GenoType MTBDR*plus*

VER 2.0

Instructions for Use

IFU-304A-09

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GenoType **MTBDR*plus*** VER 2.0
Molecular Genetic Assay for Identification of the *M. tuberculosis* Complex and its Resistance to Rifampicin and Isoniazid from Clinical Specimens and Cultivated Samples

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*plus*** VER 2.0 is a qualitative in vitro test for the identification of the *Mycobacterium tuberculosis* complex and its resistance to rifampicin (RMP) and/or isoniazid (INH) from pulmonary smear-positive or -negative clinical specimens and cultivated samples. The following species are included in the tuberculosis (TB)-causing *M. tuberculosis* complex: *M. tuberculosis*, *M. africanum*, *M. bovis* subsp. *bovis*, *M. bovis* subsp. *caprae*, *M. bovis* BCG, *M. microti*, *M. canettii*, and *M. pinnipedii*. The identification of RMP resistance is enabled by the detection of the most significant associated mutations of the *rpoB* gene (coding for the β-subunit of the RNA polymerase). For detection of INH resistance, the *katG* gene (coding for the catalase peroxidase) and the promoter region of the *inhA* gene (coding for the NADH-enoyl-ACP reductase) are examined.
The test is indicated as an aid for diagnosis and intended for use in medical laboratories.

Summary and Explanation

Tuberculosis (TB) is a bacterial infectious disease passed on by droplet infection. In 2017, there were an estimated 10.0 million incident cases of TB globally, and an estimated 1.3 million TB deaths [1]. TB treatment requires a therapy over several months. Emergence and spread of multidrug-resistant tuberculosis (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 2013, there were an estimated 480,000 cases of MDR-TB among the world's 11 million prevalent cases of TB [1].
As long as MDR-TB is not verified, use of inadequate and hence ineffective antibiotics may lead to further spread of resistant bacteria and amplification of resistance. Therefore, rapid diagnosis and identification of MDR-TB is a prerequisite for appropriate treatment.

Principles of the Procedure

The **GenoType MTBDR*plus*** VER 2.0 is based on the **DNA•STRIP** technology. The whole procedure is divided into three steps: (i) DNA extraction from clinical specimens (pulmonary, decontaminated) 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**	**304A** **12**	**30496A** **96**
Kit Component 1 of 2 (store at 2°C to 8°C)		
Membrane strips coated with specific probes (MTBDRplus 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 MTBDRplus VER 2.0) contains buffer, nucleotides, Taq polymerase	120 µl	4x 240 µl
Amplification Mix B (AM-B GT MTBDRplus VER 2.0) contains salts, specific primers, dye	420 µl	4x 840 µl

Storage, handling, 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 (>4x) freezing and thawing 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 and gloves.
When handling kit reagents, the following special safety measures must be applied:

Hybridization Buffer **(HYB)** and Substrate Concentrate **(SUB-C)** are not classified as hazardous. Due to their ingredients, however, hazard statement EUH210 applies: Safety data sheet available on request.

Denaturation Solution **(DEN)** contains <2% sodium hydroxide.
Warning!
H315: Causes skin irritation. H319: Causes serious eye irritation.
P280: Wear protective gloves/protective clothing/eye protection. P305+351+338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P313: Get medical advice/attention.

For additional information, 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®** or **GXT DNA/RNA Extraction Kit**, see chapter Ordering Information) as well as necessary equipment
- 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*** automated hybridization instrument
- Thermal cycler
- 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 5 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
- three Locus Control zones (*rpoB*, *katG*, and *inhA*) 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 negative control sample for detection of possible contamination events containing water (molecular biology grade) instead of DNA should be part of each test run; the respective test strip should show the bands CC and AC only.

Specimen Requirements

Decontaminated pulmonary smear-positive or -negative patient specimens such as sputum (induction or expectoration), bronchial material (e.g. bronchoalveolar lavages), or aspirates (e.g. pleural aspirate) as well as cultivated samples (solid/liquid medium) can be used as starting material for DNA extraction. Until the present edition of the instructions on hand, the performance of the test has not been validated with other sample materials than those mentioned above.

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 [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)) 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 [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 room temperature has to be carried out as soon as possible and should be done within 1-2 days [7,8]. 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.

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)).

Handling of potentially infectious specimens must be carried out in a class II safety cabinet.

DNA Extraction

Decontaminated patient samples as well as bacteria grown on solid medium (e.g. Loewenstein-Jensen, Middlebrook) or in liquid medium (e.g. MGIT (BD Diagnostics, Franklin Lakes, USA)) may be used as starting material for DNA extraction.

For DNA extraction from NALC-NaOH-decontaminated clinical specimens or cultured material the **GenoLyse®** kit (see chapter Ordering Information) is used. For automated DNA extraction from patient specimens, also the **GenoXtract®** in combination with the **GXT DNA/RNA Extraction Kit** (see chapter Ordering Information) can be used. For handling instructions, please refer to the respective instructions for use.

The methods mentioned here were used for performance evaluation of the **GenoType MTBDR*plus*** VER 2.0. Until the present edition of the instructions on hand, the performance of the test has not been validated with other DNA extraction methods.

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:

After DNA extraction with **GenoLyse®**
- 10 µl AM-A (see Kit Component 2)
- 35 µl AM-B (see Kit Component 2)
- 5 µl DNA solution
  Final volume: 50 µl

After DNA extraction with **GXT DNA/RNA Extraction Kit**
- 10 µl AM-A (see Kit Component 2)
- 35 µl AM-B (see Kit Component 2)
- 10 µl DNA solution
  Final volume: 55 µl

Determine the total number of samples (number of samples to be analyzed plus control samples). Prepare the number of 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. Aliquot 45 µl into each of the prepared PCR tubes and add 5 or 10 µl water (molecular biology grade) to one aliquot (negative control sample). In a separate working area, add 5 or 10 µl DNA solution to each aliquot (except for negative control).

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.

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

The heated lid must be switched on during the entire program.

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

Hybridization

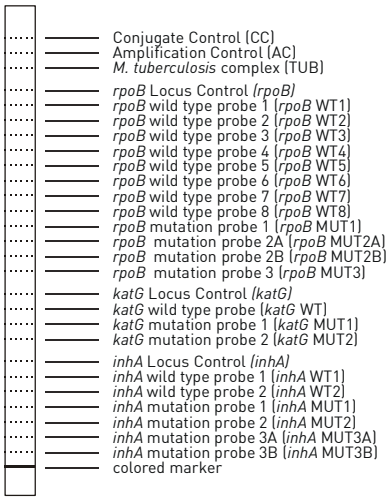
When using a hybridization instrument from Hain Lifescience, please refer to the document "Overview equipment programs" available on www.hain-lifescience.com for the name of the hybridization protocol to be used.
The following protocol describes the manual hybridization using a water bath or a **TwinCubator**.

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 in the subsequent chapter. Each strip has a total of 27 reaction zones (see figure).



Note: The strip is not displayed in original size.

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 not valid and the test has to be repeated with the respective sample. In case of overall strong signal intensities but only weak staining or absence of the Amplification Control band, a single wild type band showing significantly weaker staining than the other wild type bands of the respective locus (or Locus Control band for *katG*) is to be considered negative.

***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. In rare cases, the TUB zone may be negative while an evaluable resistance pattern is developed. If so, the presence of a strain belonging to the *M. tuberculosis* complex must be suspected and the test should be repeated (see below, "special case" no. 3).

Locus Controls (*rpoB*, *katG*, and *inhA*)
The Locus Control zones detect a gene region 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.

Wild type probes
The wild type probes comprise the most important resistance regions of the respective genes (see figure 1, as well as tables 1, 2, and 3). When all wild type probes of a gene stain positive, there is no detectable mutation within the examined regions. This indicates that the strain tested is sensitive to the respective antibiotic. In case of a mutation, the respective amplicon cannot bind to the corresponding wild type probe. The absence of a signal for at least one of the wild type probes indicates a resistance of the tested strain to the respective antibiotic.
Each pattern deviating from the wild type pattern indicates a resistance of the tested strain. The banding pattern obtained with the *rpoB* probes allows drawing a conclusion about an RMP resistance of the strain tested, the *katG* and the *inhA* banding pattern about an INH resistance.

Mutation probes
The mutation probes detect some of the most common resistance-mediating mutations (see tables 1, 2, and 3). Compared to the other probes, positive signals of the mutation probes *rpoB* MUT2A and MUT2B may show a lower signal strength.
In rare cases, when the *rpoB* MUT3 band is positive, weak staining may be detected at the *rpoB* WT8 band which is to be considered negative.
Each pattern deviating from the wild type pattern indicates a resistance of the tested strain. The banding pattern obtained with the *rpoB* probes allows drawing a conclusion about an RMP resistance of the strain tested, the *katG* and the *inhA* banding pattern about an INH resistance.

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

Note the following special cases:

1. There is a possibility that the specimen tested contains a heteroresistant strain. In case of a heteroresistance, a mutated as well as a wild type sequence can be detected in the respective strain; hence, one of the mutation probes as well as the corresponding wild type probe may stain positive on the respective strip. Whether the respective resistance becomes phenotypically evident depends on the ratio of mutated and nonmutated sequences at investigation.
2. There is a possibility that the tested specimen contains more than one *M. tuberculosis* complex strain (due to mixed culture or contamination). If at least one of these strains harbors a mutation, one of the mutation probes as well as the corresponding wild type probe may stain positive. Whether the respective resistance becomes phenotypically evident, depends on the ratio of resistant and sensitive strain at investigation.
3. There is a possibility that due to a mixed infection the tested specimen contains both an *M. tuberculosis* complex strain and a nontuberculous mycobacterium. In rare cases, the TUB band may be missing due to competition of the single amplification reactions during PCR. However, when an evaluable resistance pattern is developed, the presence of a strain belonging to the *M. tuberculosis* complex must be suspected and the test should be repeated.
4. 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 in the sample below the limit of detection or the presence of interfering substances in the sample. Such a banding pattern cannot be evaluated and the test must be repeated.
   If a cultivated sample generates a result with the complete *katG* locus missing, this indicates an INH resistance of the strain tested.

Resistance regions and common resistance-mediating mutations

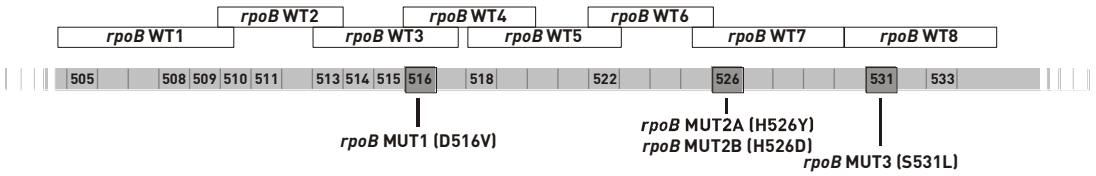


Figure 1: RMP resistance region of the *rpoB* gene
rpoB WT1-8: *rpoB* wild type probes; *rpoB* MUT1-3: *rpoB* mutation probes. The numbers specify the positions of the amino acids (codons) for all mutations listed in the table. The codons for which mutation probes were designed are highlighted.

Table 1: Mutations in the *rpoB* gene and the corresponding wild type and mutation bands (according to [9])

Failing wild type band(s)	Codons analyzed	Developing mutation band	Mutation
rpoB WT1	505-509		F505L
			T508A
			S509T
rpoB WT2	510-513		Q510H
			L511P*
rpoB WT2/WT3	510-517		Q513L*
			Q513P
			del514-516
rpoB WT3/WT4	513-519	*rpoB* MUT1	D516V
			D516Y
			del515
rpoB WT4/WT5	516-522		del518*
			N518I
rpoB WT5/WT6	518-525		S522L
			S522Q
rpoB WT7	526-529	*rpoB* MUT2A	H526Y
		rpoB MUT2B	H526D
			H526R
			H526P*
			H526Q*
			H526N
			H526L
			H526S
			H526C
rpoB WT8	530-533	*rpoB* MUT3	S531L
			S531Q*
			S531W
			L533P

* These rare mutations have only been detected theoretically (in silico).

Table 2: Mutations in the *katG* gene and the corresponding wild type and mutation bands

Failing wild type band	Codon analyzed	Developing mutation band	Mutation
katG WT	315	*katG* MUT1	S315T1
		katG MUT2	S315T2

Table 3: Mutations in the *inhA* promoter region and the corresponding wild type and mutation bands

Failing wild type band	Analyzed nucleic acid position	Developing mutation band	Mutation
inhA WT1	–15	*inhA* MUT1	C–15T
	–16	*inhA* MUT2	A–16G
inhA WT2	–8	*inhA* MUT3A	T–8C
		inhA MUT3B	T–8A

Evaluation Examples

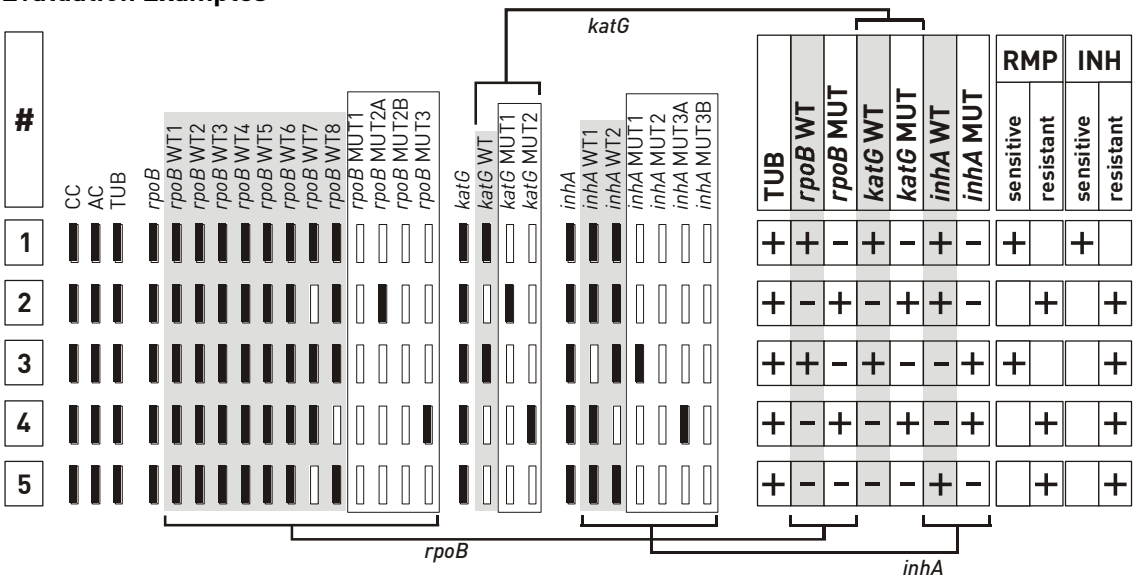


Figure 2: Examples for banding patterns and their evaluation with respect to RMP and/or INH resistance

If all wild type bands display a signal, this is classified as positive and marked in the WT column of the respective gene as "+". If at least one of the wild type bands is absent, this is classified as negative and marked in the WT column as "–". Negative entries are only made to the mutation columns when none of the mutation bands displays a coloration. If at least one of the mutation bands displays a coloration, this is classified as positive and the MUT column of the respective gene is marked with a "+".

Below, two of the examples shown above are explicated:

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 three wild type columns and "–" in the three mutation columns. Accordingly, the boxes "RMP sensitive" and "INH sensitive" are marked with a "+".

In **example 5**, one of the *rpoB* and the *katG* wild type probes are missing; hence, the boxes for "*rpoB* WT" and "*katG* WT" are marked with a "–". As none of the mutation probes are developed, these boxes are also marked with a "–". The *inhA* promoter region does not deviate from the wild type pattern. The strain is evaluated as resistant to RMP and INH.

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.

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. A silent mutation in codon 514 of the *rpoB* gene leading to the absence of the *rpoB* WT3 band was observed in rare cases [10]. Hence, if an RMP resistance is detected solely by a missing *rpoB* WT3 band, results of phenotypic drug susceptibility testing should be considered.

Additional mutations within the tested *rpoB* gene region causing RMP resistance have been published [11]. As these mutations are very rare, they were not accessible for validation purposes of this test system but were only detected in silico.
The **GenoType MTBDR*plus*** VER 2.0 only detects those resistances that have their origins in the *rpoB*, *katG*, and *inhA* regions examined here. Resistances originating from mutations of other genes or gene regions as well as other RMP and INH resistance mechanisms will not be detected by this test.
Theoretically, a resistance can exist in spite of a wild type pattern. If the sample contains a strain that has developed a heteroresistance and the resistance is caused by a mutation not covered by the mutation probes, the wild type pattern will appear. Similarly, if the sample contains more than one *M. tuberculosis* complex strain (due to mixed culture or contamination) and one of these harbors a mutation not covered by the mutation probes, the wild type pattern will also appear.

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 **GenoType MTBDR*plus*** 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.
The presence of multiple bacterial species in the sample to be analyzed might hamper the interpretation of the test.
The members of the MTB complex cannot be differentiated.

The test only works within the limits of the genomic regions the primers and probes were chosen from.
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.

Performance evaluation of this assay was carried out using the **GenoLyse®** kit for DNA extraction from decontaminated pulmonary smear-positive and smear-negative clinical specimens as well as from cultivated samples and using the **GXT DNA/RNA Extraction Kit** for automated DNA extraction from decontaminated clinical specimens. Until the present edition of the instructions on hand, the performance of the test has not been validated with other DNA extraction methods or sample materials.

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 results by phenotypic drug susceptibility testing may be necessary.

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 a new specimen and repeat test.
- Error during DNA extraction. Repeat extraction.

Ordering Information

Hain Lifescience	**Order no.**
GenoType MTBDR*plus* VER 2.0 (kit for analysis of 12 samples)	304A
GenoType MTBDR*plus* VER 2.0 (kit for analysis of 96 samples)	30496A
GenoLyse® (kit for manual DNA extraction of 12 samples)	51612
GenoLyse® (kit for manual DNA extraction of 96 samples)	51610
GXT DNA/RNA Extraction Kit (kit for automated DNA/RNA extraction of 96 samples using the **GenoXtract®**)	12.01.02
GenoXtract® (instrument for nucleic acid extraction of up to 12 samples)	8.31.01

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

Pulmonary clinical specimens

Diagnostic performance characteristics of the **GenoType MTBDR*plus*** VER 2.0 were determined in a study [12] with 338 specimens (including sputum, bronchoalveolar lavages, and pleural aspirates) compared to culture (successful cultivation on Loewenstein-Jensen solid medium or in MGIT (BD Diagnostics, Franklin Lakes, USA) and subsequent speciation using the **GenoType Mycobacterium CM** VER 1.0) and phenotypic drug susceptibility testing (DST). Additionally, the samples were examined by microscopy. Clinical data of the patients were included in the evaluation.

The study site was located in a high MDR-TB burden country. Microscopy and cultivation methods were conducted on site. Aliquots of the NALC-decontaminated sputum specimens were shipped to a second laboratory to perform DNA extraction and the **GenoType MTBDR*plus*** VER 2.0. Manual DNA extraction was performed with the **GenoLyse®** kit (162 of the 338 sputum samples), automated DNA extraction was carried out on the **GenoXtract®** using the **GXT DNA/RNA Extraction Kit** (176 of the 338 sputum samples) according to the respective instructions for use.

A congruent **GenoType MTBDR*plus*** VER 2.0 positive, clinical positive result was defined either by positivity of culture and **GenoType MTBDR*plus*** VER 2.0 or when only **GenoType MTBDR*plus*** VER 2.0 was positive and culture negative, but TB was indicated by previous culture-based findings of the respective patient. A discrepant result (**GenoType MTBDR*plus*** VER 2.0 positive and culture negative) does not exclude in all cases a TB infection of the patient as for some patients' histories of a probable TB infection were not available.

Table 1: Performance characteristics of the **GenoType MTBDR*plus*** VER 2.0 for detection of MTBC from pulmonary clinical specimens compared to culture/**GenoType Mycobacterium CM** VER 1.0 (GT Myco CM) and clinical findings

		Smear-positive			Smear-negative		
		Culture/GT Myco CM and clinic		Sens: 100%	Culture/GT Myco CM and clinic		Sens: 80.3%
		Positive	Negative	Spec: /*	Positive	Negative	Spec: 98.4%
GenoLyse®	**GenoType MTBDR*plus*** VER 2.0 Positive	39	0	PPV: 100%	49	1	PPV: 98.0%
	Negative	0	1	NPV: /*	12	60	NPV: 83.3%
		Culture/GT Myco CM and clinic		Sens: 97.5%	Culture/GT Myco CM and clinic		Sens: 78.3%
		Positive	Negative	Spec: /*	Positive	Negative	Spec: 96.0%
GXT	**GenoType MTBDR*plus*** VER 2.0 Positive	39	1	PPV: 97.5%	47	3	PPV: 94.0%
	Negative	1	0	NPV: /*	13	72	NPV: 84.7%

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 156 samples (78 **GenoLyse®** isolates and 78 **GXT** isolates) were used which were MTBC-positive in both culture and **GenoType MTBDR*plus*** VER 2.0.

Table 2: Performance characteristics of the **GenoType MTBDR*plus*** VER 2.0 for detection of RMP resistance from pulmonary clinical specimens compared to culture/DST

		Smear-positive			Smear-negative		
		Culture/DST		Sens: 100%	Culture/DST		Sens: 96.0%
		RMP-R	RMP-S	Spec: 92.3%	RMP-R	RMP-S	Spec: 93.3%
GenoLyse®	**GenoType MTBDR*plus*** VER 2.0 RMP-R	25	1	PPV: 96.2%	24	1	PPV: 96.0%
	RMP-S	0	12	NPV: 100%	1	14	NPV: 93.3%
		Culture/DST		Sens: 96.3%	Culture/DST		Sens: 86.2%
		RMP-R	RMP-S	Spec: 100%	RMP-R	RMP-S	Spec: 100%
GXT	**GenoType MTBDR*plus*** VER 2.0 RMP-R	26	0	PPV: 100%	25	0	PPV: 100%
	RMP-S	1	12	NPV: 92.3%	4	10	NPV: 71.4%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value;
RMP-R, resistant to rifampicin; RMP-S, sensitive to rifampicin

Table 3: Performance characteristics of the **GenoType MTBDR*plus*** VER 2.0 for detection of INH resistance from pulmonary clinical specimens compared to culture/DST

		Smear-positive			Smear-negative		
		Culture/DST		Sens: 96.7%	Culture/DST		Sens: 96.7%
		INH-R	INH-S	Spec: 87.5%	INH-R	INH-S	Spec: 90.0%
GenoLyse®	**GenoType MTBDR*plus*** VER 2.0 INH-R	29	1	PPV: 96.7%	29	1	PPV: 96.6%
	INH-S	1	7	NPV: 87.5%	1	9	NPV: 90.0%
		Culture/DST		Sens: 100%	Culture/DST		Sens: 90.6%
		INH-R	INH-S	Spec: 100%	INH-R	INH-S	Spec: 71.4%
GXT	**GenoType MTBDR*plus*** VER 2.0 INH-R	28	0	PPV: 100%	29	2	PPV: 93.5%
	INH-S	0	11	NPV: 100%	3	5	NPV: 62.5%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value;
INH-R, resistant to isoniazid; INH-S, sensitive to isoniazid

Cultured material

The diagnostic performance characteristics of the **GenoType MTBDR*plus*** VER 2.0 were determined in a study with 74 cultured samples compared to **GenoType Mycobacterium CM** VER 1.0 and phenotypic drug susceptibility testing (DST). The study site was located in a low MDR-TB burden country. Manual DNA extraction was performed using the **GenoLyse®** kit according to the instructions for use. From 74 cultures, 49 were positive for *M. tuberculosis* complex (MTBC) and 25 cultures showed growth of nontuberculous mycobacteria. Hence, for resistance detection in cultured material, 49 isolates were available.

Table 4: Performance characteristics of the **GenoType MTBDR*plus*** VER 2.0 for detection of MTBC from cultured material compared to culture/**GenoType Mycobacterium CM** VER 1.0 (GT Myco CM)

		Culture/GT Myco CM		Sens: 100%
		Positive	Negative	Spec: 100%
GenoType MTBDR*plus*	Positive	49	0	PPV: 100%
VER 2.0	Negative	0	25	NPV: 100%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value

Table 5: Performance characteristics of the **GenoType MTBDR*plus*** VER 2.0 for detection of RMP resistance from cultured material compared to culture/DST

		Culture/DST		Sens: /*
		RMP-R	RMP-S	Spec: 100%
GenoType MTBDR*plus*	RMP-R	0	0	PPV: /*
VER 2.0	RMP-S	0	49	NPV: 100%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value;
RMP-R, resistant to rifampicin; RMP-S, sensitive to rifampicin
* no value due to low sample number

Table 6: Performance characteristics of the **GenoType MTBDR*plus*** VER 2.0 for detection of INH resistance from cultured material compared to culture/DST

		Culture/DST		Sens: /*
		INH-R	INH-S	Spec: 100%
GenoType MTBDR*plus*	INH-R	3	0	PPV: /*
VER 2.0	INH-S	0	46	NPV: 100%

Sens, diagnostic sensitivity; Spec, diagnostic specificity; PPV, positive predictive value; NPV, negative predictive value;
INH-R, resistant to isoniazid; INH-S, sensitive to isoniazid
* no value due to low sample number

Analytical performance

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 was determined with 61 DNA isolates including the following MTBC strains: *M. tuberculosis*, *M. africanum*, *M. bovis, M. canettii, M. microti*, and *M. pinnipedii* (all RMP- and INH-sensitive). The following strains not detectable with the test system were analyzed: *Actinomyces naeslundii, Aggregatibacter actinomycetemcomitans, Bacillus cereus, Corynebacterium ammoniagenes, C. bovis, C. durum, Escherichia coli, Gordona rubropertinctus, Klebsiella oxytoca, Mycobacterium abscessus, M. alvei, M. asiaticum, M. avium, M. celatum, M. chelonae, M. chimaera, M. fortuitum* (2 sequevars), *M. frederiksbergense, M. gastri, M. genavense, M. goodii, M. gordonae, M. heckeshornense, M. immunogenum, M. interjectum, M. intermedium, M. intracellulare, M. lentiflavum, M. marinum, M. mucogenicum, M. palustre, M. peregrinum, M. scrofulaceum, M. shimoidei, M. simiae, M. smegmatis, M. szulgai, M. triplex, M. ulcerans, M. xenopi,* MRSA, *Nocardia abscessus, N. africana, N. amarae, N. asteroides, N. farcinica, Porphyromonas gingivalis, Prevotella intermedia, Rhodococcus erythropolis, Saccharomonospora glauca, Tannerella forsythia, Treponema denticola, Tsukamurella inchonensis, T. pulmonis.*

The six MTBC isolates were correctly identified as RMP- and INH-sensitive MTBC strains. All other 55 isolates displayed no TUB band and no evaluable band pattern for RMP and INH resistances. Hence, an analytical specificity of 100% was achieved.

Analytical sensitivity (limit of detection, LOD)

For determination of analytical sensitivity of the **GenoType MTBDR*plus*** VER 2.0 for clinical samples, ten parallel BCG cultures were diluted and spiked into MTBC-negative sputum samples (final concentrations in sputum samples: 1000, 500, 160, and 100 CFU/ml). Including a negative control, DNA was extracted once using the **GenoLyse®** kit and once using the **GXT DNA/RNA Extraction Kit**, and analyzed with the **GenoType MTBDR*plus*** VER 2.0 applying the "MDR DIR" PCR protocol. A limit of detection of 160 CFU/ml was determined with both extraction methods.

For determination of analytical sensitivity of the **GenoType MTBDR*plus*** VER 2.0 for culture samples, four BCG cultures (RMP- and INH-sensitive, 1.6×10^4, 1.6×10^3, 1.6×10^2, and 100 CFU/ml) were set up in triplicate. Including a negative control, DNA was extracted using the **GenoLyse®** kit and analyzed with the **GenoType MTBDR*plus*** VER 2.0 applying the "MDR CUL" PCR protocol. A limit of detection of 1.6×10^4 CFU/ml was determined.

Reproducibility

In order to determine the intra-assay precision of the **GenoType MTBDR*plus*** VER 2.0, two BCG cultures (RMP- and INH-sensitive, 1,500 and 150 CFU/ml) and an *M. avium* culture (10,000 CFU/ml) were set up in triplicate and spiked into negative sputum specimens. These samples and a negative control were tested with the **GenoType MTBDR*plus*** VER 2.0 under identical conditions, applying the "MDR DIR" PCR protocol. DNA extraction was performed once using the **GenoLyse®** DNA extraction kit, and once using the **GXT DNA/RNA Extraction Kit**. All parallels showed identical and correct banding patterns and comparable signal strengths. Additionally, signal strengths between the two DNA extraction methods and between different dilutions of the same samples were comparable. Hence, an intra-assay precision of 100% was achieved.

In order to determine the inter-assay precision of the **GenoType MTBDR*plus*** VER 2.0, two BCG cultures (RMP- and INH-sensitive, 1,500 and 150 CFU/ml) and an *M. avium* culture (10,000 CFU/ml) were set up in triplicate and spiked into negative sputum specimens. These samples and a negative control were tested in nine runs: on three different days, using three different sets of instruments, and conducted by three different operators. DNA extraction was performed once using the **GenoLyse®** DNA extraction kit and once using the **GXT DNA/RNA Extraction Kit**. The "MDR DIR" PCR protocol was applied for PCR. Apart from the varied parameter, all other testing conditions were identical. No deviations were detected between parallel samples, that is between runs banding patterns were identical and correct and signal strengths were comparable. Moreover, signal strengths were comparable between different DNA extraction methods and different bacterial concentrations. 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*plus*** VER 2.0, 6 different *M. tuberculosis* complex samples (4 RMP- and INH-resistant, 2 RMP- and INH-sensitive) were cultured in 4 different media (solid media: Loewenstein-Jensen, Stonebrink, and Middlebrook-7H10, liquid medium: MGIT (BD Diagnostics, Franklin Lakes, USA)). DNA was extracted from the culture samples using the **GenoLyse®** DNA extraction kit and then tested with the **GenoType MTBDR*plus*** VER 2.0. 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*plus*** VER 2.0.

Interfering substances may also be carried over from the sample material. Hence, the substances indicated in table 7 were tested in order to assess a potential interference of the **GenoType MTBDR*plus*** VER 2.0. Defined BCG culture dilutions above and at the detection limit were spiked with various amounts of the potential inhibitors. From all samples, DNA extraction was performed using the **GenoLyse®** DNA extraction kit. Then the culture dilutions were tested with the **GenoType MTBDR*plus*** VER 2.0.

Table 7: Tested potential interferents of the **GenoType MTBDR*plus*** VER 2.0

Substance/class	Description/active ingredient	Test concentration(s)
Allergy relief medicines	Tea tree oil	0.008 % v/v to 0.5 % v/v
Anesthetics (endotracheal intubation)	Lidocaine HCl 4%	20% v/v; 30% v/v
Anesthetics (oral)	Benzocaine 20%	5% w/v
Antibiotics (nasal ointment)	Mupirocin	1.2 mg/ml; 2.4 mg/ml
Antibiotics (systemic)	Amoxicillin	2.2 µg/ml
Anti-tuberculosis drugs	Isoniazid 1 mg/ml	50 µg/ml
Anti-tuberculosis drugs	Rifampicin 1 mg/ml	25 µg/ml
Anti-tuberculosis drugs	Pyrazinamide 10 mg/ml	100 µg/ml
Anti-tuberculosis drugs	Ethambutol 1 mg/ml	5 µg/ml; 50 µg/ml
Anti-tuberculosis drugs	Streptomycin 1 mg/ml	25 µg/ml
Anti-viral drugs	Zanamivir	800 µg/ml
Blood	Whole blood	0.2% v/v to 5% v/v
Blood	Hemoglobin	0.05% v/v to 0.3% v/v
Bronchodilators	Theophylline	222 pmol/ml
DNA (human)		10 µg/ml
Expectorants (oral)	Guaifenesin 400 mg/pill	2.5 mg/ml; 5 mg/ml
Gastric acid	0.5% HCl, 0.1 M KCl, 0.1 M NaCl, pH 1-2	5% v/v
Influenza vaccine as nasal spray (FluMist®)	Live attenuated influenza vaccine	5% v/v
Inhaled bronchodilators	Salbutamol sulfate 2.5 mg/3 ml	50 µg/ml; 100 µg/ml
Mouthwash/gargle solutions	Listerine (eucalyptol 0.029%, menthol 0.042%, methyl-salicylate 0.06%, thymol 0.064%, denatured alcohol 20%)	20% v/v
Mucin: bovine submaxillary gland, type I-S	Purified mucin protein 5% w/v	1.5% w/v; 5% w/v
Nasal corticosteroids	Dexamethasone	1.52 pmol/ml
Nasal gel (homeopathic)	Sulfur	5% w/v
Nasal sprays or drops	Phenylephrine 0.5%	25% v/v; 100% v/v
Nebulizing solutions (hypertonic saline)	NaCl	3% w/v; 5% w/v
Physiologic saline	NaCl (0.9%)	0.9% w/v
Pneumocystis jiroveci medications	Pentamidine	300 ng/ml
Pus		0.2% v/v to 5% v/v
Specimen processing reagents	NALC-NaOH (N-acetyl-l-cysteine-sodium hydroxide)	0.5% v/v; 1% v/v
Tobacco	Nicogel (40% tobacco extract)	0.5% w/v

Inhibition of the **GenoType MTBDR*plus*** VER 2.0 (invalid test result) was observed in the presence of the following substances in the concentrations as indicated: whole blood at 0.6%, hemoglobin at 0.1%, pus at 2%, lidocaine at 30%, mupirocin at 2.4 mg/ml, tea tree oil at 0.5%, and guaifenesin at 5 mg/ml.

Stability

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

References

1. World Health Organization. Global tuberculosis report 2018. WHO/CDS/TB/2018.20. World Health Organization, Geneva, Switzerland 2018.
2. Zhang Y, Yew WW. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis* 2009; 13: 1320–1330.
3. Biosafety in microbiological and biomedical laboratories, 5th edition. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, USA 2009.
4. Protection of laboratory workers from occupationally acquired infections. Approved guideline. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards), USA, Document M29 (please refer to the latest version).
5. Kent PT, Kubica GP. Public health mycobacteriology: a guide for the level III laboratory. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, USA 1985.
6. Isenberg HD. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C., USA 1992.
7. Richter E, Beer J, Diel R, Hillemann D, Hoffmann H, Klotz M, Mauch H, Rüsch-Gerdes S. MiQ 5, Tuberkulose, Mykobakteriose. In: Podbielski A, Herrmann M, Kniehl E, Mauch H, Rüssmann H (eds): Mikrobiologisch-infektiologische Qualitätsstandards. Elsevier, Munich, Germany 2010.
8. DIN, Deutsches Institut für Normung e.V. (ed). DIN 58943-4:2009-02: Medical microbiology - Diagnosis of tuberculosis - Part 4: Primary samples for the diagnosis of tuberculosis and mycobacteria – Qualitative and quantitative requirements, extraction, transport and storage. Beuth, Berlin, Germany 2009.
9. Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, Matter L, Schopfer K, Bodmer T. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. *Lancet* 1993, 341: 647-650.
10. Alonso M, Palacios JJ, Herranz M, Penedo A, Menéndez A, Bouza E, García de Viedma D. Isolation of *Mycobacterium tuberculosis* strains with a silent mutation in rpoB leading to potential misassignment of resistance category. *J Clin Microbiol* 2011; 49: 2688-2690.
11. Musser JM. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. *Clin Microbiol Rev* 1995; 8: 496-514.
12. Crudu V, Stratan E, Romancenco E, Allerheiligen V, Hillemann A, Moraru N. First evaluation of an improved assay for molecular genetic detection of tuberculosis as well as RMP and INH resistances. *J Clin Microbiol* 2012; Epub ahead of print, doi:10.1128/JCM.05903-11.

Important Changes in IFU-304A-09

Chapter	Change
Reagents and Instruments, Ordering Information	Freezing and thawing kit constituents more than four times should be avoided. If necessary, aliquot reagents using suitable screw cap tubes.
Evaluation and Interpretation of Results	**New**: "In case of overall strong signal intensities but only weak staining or absence of the Amplification Control band, a single wild type band showing significantly weaker staining than the other wild type bands of the respective locus (or Locus Control band for *katG*) is to be considered negative."

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