GenoType Mycobacterium CM

VER 2.0

Instructions for Use

IFU-299A-03

CE

IVD for in vitro diagnostic use only

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HAIN LIFESCIENCE

GenoType **Mycobacterium CM** VER 2.0
Molecular Genetic Assay for Identification of Clinically Relevant Mycobacterial Species from Cultured Material

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 Mycobacterium CM** VER 2.0 is a qualitative in vitro test for the identification of the *Mycobacterium tuberculosis* complex as well as the following nontuberculous mycobacteria from cultured material: *M. avium, M. chelonae, M. abscessus* complex, *M. fortuitum* group, *M. gordonae, M. intracellulare, M. scrofulaceum, M. szulgai, M. interjectum, M. kansasii, M. malmoense, M. marinum/M. ulcerans,* and *M. xenopi*.
The test is indicated as an aid for diagnosis and intended for use in medical laboratories.

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 2019, there were an estimated 10 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 (*M. tuberculosis, M. africanum, M. bovis* subsp. *bovis, M. bovis* subsp. *caprae, M. bovis* BCG, *M. microti, M. canettii, and M. pinnipedii*), (ii) *M. 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 Mycobacterium CM** permits the rapid and reliable differentiation of relevant mycobacteria and therefore the fast application of specific treatment and preventive measures. If it was not possible to identify a single species with this test, a specification may be achieved using the **GenoType Mycobacterium AS** kit (see Interpretation Chart).

Principles of the Procedure

The **GenoType Mycobacterium CM** VER 2.0 is based on the **DNA•STRIP** technology. The whole procedure is divided into three steps: (i) DNA extraction from 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 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

Order no.	**299A**	**29996A**
Tests	**12**	**96**
Kit Component 1 of 2 (store at 2°C to 8°C)		
Membrane strips coated with specific probes (Mycobacterium CM 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 Mycobacterium CM VER 2.0) contains buffer, nucleotides, Taq polymerase	120 µl	4x 240 µl
Amplification Mix B (AM-B GT Mycobacterium CM VER 2.0) contains salts, specific primers, dye	420 µl	4x 840 µl
Internal Control DNA (IC GT Mycobacterium CM VER 2.0) contains bacterial DNA	192 µl	192 µl
Control DNA (C+ GT Mycobacterium CM VER 2.0) contains specific polynucleotides	95 µl	95 µl

Storage and Disposal of Kit Constituents

1/2 Kit Component 1 of 2

2/2 Kit Component 2 of 2

Store all constituents from Kit Component 1 at 2°C to 8°C. Store all constituents from Kit Component 2 at –20°C to –18°C and keep strictly separated from contaminating DNA.
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 (>4x) 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, protective gloves, and eye protection.

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

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
- 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
- Reagents for cultivation of mycobacteria as well as necessary equipment
- 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 (exception: an amplicon generated with the **GenoType Mycobacterium CM** VER 2.0 can directly be hybridized to **GenoType Mycobacterium AS** strips). 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 during 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 during DNA extraction. A valid negative control must exclusively show the CC and IC bands.
Additionally, a positive control sample containing the provided Control DNA (C+) may be included. The Control DNA contains *M. kansasii* DNA and shows an *M. kansasii* banding pattern without IC band on the respective test strip. The amount provided is sufficient for 19 positive control samples.
IC and C+ must not be interchanged during the procedure because this may lead to erroneous results (see chapter Troubleshooting).

Sample Requirements

Mycobacteria grown on solid medium (e.g. Loewenstein-Jensen, Middlebrook) or in liquid medium (e.g. MGIT (BD Diagnostics, Franklin Lakes, USA)) are used as starting material for DNA extraction. The test must not be used for detection directly from patient specimens.

Precautions for handling samples

Culture samples 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 culture samples that may contain mycobacteria should be handled applying Biosafety Level 2 practices or, when indicated, Biosafety Level 3 practices (e.g. see [3]). Observe all federal, state, and local safety regulations.

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, transport and preparation

Transport, storage and preparation of patient specimens and culture samples should be carried out according to local, national and/or international guidelines and standards of the laboratory.

DNA Extraction

Mycobacteria grown on solid medium (e.g. Loewenstein-Jensen, Middlebrook) or in liquid medium (e.g. MGIT (BD Diagnostics, Franklin Lakes, USA)) are used as starting material for DNA extraction. The working area must be free from contaminating DNA.

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

The method described above was used for performance evaluation of the **GenoType Mycobacterium CM** VER 2.0. The performance of the test has not been validated with other DNA extraction methods or sample materials.

Amplification

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

Prepare for each sample:

- 10 µl AM-A (see Kit Component 2)
- 35 µl AM-B (see Kit Component 2)
- 5 µl DNA solution

Final volume: 50 µl

Determine the total number of samples (number of samples to be analyzed plus control samples). Prepare the number of 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. 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 with the respective preinstallation, select protocol "MDR CUL".

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

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

Hybridization

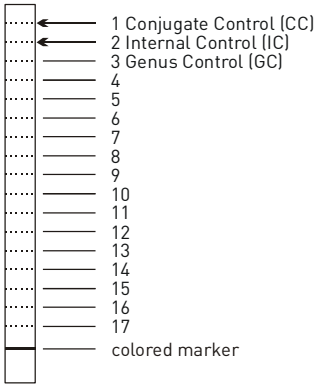
When using a hybridization instrument from Hain Lifescience, please refer to the document "Overview equipment programs" available on www.hain-lifescience.com for the name of the hybridization protocol to be used.
The following protocol describes the manual hybridization using a water bath or a **TwinCubator**.

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. Please note, that the positive control C+ does not show the IC band.
When only the CC and IC bands 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 the presence of amplification inhibitors. In this case, the test result is not valid and the test has to be repeated with the respective sample.

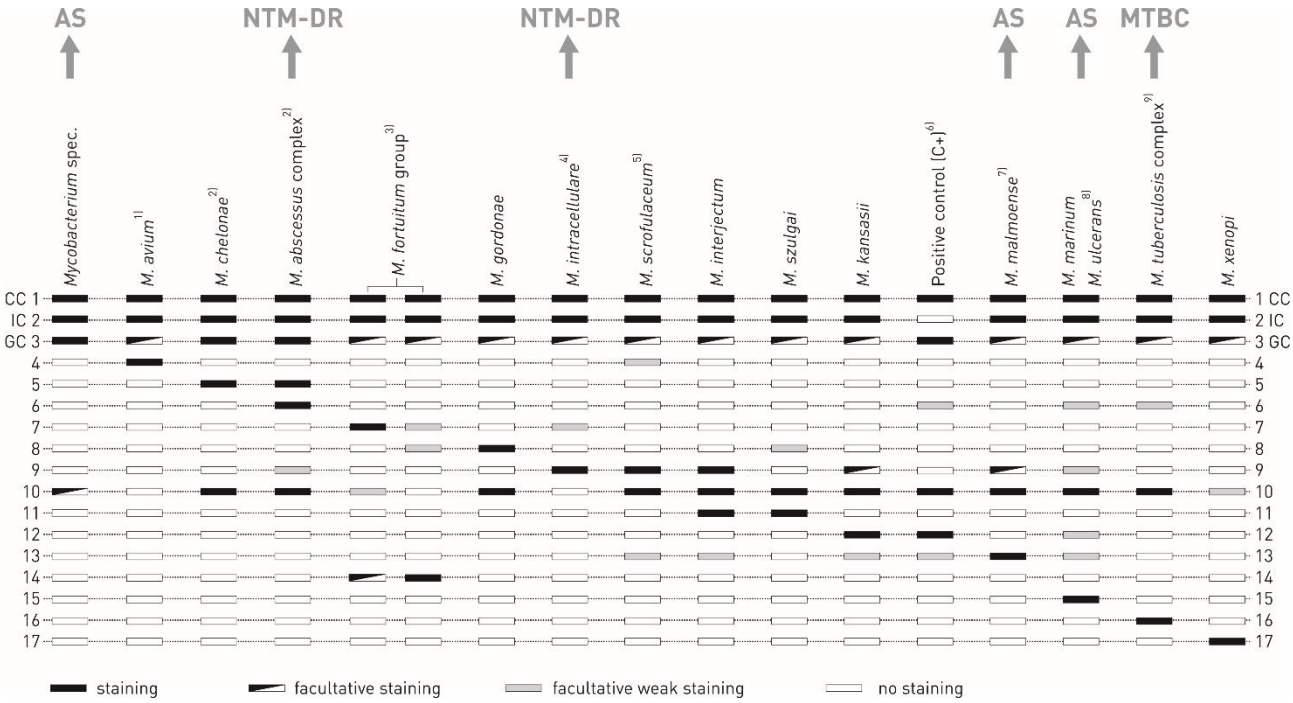
Genus Control (GC)
Staining of this zone documents the presence of a member of the genus *Mycobacterium*. The intensity of this band varies depending on the mycobacterial species.
When a species-specific banding pattern has developed, the GC band may be weak or even drop out completely due to competition of the single reactions during amplification. The test result, however, is to be assessed as valid.

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).

Additional mycobacterial species can be identified with the **GenoType Mycobacterium AS** kit (see Interpretation Chart).
An amplicon generated with the **GenoType Mycobacterium CM** VER 2.0 can directly be hybridized to **GenoType Mycobacterium AS** strips.

Interpretation Chart



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

AS: **GenoType Mycobacterium AS**
MTBC: **GenoType MTBC**
NTM-DR: **GenoType NTM-DR**

1) Does not include other species of the *M. avium* complex.
2) *M. immunogenum* shows the same banding pattern as *M. chelonae* or the *M. abscessus* complex.
Members of the *M. abscessus* complex can be differentiated with the **GenoType NTM-DR** kit.
3) Of the *M. fortuitum group*, only the following members have been tested: *M. fortuitum, M. peregrinum, M. alvei*, and *M. septicum.* It has not been tested whether other members of the *M. fortuitum* group also show this banding pattern.
M. fortuitum shows the banding pattern as depicted in the left column.
In most cases, *M. peregrinum* shows the banding pattern as depicted in the right column. In rare cases however, *M. peregrinum* may show the banding pattern as depicted in the left column.
M. alvei and *M. septicum* show the banding pattern as depicted in the right column.

Species not belonging to the *M. fortuitum* group:
M. mageritense shows the banding pattern as depicted in the left column without band 14.
Rare *M. 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.
4) *M. marseillense* and *M. 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.
5) *M. paraffinicum* and *M. parascrofulaceum* show the same banding pattern as *M. scrofulaceum*.
6) The positive control (C+) shows an *M. kansasii* banding pattern without IC band.
7) *M haemophilum*, *M. palustre*, and *M. nebraskense* show the same banding pattern as *M. malmoense*.
M. haemophilum/*M. nebraskense* can be identified with the **GenoType Mycobacterium AS** kit.
8) *M. ulcerans* can be identified with the **GenoType Mycobacterium AS** kit.
9) Members of the *M. tuberculosis* complex can be differentiated with the **GenoType MTBC** kit.

Important notes for evaluation
M. chelonae and the *M. abscessus* complex cannot be differentiated if the amplicon hybridizing to both the Genus Control and to band 6 is suppressed due to competition of the single reactions during amplification. Therefore, a developed GC band is required for identification of *M. chelonae* and of the *M. abscessus* complex.

M. scrofulaceum and *M. malmoense* can only 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*. 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*.

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

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.

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 Mycobacterium CM** 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.

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 with the **GenoLyse®** kit for DNA extraction from cultured material. The performance of the test has not been validated with other DNA extraction methods or sample materials.

Troubleshooting

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

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

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

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

Unexpected result
- Wrong incubation temperature.
- Hybridization Buffer and/or Stringent Wash Solution were not properly prewarmed or mixed.
- Contamination of neighboring wells by spillage during addition of Hybridization Buffer.
  Repeat reverse hybridization.
- Contamination of extracted DNA with previously extracted or amplified DNA. Repeat extraction.
- Contamination of amplification reagents. In this case, a negative control sample shows additional bands besides CC and IC. 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.
- Error during DNA extraction. Repeat extraction.
- IC and C+ interchanged. In this case, the negative control and negative samples show an *M. kansasii* banding pattern without IC band and the positive control (if included) does not show the *M. kansasii* banding pattern, but only bands CC and IC. The banding pattern of positive samples is mostly not interpretable. Repeat extraction.

Ordering Information

	Order no.
GenoType Mycobacterium CM VER 2.0 (kit for analysis of 12 samples)	299A
GenoType Mycobacterium CM VER 2.0 (kit for analysis of 96 samples)	29996A
GenoLyse® (kit for manual DNA extraction of 12 samples)	51612
GenoLyse® (kit for manual DNA extraction of 96 samples)	51610
GenoType Mycobacterium AS (kit for analysis of 12 samples)	298
GenoType Mycobacterium AS (kit for analysis of 96 samples)	29896
GenoType MTBC (kit for analysis of 12 samples)	301
GenoType MTBC (kit for analysis of 96 samples)	30196
GenoType NTM-DR (kit for analysis of 12 samples)	29712
GenoType NTM-DR (kit for analysis of 96 samples)	29796

Performance Characteristics

For the performance evaluation of the **GenoType Mycobacterium CM** VER 2.0, the test was carried out according to the instructions on hand.

Diagnostic performance

Diagnostic performance characteristics of the **GenoType Mycobacterium CM** VER 2.0 were determined with 114 cultivated samples.
The **GenoType Mycobacterium CM** VER 2.0 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).
DNA extraction was performed with the **GenoLyse®** kit according to the instructions for use.

Test results were rated true positive if the result of the **GenoType Mycobacterium CM** VER 2.0 was in agreement with that obtained with culture/**GenoType Mycobacterium CM** VER 1.0.

Table 1: Performance characteristics of the **GenoType Mycobacterium CM** VER 2.0 for detection of mycobacteria from cultured material compared to culture/**GenoType Mycobacterium CM** VER 1.0 (GT Myco CM V1)

		Culture/GT Myco CM V1		
		Positive	Negative	Diagnostic sensitivity: 100%
				Diagnostic specificity: 100%
GenoType Mycobacterium CM VER 2.0	Positive	105[1)]	0	Positive predictive value: 100%
	Negative	0	9	Negative predictive value: 100%

[1)] The 105 samples were identified as 7x *M. abscessus* complex, 15x *M. avium*, 6x *M. fortuitum* group *(M. fortuitum)*, 8x *M. gordonae*, 16x *M. intracellulare*, 6x *M. kansasii*, 6x *M. malmoense*, 5x *M. marinum/M. ulcerans*, 2x *M. scrofulaceum*, 1x *M. tuberculosis* complex, 5x *M. xenopi*, 5x *M. chelonae*, and 23x *Mycobacterium* spec.

Analytical performance

Analytical specificity

The specificity of the **GenoType Mycobacterium CM** VER 2.0 is ensured by the accurate design of specific primers and probes which considers, among others, homology comparisons of the sequences published in gene databases, and by stringent reaction conditions.
The analytical specificity of the **GenoType Mycobacterium CM** VER 2.0 was determined with strains of all mycobacteria identifiable by this test, as well as with strains of mycobacterial and non-mycobacterial species that are not detectable with the test system.

All mycobacteria identifiable with 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 Mycobacterium CM** VER 2.0 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 identifiable with the **GenoType Mycobacterium CM** VER 2.0 were tested:

M. avium	*M. szulgai*	*M. abscessus* complex	*M. tuberculosis* complex
M. chelonae	*M. kansasii*	*(M. abscessus* subsp. *abscessus,*	*(M. tuberculosis*,
M. immunogenum	*M. malmoense*	*M. abscessus* subsp. *bolletii,*	*M. bovis* subsp. *bovis*,
M. mageritense	*M. haemophilum*	*M. abscessus* subsp. *massiliense)*	*M. bovis* subsp. *caprae*,
M. gordonae	*M. marseillense*		*M. bovis* BCG,
M. intracellulare	*M. palustre*	*M. fortuitum* group	*M. africanum*,
M. chimaera	*M. nebraskense*	*(M. fortuitum,*	*M. microti*,
M. scrofulaceum	*M. marinum*	*M. peregrinum,*	*M. canettii,*
M. parascrofulaceum	*M. ulcerans*	*M. alvei,*	*M. pinnipedii)*
M. paraffinicum	*M. xenopi*	*M. septicum)*	
M. interjectum			

Tested mycobacterial species that are not identifiable with the **GenoType Mycobacterium CM** VER 2.0:

M. asiaticum	*M. goodii*	*M. mucogenicum*	*M. simiae*
M. celatum	*M. heckeshornense*	*M. phlei*	*M. smegmatis*
M. gastri	*M. intermedium*	*M. shimoidei*	*M. triplex*
M. genavense	*M. lentiflavum*		

Tested non-mycobacterial species that are not identifiable with the **GenoType Mycobacterium CM** VER 2.0:

Bordetella pertussis	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Streptococcus pneumoniae*
Corynebacterium ulcerans	*Nocardia amarae*	*Rhodococcus erythropolis*	*Streptomyces somaliensis*
Corynebacterium xerosis	*Nocardia asteroides*	*Rhodococcus rhodochrous*	*Tsukamurella inchonensis*
Escherichia coli	*Nocardia farcinica*	*Rhodococcus ruber*	*Tsukamurella paurometabola*
Haemophilus influenzae	*Nocardia otidiscaviarum*	*Staphylococcus aureus*	*Tsukamurella pulmonis*

Analytical sensitivity (limit of detection, LOD)

For determination of analytical sensitivity of the **GenoType Mycobacterium CM** VER 2.0 for cultured material, four BCG culture dilutions (1.65x 10^6, 1.65x 10^5, 1.65x 10^4 and 1.65x 10^3 CFU/ml) were set up in triplicate. Including a negative control, DNA was extracted using the **GenoLyse®** kit and analyzed with the **GenoType Mycobacterium CM** VER 2.0 applying the "MDR CUL" PCR protocol. The limit of detection was 1.65x 10^5 CFU/ml.

Reproducibility

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

- BCG culture dilution above the limit of detection
- BCG culture dilution at the limit of detection
- *Bordetella pertussis*-positive DNA sample
- Negative control

DNA was extracted using the **GenoLyse®** kit and the isolates were analyzed with the **GenoType Mycobacterium CM** VER 2.0 applying the "MDR CUL" PCR protocol.

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 in 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 culture medium. In order to assess if the medium influences the **GenoType Mycobacterium CM** VER 2.0, six different *M. tuberculosis* complex strains were cultured in four different media (solid media: Loewenstein-Jensen, Stonebrink, and Middlebrook-7H10, liquid medium: MGIT (BD Diagnostics, Franklin Lakes, USA)). Then the DNA was extracted using the **GenoLyse®** kit and the isolates were analyzed with the **GenoType Mycobacterium CM** VER 2.0 applying the "MDR CUL" PCR protocol.
All samples showed the correct results with all tested media. Hence, it can be excluded that the tested media import inhibitors into the **GenoType Mycobacterium CM** VER 2.0 test.

Stability

Shelf life of the **GenoType Mycobacterium CM** VER 2.0 when stored as recommended: see box label.
Stability is determined according to DIN EN ISO 23640.

References

1. World Health Organization. Global tuberculosis report 2020. Licence: CC BY-NC-SA 3.0 IGO. World Health Organization, Geneva, Switzerland 2020.
2. Falkinham JO 3rd. Epidemiology of infection by nontuberculous mycobacteria. *Clin Microbiol Rev* 1996; 9: 177-215.
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).

Important Changes in IFU-299A-03

Chapter	Change
Reagents and Instruments	The former chapters "Kit Contents", "Storage and Disposal of Kit Constituents", "Precautions for Handling Kit Constituents", and "Material Required but not Included in the Kit" are now subchapters of the new heading "Reagents and Instruments".
Precautions for Handling Kit Constituents	The hazard labeling now refers to safety data sheets. For additional information on the hazardous substances included in the kit, please refer to the safety data sheets which can be downloaded from: www.hain-lifescience.com/products/msds.html
Sample Requirements	The former chapter "Specimen Requirements" was renamed "Sample Requirements". **New**: "All culture samples that may contain mycobacteria should be handled applying Biosafety Level 2 practices or, when indicated, Biosafety Level 3 practices (e.g. see [3]). Observe all federal, state, and local safety regulations." **New**: "Transport, storage and preparation of patient specimens and culture samples should be carried out according to local, national and/or international guidelines and standards of the laboratory."

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