with loss-of-function mutations in the *eis* reading frame (e.g. g3a mutation in the valine start codon) resulting in phenotypic susceptibility [16]."

CE IVD

Hain Lifescience GmbH
Hardwiesenstraße 1, 72147 Nehren, Germany
www.hain-lifescience.de, +49 (0) 74 73- 94 51- 0

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Hardwiesenstr. 1, 72147 Nehren, GERMANY

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Hain Lifescience GmbH
Pfaffenwiesen 4, 72147 Nehren, GERMANY

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Pfaffenwiesen 10, 72147 Nehren, GERMANY

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