GenoType CMdirect
VER 1.0

Instructions for Use

IFU-295-01

for in vitro diagnostic use only

09/2017
**GenoType C**Mdirect VER 1.0

**Molecular Genetic Assay for Identification of Clinically Relevant Mycobacterial Species from Clinical Specimens**

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**

**Summary and Explanation**
Mycobacterioses are infectious diseases caused by bacteria of the genus *Mycobacterium*. The most significant is tuberculosis (TB) caused by the members of the *Mycobacterium tuberculosis* complex. In 2015, there were an estimated 10.4 million incident cases of TB globally, and an estimated 1.4 million TB deaths [1]. The TB pathogens are immobile, obligate aerobic, acid-fast bacilli belonging to the family of Mycobacteriaceae. They are gram-positive with a high genomic G+C content (59-66%). The genus *Mycobacterium* comprises numerous species which are divided into three groups: (i) the *Mycobacterium tuberculosis* complex (*Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis* subsp. *bovis*, *Mycobacterium bovis* subsp. *caprae*, *Mycobacterium bovis* BDQ, *Mycobacterium canetti*, *Mycobacterium microti*, and *Mycobacterium pinnipedii*), (ii) *Mycobacterium leprae* causing leprosy, and (iii) atypical or nontuberculous mycobacteria (NTM). In view of the varying pathogenicity and apathogenicity of some species, a fast and certain identification of the *M. tuberculosis* complex and hence its differentiation from the NTM is most essential.

NTM can cause chronic mycobacterioses. Infectiousness and symptoms vary in a broad range and depend both on the pathogen as well as on the immunocompetence of the person affected [2]. Immunocompromised persons such as HIV or leukemia patients are most likely to develop a severe mycobacteriosis.

The GenoType **C**Mdirect VER 1.0 permits the rapid and reliable differentiation of relevant mycobacteria and therefore the fast application of specific treatment and preventive measures.

**Principles of the Procedure**
The GenoType **C**Mdirect VER 1.0 test is based on the DNA+STRIP technology. The whole procedure is divided into three steps: (i) DNA extraction from decontaminated clinical specimens (the necessary reagents are not included in the kit), (ii) 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 the different sequences of the bacterial species. 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

<table>
<thead>
<tr>
<th>Order no.</th>
<th>Tests</th>
<th>295</th>
<th>29596</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kit Component 1 of 2</strong> (store at 2°C to 8°C)</td>
<td>Membrane strips [CMdirect VER 1.0 STRIPS] coated with specific probes</td>
<td>12</td>
<td>2x 48</td>
</tr>
<tr>
<td>Denaturation Solution (DEN) contains &lt;2% NaOH, dye</td>
<td>240 µl</td>
<td>2x 960 µl</td>
<td></td>
</tr>
<tr>
<td>Hybridization Buffer (HYB) contains &lt;10% anionic tenside, dye</td>
<td>12 ml</td>
<td>96 ml</td>
<td></td>
</tr>
<tr>
<td>Stringent Wash Solution (STR) contains &gt;25% of a quaternary ammonium compound, &lt;1% anionic tenside, dye</td>
<td>12 ml</td>
<td>96 ml</td>
<td></td>
</tr>
<tr>
<td>Rinse Solution (RIN) contains buffer, &lt;1% NaCl, &lt;1% nonionic tenside</td>
<td>36 ml</td>
<td>3x 96 ml</td>
<td></td>
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<tr>
<td>Conjugate Concentrate (CON-C) contains streptavidin-conjugated alkaline phosphatase, dye</td>
<td>120 µl</td>
<td>960 µl</td>
<td></td>
</tr>
<tr>
<td>Conjugate Buffer (CON-D) contains buffer, &lt;1% blocking reagent, &lt;1% NaCl</td>
<td>12 ml</td>
<td>96 ml</td>
<td></td>
</tr>
<tr>
<td>Substrate Concentrate (SUB-C) contains &gt;70% dimethyl sulfoxide, &lt;10% 4-nitro blue tetrazolium chloride, &lt;10% 5-bromo-4-chloro-3-indolyl phosphate</td>
<td>120 µl</td>
<td>960 µl</td>
<td></td>
</tr>
<tr>
<td>Substrate Buffer (SUB-D) contains buffer, &lt;1% MgCl₂, &lt;1% NaCl</td>
<td>12 ml</td>
<td>96 ml</td>
<td></td>
</tr>
<tr>
<td>Tray, evaluation sheet</td>
<td>1 of each</td>
<td>4 of each</td>
<td></td>
</tr>
<tr>
<td>Instructions for use, template</td>
<td>1 of each</td>
<td>1 of each</td>
<td></td>
</tr>
<tr>
<td>Lot label</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

| **Kit Component 2 of 2** (store at −20°C to −18°C) | Amplification Mix A (AM-A GT CMdirect VER 1.0) contains buffer, nucleotides, Taq polymerase | 120 µl | 4x 240 µl |
| Amplification Mix B (AM-B GT CMdirect VER 1.0) contains salts, specific primers, dye | 420 µl | 4x 840 µl |
| Internal Control DNA (IC GT CMdirect VER 1.0) contains specific polynucleotides | 192 µl | 192 µl |
| Control DNA (C+ GT CMdirect VER 1.0) contains specific polynucleotides | 95 µl | 95 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.

Store Internal Control DNA (IC) at −20°C to −18°C in the same room where the DNA is extracted.

Store Control DNA (C+) at −20°C to −18°C in the same room where the DNA is added to the tubes containing the aliquoted master mix.

Refreeze AM-A, AM-B, IC, and C+ immediately after use.

Avoid repeated freezing and thawing of AM-A, AM-B, IC, and C+; when processing only small sample numbers per run, aliquot AM-A, AM-B, IC, and C+.

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.

For additional information, please refer to the safety data sheets which can be downloaded from: [www.hain-lifescience.com/products/msds.html](http://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]).

Material required but not included in the kit
- Absorbent paper
- Adjustable pipettes for 10, 20, 200, and 1000 µl
- Class II safety cabinet
- Disposable gloves
- Disposable sterile pipette tips with filter
- DNA extraction kit [GenoLyse®], see chapter Ordering Information as well as necessary equipment
- Graduated cylinder
- PCR tubes, DNase- and RNase-free
- 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)

Quality Control
In order to control the correct performance of the test and the proper functioning of kit constituents, each strip includes 3 control zones:
- a Conjugate Control zone (CC) to check the binding of the conjugate on the strip and a correct chromogenic reaction
- an Internal Control zone (IC) which documents a successful DNA extraction and amplification reaction
- a Genus Control zone (GC) which documents the presence of a member of the genus Mycobacterium

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, controls, 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.

The kit includes an Internal Control DNA (IC) which is added to each sample prior to DNA extraction. The amplicon of the Internal Control DNA binds to the Internal Control zone on the strip (see above).

A negative control sample for detection of possible contamination events should be part of each run and is included in the sample set during DNA extraction (see respective instructions for use). A valid negative control must show the bands CC and IC. In addition to bands CC and IC only band 10 may be developed. Additionally, a positive control sample containing the provided Control DNA (C+) may be included in the sample set during amplification. The C+ contains specific polymynucleotides and shows an M. kansasii banding pattern without 1C band on the respective test strip (see chapter Interpretation Chart). The amount of C+ provided is sufficient for 19 positive control samples.

Specimen Requirements
NALC-NaOH-decontaminated sputum samples 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.

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 including Hepatitis B and Human Immunodeficiency Virus (HIV) must always be labeled and handled under suitable safety conditions according to institutional guidelines.

Patient specimens must be centrifuged in a class II safety cabinet or in an aerosol-tight rotor. Open aerosol-tight rotor in safety cabinet only. For inactivated samples, a standard rotor can be used for centrifugation outside the safety cabinet.

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 takes place, 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]. The 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 may 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.

DNA Extraction
NALC-NaOH-decontaminated sputum samples can be used as starting material for DNA extraction.

For DNA extraction the GenoLyse® kit (see chapter Ordering Information) is used.

The method described above was used for performance evaluation of the GenoType CMdirect VER 1.0. 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 has to 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 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 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 of the master mix into each of the prepared PCR tubes. In a separate working area, add 5 µl DNA solution (or C+ for a positive control) to each aliquot. Refreeze AM-A, AM-B, and C+ immediately after use.

Amplification profile:
When using a thermal cycler from Hain Lifescience, select protocol “MDR DIR”.

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>95°C</td>
<td>1 cycle</td>
</tr>
<tr>
<td>30 sec</td>
<td>95°C</td>
<td>20 cycles</td>
</tr>
<tr>
<td>2 min</td>
<td>65°C</td>
<td></td>
</tr>
<tr>
<td>25 sec</td>
<td>95°C</td>
<td>30 cycles</td>
</tr>
<tr>
<td>40 sec</td>
<td>50°C</td>
<td></td>
</tr>
<tr>
<td>40 sec</td>
<td>70°C</td>
<td>1 cycle</td>
</tr>
<tr>
<td>8 min</td>
<td>70°C</td>
<td></td>
</tr>
<tr>
<td>Heating rate</td>
<td>0.2°C/sec</td>
<td></td>
</tr>
</tbody>
</table>

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 IC with the respective lines on the sheet. Note down positive signals in the last but one column, determine species with the help of the interpretation chart and enter name of the identified species in the last column. The supplied template also serves as an aid for evaluation and must be aligned with the bands CC and IC of the strip as well. Each strip has a total of 17 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.

**Internal Control (IC)**
When the test is performed correctly, a control amplicon will bind to the Internal Control zone.
In case of a positive test result, the signal of the Internal 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 bands CC, IC, and possibly band 10 are developed, this represents a valid negative result. A missing IC band in case of a negative test result indicates mistakes during DNA extraction or 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.

**Genus Control (GC)**
Staining of this zone documents the presence of a member of the genus *Mycobacterium*. The intensity of this band varies depending on the mycobacterial species. A species-specific banding pattern can only be interpreted, if the GC band is developed.

**Other bands**
Specific probes, for evaluation see interpretation chart.

Please note:
Not all bands of a strip have to show the same signal strength. Generally, only those bands whose intensities are about as strong as or stronger than that of the Internal Control zone (IC) are to be considered (exceptions: see chapter Interpretation Chart).

Only banding patterns described in chapter Interpretation Chart can be interpreted. Other banding patterns are not interpretable.
### Interpretation Chart

<table>
<thead>
<tr>
<th>AS</th>
<th>NTM-DR</th>
<th>NTM-DR</th>
<th>AS</th>
<th>MTBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mycobacterium species</strong></td>
<td><strong>M. avium</strong></td>
<td><strong>M. chelonae</strong></td>
<td><strong>M. abscessus complex</strong></td>
<td><strong>M. fortuitum group</strong></td>
</tr>
<tr>
<td>1</td>
<td>CC</td>
<td>I</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
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<td>13</td>
</tr>
<tr>
<td>14</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td><strong>NTM</strong></td>
</tr>
<tr>
<td><strong>M. fortuitum</strong></td>
<td><strong>M. intracellulare</strong></td>
<td><strong>M. chimaera</strong></td>
<td><strong>M. abscessus</strong></td>
<td><strong>M. malmoense</strong></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
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<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>17</td>
<td><strong>NTM-DR</strong></td>
<td><strong>AS</strong></td>
<td><strong>MTBC</strong></td>
</tr>
</tbody>
</table>

- **CC**: Conjugate Control
- **IC**: Internal Control
- **AS**: GenoType Mycobacterium AS (from cultured material)
- **NTM-DR**: GenoType NTM-DR (from cultured material)
- **MTBC**: GenoType MTBC (from cultured material)

**Band No. 1 (CC):** Conjugate Control
**Band No. 2 (IC):** Internal Control
**Band No. 3 (GC):** Genus Control

**Species not belonging to the M. fortuitum group:**
Mycobacterium magerilense shows the banding pattern as depicted in the right column without band 14. Rare Mycobacterium smegmatis variants may also show the banding pattern as depicted in the left column without band 14. In this case, however, band 7 shows only a weak signal.

**M. avium complex** shows the same banding pattern as M. chelonae or the M. abscessus complex.

**M. intracellulare** and M. chimaera can be differentiated with the GenoType NTM-DR kit from cultured material.

**Mycobacterium marseillense** and **Mycobacterium chimaera** (both members of the M. avium complex) show the same banding pattern as M. intracellulare. **M. intracellulare** and M. chimaera can be differentiated with the GenoType NTM-DR kit from cultured material.

**Mycobacterium abscessus** can be identified with the GenoType NTM-DR kit from cultured material.

**M. scrofulaceum** and **M. intracellulare** can be differentiated by means of the intensity of band 13 when the specific bands 9, 10, and 13 are developed. If the intensity of band 13 is weaker than that of the IC band, the banding pattern indicates the presence of **M. scrofulaceum/M. intracellulare**. If the intensity of band 13 is as strong as or stronger than the IC band, the banding pattern indicates the presence of **M. malmoense**.

**Mycobacterium haemophilum**, **Mycobacterium palustre**, and **Mycobacterium nebraskense** show the same banding pattern as M. malmoense. **M. haemophilum/M. nebraskense** can be identified with the GenoType Mycobacterium AS kit from cultured material.

**For M. szulgai,** the intensity of band 11 may be weaker than that of the IC band.

**M. ulcerans** can be identified with the GenoType Mycobacterium AS kit from cultured material.

**For the M. tuberculosis complex,** the intensity of bands 10 and/or 16 may be weaker than that of the IC band. If band 15 has also stained positive, additional detection methods must be applied.

Members of the M. tuberculosis complex can be differentiated with the GenoType MTBC kit from cultured material.
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 results of this test may only be interpreted in combination with additional laboratory and clinical data available to the responsible physician. The test reflects the current state of knowledge of Hain Lifescience.

Members of the *M. tuberculosis* complex cannot be differentiated. Likewise, members of the *M. abscessus* complex cannot be differentiated. If more than one species is assigned to a banding pattern, these species cannot be discriminated with this test system. In case a bacterial strain does not belong to one of the species identifiable with the GenoType CMdirect VER 1.0 but is closely related to one of them, it may, in rare cases, generate the banding pattern of the closely related species detectable with the test. The presence of multiple bacterial species in the sample to be analyzed might hamper the interpretation of the test. Application of exceed DNA concentrations may lead to overload of the test system; therefore, the test must not be used for examining cultured samples.

As any DNA detection method, the test system on hand detects DNA from viable and nonviable bacteria. Therefore, the GenoType CMdirect VER 1.0 may not be used for monitoring the progression or success of treatment of patients with antimicrobial therapy. The GenoType CMdirect VER 1.0 generates qualitative results. The intensities of the bands on a strip do not give information about the number of cells in a positive sample.

As with 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 with the GenoLyse® kit for DNA extraction from NALC-NaOH-decontaminated sputum samples. 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.

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, IC, and possibly band 10. Repeat amplification using fresh reagents.
  - Improper sampling, storage, transport, or preparation of specimen. Request new specimen and repeat test.
  - Error during DNA extraction. Repeat extraction.

**Ordering Information**

<table>
<thead>
<tr>
<th>Order no.</th>
<th>GenoType CMdirect VER 1.0 (kit for analysis of 12 samples)</th>
<th>GenoType CMdirect VER 1.0 (kit for analysis of 96 samples)</th>
<th>GenoLyse® (kit for manual DNA extraction of 12 samples)</th>
<th>GenoLyse® (kit for manual DNA extraction of 96 samples)</th>
<th>GenoType Mycobacterium AS (kit for analysis of 12 samples)</th>
<th>GenoType Mycobacterium AS (kit for analysis of 96 samples)</th>
<th>GenoType MTBC (kit for analysis of 12 samples)</th>
<th>GenoType MTBC (kit for analysis of 96 samples)</th>
<th>GenoType NTM-DR (kit for analysis of 12 samples)</th>
<th>GenoType NTM-DR (kit for analysis of 96 samples)</th>
</tr>
</thead>
</table>
Performance Characteristics
For the performance evaluation of the GenoType CMdirect VER 1.0 the test was carried out according to the instructions on hand.

Diagnostic performance
Diagnostic performance characteristics of the GenoType CMdirect VER 1.0 were determined in two studies.

The first study comprised 131 sputum samples. The GenoType CMdirect VER 1.0 was compared to culture (successful cultivation on Loewenstein-Jensen solid medium or in MGIT (BD Diagnostics, Franklin Lakes, USA) and subsequent species identification using the GenoType Mycobacterium CM VER 1.0). As a reference method for specimens with discrepant results, the respective samples were analyzed either with the GenoType MTBDRplus VER 2.0 or by sequencing. Additionally, all samples were examined by microscopy.

DNA extraction from NALC-NaOH-decontaminated sputum samples was performed with the GenoLyse® kit according to the instructions for use.

In case of culture-positive samples, test results were rated true positive if the result of the GenoType CMdirect VER 1.0 was in agreement with that obtained with GenoType Mycobacterium CM VER 1.0 from cultured samples. In case of culture-negative samples, test results were rated true positive if the GenoType MTBDRplus VER 2.0 gave a positive result (for identification of the M. tuberculosis complex) for the respective sample or if the result obtained with the GenoType CMdirect VER 1.0 was confirmed by sequencing (for identification of the other identifiable mycobacterial species).

One of the 131 samples (identified as M. intracellularis with culture/GenoType Mycobacterium CM VER 1.0) showed a non-interpretable banding pattern when analyzed with the GenoType CMdirect VER 1.0. This sample was excluded from evaluation.

Table 1: Results of the GenoType CMdirect VER 1.0 for detection of mycobacteria from sputum samples compared to culture/GenoType Mycobacterium CM VER 1.0 (GT Myco CM V1) or to culture/GT Myco CM V1 and reference methods (GenoType MTBDRplus VER 2.0 or sequencing)

<table>
<thead>
<tr>
<th>Smear-positive</th>
<th>GenoType CMdirect VER 1.0</th>
<th>Culture/GT Myco CM V1</th>
<th>Sens: 100%</th>
<th>Spec: /°</th>
<th>PPV: 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>24*</td>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smear-negative</th>
<th>GenoType CMdirect VER 1.0</th>
<th>Culture/GT Myco CM V1</th>
<th>Sens: 63.6%</th>
<th>Spec: 93.7%</th>
<th>Culture/GT Myco CM V1 reference methods</th>
<th>Sens: 75.0%</th>
<th>PPV: 92.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7°</td>
<td>Negative</td>
<td>6°</td>
<td>8°</td>
<td>Positive</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>4°</td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>4°</td>
<td>8°</td>
</tr>
</tbody>
</table>

Sens: Diagnostic sensitivity; Spec: Diagnostic specificity; PPV: Positive predictive value; NPV: Negative predictive value
* no value because no negative samples present

In case of culture-negative samples, test results were rated true positive if the result of the GenoType CMdirect VER 1.0 was in agreement with that obtained with the GenoType Mycobacterium CM VER 1.0 from cultured samples. In case of clinical data clearly indicated a mycobacterial infection of the respective patient.

The second study comprised 178 sputum samples. The GenoType CMdirect VER 1.0 was compared to culture (successful cultivation on Loewenstein-Jensen solid medium or in MGIT (BD Diagnostics, Franklin Lakes, USA) and subsequent species identification using the GenoType Mycobacterium CM VER 1.0). For specimens with discrepant results, clinical data of the patients were included in the evaluation. Additionally, all samples were examined by microscopy.

DNA extraction from NALC-NaOH-decontaminated sputum samples was performed with the GenoLyse® kit according to the instructions for use.

In case of culture-positive samples, test results were rated true positive if the result of the GenoType CMdirect VER 1.0 was in agreement with that obtained with the GenoType Mycobacterium CM VER 1.0 from cultured samples. In case of culture-negative samples, test results were rated true positive if clinical data clearly indicated a mycobacterial infection of the respective patient.
### Table 2: Results of the GenoType CMdirect VER 1.0 for detection of mycobacteria from sputum samples compared to culture/GenoType Mycobacterium CM VER 1.0 (GT Myco CM V1) or to culture/GT Myco CM V1 and clinical data

<table>
<thead>
<tr>
<th>smear-positive</th>
<th>Culture/GT Myco CM V1</th>
<th>Sens: 100%</th>
<th>Spec: *&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GenoType CMdirect VER 1.0</td>
<td>Positive</td>
<td>14&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>smear-negative</th>
<th>Culture/GT Myco CM V1</th>
<th>Sens: 54.5%</th>
<th>Spec: 98.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>GenoType CMdirect VER 1.0</td>
<td>Positive</td>
<td>6&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>5&lt;sup&gt;2&lt;/sup&gt;</td>
<td>150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Culture/GT Myco CM V1 + clinical data</th>
<th>Sens: 64.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>5&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Sens: Diagnostic sensitivity; Spec: Diagnostic specificity; PPV: Positive predictive value; NPV: Negative predictive value

* no value because no negative samples present

<sup>1</sup> These 14 samples were identified as 1x M. abscessus complex and 13x M. tuberculosis complex.

<sup>2</sup> These 6 samples were identified as 2x M. abscessus complex, 1x M. chelonae, and 3x M. tuberculosis complex.

<sup>3</sup> 4 out of 5 samples (identified as 1x M. avium, 1x M. chelonae, 1x M. intracellulare, and 1x Mycobacterium spec. with culture/GT Myco CM V1) were below the detection limit of the GenoType CMdirect VER 1.0.

<sup>4</sup> 1 out of 5 samples identified as M. tuberculosis complex with culture/GT Myco CM V1) was identified as Mycobacterium spec. with the GenoType CMdirect VER 1.0.

<sup>5</sup> The 3 culture-negative samples were identified as 2x M. avium and 1x Mycobacterium spec. with the GenoType CMdirect VER 1.0. Due to clinical data indicating an infection with M. avium or mycobacteria in general, respectively, these samples were rated as true positive.

### Analytical performance

#### Analytical specificity

The specificity of the GenoType CMdirect VER 1.0 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 of the GenoType CMdirect VER 1.0 was determined with strains of all mycobacteria detectable by this test, as well as with strains of mycobacterial and non-mycobacterial species that are not identifiable with the test system.

All mycobacteria detectable by this assay generated the correct specific banding pattern. Isolates of the mycobacterial species not identifiable with the test system and of all tested non-mycobacterial species displayed no specific banding pattern. Hence, the analytical specificity for the specific probes of the GenoType CMdirect VER 1.0 strip was 100%.

The same samples were also evaluated for performance of the Genus-specific probe (GC). An analytical specificity of 98.6% was determined for this probe.

#### Strains of all mycobacteria detectable with the GenoType CMdirect VER 1.0 were tested:

- **M. avium**
- **M. chelonae**
- **M. chimaera**
- **M. gordonae**
- **M. haemophilum**
- **M. immunogenum**
- **M. interjectum**
- **M. intracellulare**
- **M. kansasi**
- **M. mageritense**
- **M. malmoense**
- **M. marinum**
- **M. marseillense**
- **M. nebraskense**
- **M. palustre**
- **M. paraffinicum**
- **M. parascrofulaceum**
- **M. rorippae**
- **M. szulcii**
- **M. ulcerans**
- **M. xenopi**
- **M. septicum**

- **M. abscessus complex**
- **M. abscessus subsp. abscessus**
- **M. abscessus subsp. bolletii**
- **M. abscessus subsp. massiliense**
- **M. fortuitum**
- **M. fortuitum subsp. fortuitum**
- **M. fortuitum subsp. massiliense**
- **M. peregrinum**
- **M. alvei**

#### Tested mycobacterial species that are not identifiable with the GenoType CMdirect VER 1.0:

- **Mycobacterium asiaticum**
- **Mycobacterium goodii**
- **Mycobacterium mucogenicum**
- **Mycobacterium simiae**
- **Mycobacterium celatum**
- **Mycobacterium heckeshornense**
- **Mycobacterium phlei**
- **Mycobacterium smegmatis**
- **Mycobacterium gastri**
- **Mycobacterium intermedium**
- **Mycobacterium shimoidei**
- **Mycobacterium triplex**
- **Mycobacterium genavense**
- **Mycobacterium lentiflavum**

#### Tested non-mycobacterial species that are not detectable with the GenoType CMdirect VER 1.0:

- **Klebsiella pneumoniae**
- **Pseudomonas aeruginosa**
- **Streptococcus pneumoniae**
- **Nocardia asteroides**
- **Rhodococcus rhodochrous**
- **Tsukamurella inchonensis**
- **Escherichia coli**
- **Nocardia farcinica**
- **Rhodococcus ruber**
- **Tsukamurella pauronometabolica**
- **Haemophilus influenzae**
- **Nocardia otitiscaviarum**
- **Staphylococcus aureus**
- **Tsukamurella palpebralis**

### Analytical sensitivity (limit of detection, LOD)

For determination of the LOD, cultures of mycobacterial species detectable with this test were prepared. Different concentrations of these cultures were added to negative NALC-NaOH-decontaminated sputum samples. DNA was extracted from each sample in 20 parallels with the GenoLyse<sup>®</sup> kit and the isolates were analyzed with the GenoType CMdirect VER 1.0. The LOD values determined for the different mycobacterial species are given in Table 3.

For determination of the LOD for *M. ulcerans*, plasmid DNA containing the respective *M. ulcerans*-specific DNA sequences was used. Dilutions with different concentrations of the plasmid DNA were prepared in 20 parallels each and analyzed directly with the GenoType CMdirect VER 1.0.
To assess a potential interference, please refer to the latest version.

Interfering substances

There are substances that may inhibit PCR reactions. Such inhibitors may, for example, originate from the sample material.

Table 3: LOD for mycobacteria detectable with the GenoType CMdirect VER 1.0

<table>
<thead>
<tr>
<th>Tested mycobacteria</th>
<th>LOD$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. bovis BCG (M. tuberculosis complex)</td>
<td>1,500 CFU/ml</td>
</tr>
<tr>
<td>M. abscessus (M. abscessus complex)</td>
<td>1,500 CFU/ml</td>
</tr>
<tr>
<td>M. avium</td>
<td>15,000 CFU/ml</td>
</tr>
<tr>
<td>M. chelonae</td>
<td>1,500 CFU/ml</td>
</tr>
<tr>
<td>M. fortuitum (M. fortuitum-group)</td>
<td>750 CFU/ml</td>
</tr>
<tr>
<td>M. gordonae</td>
<td>15,000 CFU/ml</td>
</tr>
<tr>
<td>M. interjectum</td>
<td>15,000 CFU/ml</td>
</tr>
<tr>
<td>M. intracellulare</td>
<td>50,000 CFU/ml</td>
</tr>
<tr>
<td>M. kansasi</td>
<td>15,000 CFU/ml</td>
</tr>
<tr>
<td>M. malmoense</td>
<td>7,500 CFU/ml</td>
</tr>
<tr>
<td>M. marinum</td>
<td>7,500 CFU/ml</td>
</tr>
<tr>
<td>M. peregrinum (M. fortuitum group)</td>
<td>1,500 CFU/ml</td>
</tr>
<tr>
<td>M. scrofulaceum</td>
<td>15,000 CFU/ml</td>
</tr>
<tr>
<td>M. szulgai</td>
<td>15,000 CFU/ml</td>
</tr>
<tr>
<td>M. xenopi</td>
<td>7,500 CFU/ml</td>
</tr>
<tr>
<td>M. ulcerans</td>
<td>50 DNA copies/PCR</td>
</tr>
</tbody>
</table>

$^a$ Lowest tested bacterial or plasmid concentration at which at least 19 out of 20 replicates showed a positive test result.

Reproducibility

The intra-assay precision and the inter-assay precision were determined with the following four samples:

- M. bovis BCG culture dilution above the LOD in negative NALC-NaOH-decontaminated sputum
- M. bovis BCG culture dilution at the LOD in negative NALC-NaOH-decontaminated sputum
- Bordetella pertussis-positive DNA sample
- Negative control

DNA was extracted using the GenoLyse$^b$ kit and the isolates were analyzed with the GenoType CMdirect VER 1.0.

In order to determine the intra-assay precision, the samples were set up in four parallels and analyzed under identical conditions (same kit lot, same instrument, same operator, same point of time, etc.) in one PCR run. All parallels showed identical and correct banding patterns and comparable signal strengths. Hence, an intra-assay precision of 100% was achieved.

In order to determine the inter-assay precision, the samples were analyzed on three different days. The other experimental conditions (kit lot, instrument, operator, etc.) were identical. All parallels showed identical and correct banding patterns and comparable signal strengths. Hence, an inter-assay precision of 100% was achieved.

Interfering substances

There are substances that may inhibit PCR reactions. Such inhibitors may, for example, originate from the sample material. Hence, the substances indicated in table 4 were tested in order to assess a potential interference with the GenoType CMdirect VER 1.0. Defined M. bovis BCG culture dilutions above, at, and below the LOD were spiked with various amounts of the potential inhibitors. Then the DNA was extracted using the GenoLyse$^b$ kit and the isolates were analyzed with the GenoType CMdirect VER 1.0.

Table 4: Tested potential interferents of the GenoType CMdirect VER 1.0.

<table>
<thead>
<tr>
<th>Substance/class</th>
<th>Description/active ingredient</th>
<th>Substance concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Whole blood</td>
<td>2.5% v/v to 90% v/v</td>
</tr>
<tr>
<td>Blood</td>
<td>Hemoglobin</td>
<td>1% v/v to 13.5% v/v</td>
</tr>
<tr>
<td>Pus</td>
<td></td>
<td>2.5% v/v to 90% v/v</td>
</tr>
</tbody>
</table>

Interference of the GenoType CMdirect VER 1.0 was observed in samples containing concentrations greater than 25% whole blood, 10% hemoglobin, and 12% pus.

Stability

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

Stability is determined according to DIN EN ISO 23640.

References

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].