Konformitätserklärung / Déclaration de Conformité / Declaration of Conformity / Dichiarazione di Conformità / Declaración de Conformidad

Wir / Nous / We / Noi / Nosotros

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

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erklären in alleiniger Verantwortung, dass
déclarons sous notre propre responsabilité que
declare on our own responsibility that
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das Medizinprodukt
le dispositif médical
the medical device
il dispositivo medico
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Name / nome / name / nome / nombre
Type / type ou modèle / type or model / tipo o modello / tipo o modelo
Artikelnummer (Anzahl der Teste) / numéro d'article (nombre de tests) / order no. (number of tests) / numero dell' articolo (numero del test) / número de artículo (número de tests)

GenoType CM*direct*
VER 1.0

295 (12)
29596 (96)

allen anwendbaren Anforderungen der Richtlinie 98/79/EG entspricht.
remplit toutes les exigences applicables de la Directive 98/79/CE.
meets all the applicable provisions of the Directive 98/79/EC.
adempie a tutte le applicabile esigenze della Direttiva 98/79/CE.
cumple todas las exigencias aplicables de la Directiva 98/79/CE.

Angewandte harmonisierte Normen:
Normes harmonisées appliquées:
Applied harmonized standards:
Norme armonizzate applicate:
Normativas armonizadas aplicables:

DIN EN ISO 13485, DIN EN ISO 14971, DIN EN ISO 15223-1, DIN EN ISO 18113-1, DIN EN ISO 18113-2, DIN EN ISO 23640, DIN EN 13612, DIN EN 13641

Angewandte nationale Normen:
Normes nationales appliquées:
Applied nationalized standards:
Norme nazionali applicate:
Normas nacionales aplicables:

Andere normative Dokumente:
Autres documents normatifs:
Other normative documents:
Altri documenti normativi:
Otras normas y estándares:

Benannte Stelle (falls zutreffend):
Organisme notifié (le cas échéant):
Notified body (if applicable):
Organo notificato (se il caso):
Organismo notificado (si es aplicable):

Konformitätsbewertungsverfahren:
Procédure d'évaluation de la conformité:
Conformity assessment procedure:
Procedimento d'evaluazione della conformita:
Procedimiento de evaluación de la conformidad:

Council Directive 98/79/EC Annex III

gültig bis / valide jusqu'à / valid until / valido fino a / valido hasta:

May 26, 2026

Nehren, May 23, 2022

Ort, Datum / lieu, date / place, date / luogo, data / lugar, fecha

Unterschrift Qualitätsmanager / signature manager de la qualité / signature quality management / firma responsabile qualità / firma gerente de calidad

GenoType CM*direct*

VER 1.0

Instructions for Use

IFU-295-01

CE

IVD for in vitro diagnostic use only

2017-09-21

HAIN LIFESCIENCE

GenoType **CM*direct*** 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

The **Geno**Type **CM*direct*** VER 1.0 is a qualitative in vitro test for identification of the *Mycobacterium tuberculosis* complex as well as the following nontuberculous mycobacteria from clinical specimens: *Mycobacterium avium, Mycobacterium chelonae, Mycobacterium abscessus* complex, *Mycobacterium fortuitum* group, *Mycobacterium gordonae, Mycobacterium intracellulare, Mycobacterium scrofulaceum/M. intracellulare, Mycobacterium szulgai, Mycobacterium interjectum, Mycobacterium kansasii, Mycobacterium malmoense, Mycobacterium marinum/Mycobacterium ulcerans,* and *Mycobacterium 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 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* BCG, *Mycobacterium canettii, 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 **Geno**Type **CM*direct*** 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 **Geno**Type **CM*direct*** 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) 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.	295	29596
Tests	**12**	**96**
Kit Component 1 of 2 (store at 2°C to 8°C)		
Membrane strips (CMdirect VER 1.0 STRIPS) coated with specific probes	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 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.

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]).

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 polynucleotides and shows an *M. kansasii* banding pattern without IC 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 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.

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 CM*direct*** 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 with the respective preinstallation, select protocol "MDR DIR".

15 min	95°C	1 cycle
30 sec	95°C	20 cycles
2 min	65°C	
25 sec	95°C	30 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**.

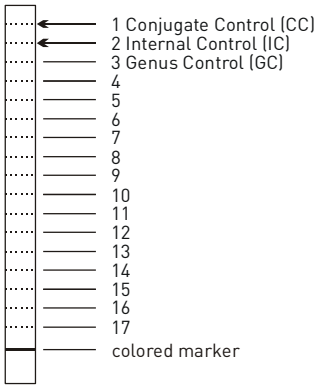
Preparation
Prewarm shaking water bath to **45°C** (the maximum tolerated deviation from the target temperature is +/–1°C) or switch on **TwinCubator**. Prewarm solutions HYB and STR to 37°C to 45°C before use. The reagents must be free from precipitates (note, however, that solution CON-D is opaque). 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.
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.

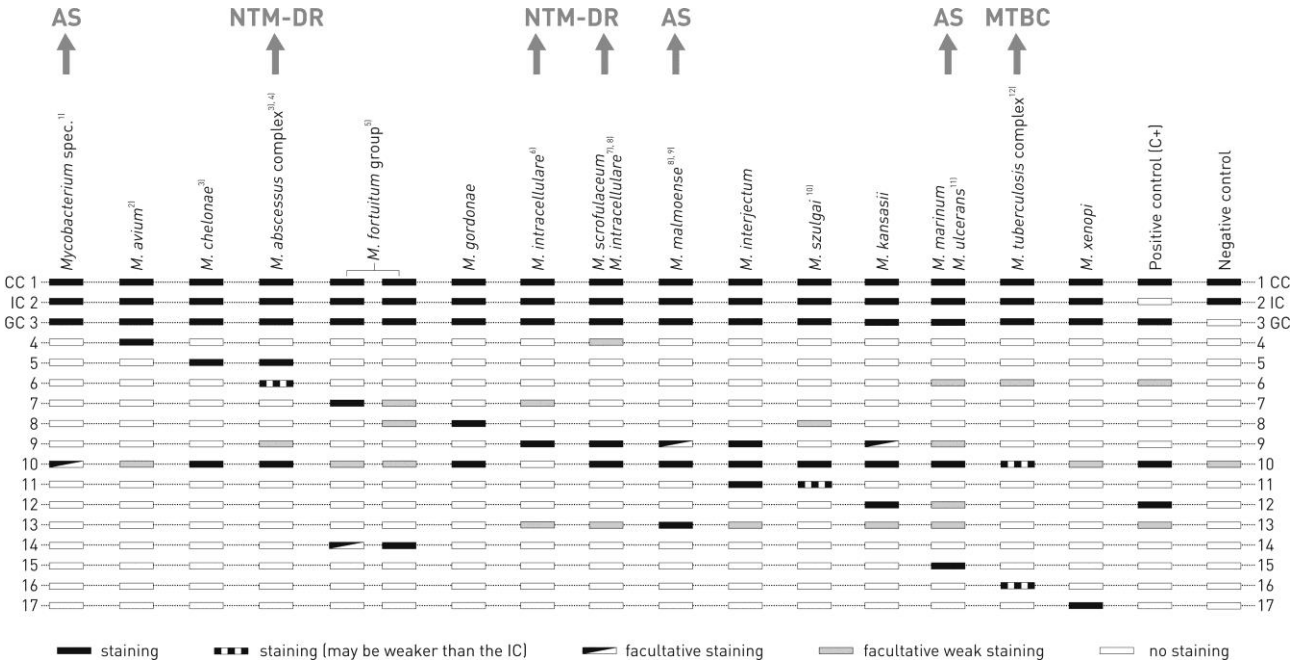
Genus Control (GC)
Staining of this zone documents the presence of a member of the genus *Mycobacterium*. The intensity of this band varies depending on the mycobacterial species. A species-specific banding pattern can only be interpreted, if the GC band is developed.

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



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

AS: **GenoType Mycobacterium AS** (from cultured material)
MTBC: **GenoType MTBC** (from cultured material)
NTM-DR: **GenoType NTM-DR** (from cultured material)

1) Mycobacterial species may be identified with the **GenoType Mycobacterium AS** kit from cultured material.
2) Does not include other species of the *Mycobacterium avium* complex.
3) *Mycobacterium immunogenum* shows the same banding pattern as *M. chelonae* or the *M. abscessus* complex.
4) For the *M. abscessus* complex, the intensity of band 6 may be weaker than that of the IC band.
Members of the *M. abscessus* complex can be differentiated with the **GenoType NTM-DR** kit from cultured material.
5) Of the *M. fortuitum group*, only the following members have been tested: *Mycobacterium fortuitum, Mycobacterium peregrinum, Mycobacterium alvei*, and *Mycobacterium 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:
Mycobacterium mageritense shows the banding pattern as depicted in the left 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.
6) *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.
7) *Mycobacterium paraffinicum* and *Mycobacterium parascrofulaceum* show the same banding pattern as *M. scrofulaceum/M. intracellulare*.
M. intracellulare can be identified with the **GenoType NTM-DR** kit from cultured material.
8) *M. scrofulaceum/M. intracellulare* 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/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*.
9) *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.
10) For *M. szulgai*, the intensity of band 11 may be weaker than that of the IC band.
11) *M. ulcerans* can be identified with the **GenoType Mycobacterium AS** kit from cultured material.
12) 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 CM*direct*** 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 exceeded 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 CM*direct*** VER 1.0 may not be used for monitoring the progression or success of treatment of patients with antimicrobial therapy.
The **GenoType CM*direct*** 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

	Order no.
GenoType CM*direct* VER 1.0 (kit for analysis of 12 samples)	295
GenoType CM*direct* VER 1.0 (kit for analysis of 96 samples)	29596
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 CM*direct*** VER 1.0 the test was carried out according to the instructions on hand.

Diagnostic performance

Diagnostic performance characteristics of the **GenoType CM*direct*** VER 1.0 were determined in two studies.

The first study comprised 131 sputum samples.
The **GenoType CM*direct*** 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 MTBDR*plus*** 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 CM*direct*** 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 MTBDR*plus*** 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 CM*direct*** VER 1.0 was confirmed by sequencing (for identification of the other identifiable mycobacterial species).

One of the 131 samples (identified as *M. intracellulare* with culture/**GenoType Mycobacterium CM** VER 1.0) showed a not interpretable banding pattern when analyzed with the **GenoType CM*direct*** VER 1.0. This sample was excluded from evaluation.

Table 1: Results of the **GenoType CM*direct*** 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 MTBDR*plus*** VER 2.0 or sequencing)

			Culture/GT Myco CM V1		
			Positive	Negative	Sens: 100%
					Spec: /*
smear-positive	**GenoType CM*direct*** VER 1.0	Positive	24[1]	0	PPV: 100%
		Negative	0	0	NPV: /*

			Culture/GT Myco CM V1				Culture/GT Myco CM V1 +reference methods		
			Positive	Negative	Sens: 63.6%		Positive	Negative	Sens: 75.0%
					Spec: 93.7%				Spec: 98.9%
smear-negative	**GenoType CM*direct*** VER 1.0	Positive	7[2]	6[4]	PPV: 53.8%	Positive	12	1	PPV: 92.3%
		Negative	4[3]	89	NPV: 95.7%	Negative	4[3]	89	NPV: 95.7%

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

* no value because no negative samples present

[1] These 24 samples were identified as 1x *M. abscessus* complex, 5x *M. avium*, 1x *M. kansasii*, and 17x *M. tuberculosis* complex.

[2] These 7 samples were identified as 2x *M. avium*, 1x *M. gordonae*, and 4x *M. tuberculosis* complex.

[3] In 3 out of 4 samples (identified as 1x *M. avium*, 1x *M. malmoense*, and 1x *M. tuberculosis* complex with culture/GT Myco CM V1), the bacterial concentration was below the detection limit of the **GenoType CM*direct*** VER 1.0.
1 out of 4 samples (identified as *M. kansasii* with culture/GT Myco CM V1) was identified as *M. gordonae* with the **GenoType CM*direct*** VER 1.0. When the cultivated sample was re-tested with the **GenoType CM*direct*** VER 1.0, it was also identified as *M. kansasii*; hence, a likely explanation for this discrepancy is a mixed infection.

[4] 5 out of the 6 culture-negative samples showed a positive result when analyzed with the **GenoType MTBDR*plus*** VER 2.0 as reference method and were identified as *M. tuberculosis* complex with the **GenoType CM*direct*** VER 1.0 as well as with the reference method.

The second study comprised 178 sputum samples.
The **GenoType CM*direct*** 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 CM*direct*** 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 CM*direct*** 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

			Culture/GT Myco CM V1		
			Positive	Negative	Sens: 100%
smear-positive	**GenoType CM*direct*** VER 1.0	Positive	14[1)]	0	Spec: /*
		Negative	0	0	PPV: 100%
					NPV: /*

			Culture/GT Myco CM V1			Culture/GT Myco CM V1 + clinical data		
			Positive	Negative	Sens: 54.5%	Positive	Negative	Sens: 64.3%
					Spec: 98.0%			Spez: 100%
smear-negative	**GenoType CM*direct*** VER 1.0	Positive	6[2)]	3[4)]	PPV: 66.7%	9	0	PPV: 100%
		Negative	5[3)]	150	NPV: 96.8%	5[3)]	150	NPV: 96.8%

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

* no value because no negative samples present

1) These 14 samples were identified as 1x *M. abscessus* complex and 13x *M. tuberculosis* complex.

2) These 6 samples were identified as 2x *M. abscessus* complex, 1x *M. chelonae*, and 3x *M. tuberculosis* complex.

3) 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 CM*direct*** VER 1.0.
1 out of 5 samples (identified as *M. tuberculosis* complex with culture/GT Myco CM V1) was identified as *Mycobacterium* spec. with the **GenoType CM*direct*** VER 1.0.

4) The 3 culture-negative samples were identified as 2x *M. avium* and 1x *Mycobacterium* spec. with the **GenoType CM*direct*** 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 CM*direct*** 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 CM*direct*** 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 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 CM*direct*** 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 CM*direct*** VER 1.0 were tested:

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

Tested mycobacterial species that are not identifiable with the **GenoType CM*direct*** 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 CM*direct*** VER 1.0:

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 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®** kit and the isolates were analyzed with the **GenoType CM*direct*** 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 CM*direct*** VER 1.0.

Table 3: LOD for mycobacteria detectable with the **GenoType CM*direct*** VER 1.0

Tested mycobacteria	LOD[1)]
M. bovis BCG (*M. tuberculosis* complex)	1,500 CFU/ml
M. abscessus (*M. abscessus* complex)	1,500 CFU/ml
M. avium	15,000 CFU/ml
M. chelonae	1,500 CFU/ml
M. fortuitum (*M. fortuitum*-group)	750 CFU/ml
M. gordonae	15,000 CFU/ml
M. interjectum	15,000 CFU/ml
M. intracellulare	50,000 CFU/ml
M. kansasii	15,000 CFU/ml
M. malmoense	7,500 CFU/ml
M. marinum	7,500 CFU/ml
M. peregrinum (*M. fortuitum* group)	1,500 CFU/ml
M. scrofulaceum	15,000 CFU/ml
M. szulgai	15,000 CFU/ml
M. xenopi	7,500 CFU/ml
M. ulcerans	50 DNA copies/PCR

[1)] 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®** kit and the isolates were analyzed with the **GenoType CM*direct*** 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 CM*direct*** 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®** kit and the isolates were analyzed with the **GenoType CM*direct*** VER 1.0.

Table 4: Tested potential interferents of the **GenoType CM*direct*** VER 1.0.

Substance/class	Description/active ingredient	Substance concentrations
Blood	Whole blood	2.5% v/v to 90% v/v
Blood	Hemoglobin	1% v/v to 13.5% v/v
Pus		2.5% v/v to 90% v/v

Interference of the **GenoType CM*direct*** VER 1.0 was observed in samples containing concentrations greater than 25% whole blood, 10% hemoglobin, and 10% 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

1. World Health Organization. Global tuberculosis report 2016. WHO/HTM/TB/2016.13. World Health Organization, Geneva, Switzerland 2016.
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).
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.

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Christoph Dicks
Head of Certification/Notified Body

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