GenoType MTBC

VER 1.X

Instructions for Use

IFU-301-10

CE

IVD for in vitro diagnostic use only

06/2015

HAIN LIFESCIENCE

GenoType MTBC
Molecular Genetic Assay for Differentiation of the *Mycobacterium tuberculosis* Complex 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 MTBC** test is a qualitative in vitro test from cultured material for the differentiation of the following species/strains belonging to the *Mycobacterium tuberculosis* complex (MTBC): *M. tuberculosis/M. canettii, M. africanum, M. microti*, *M. bovis* subsp. *bovis, M. bovis* subsp. *caprae*, and *M. bovis* BCG.
The test is indicated as an aid for diagnosis and intended for use in medical laboratories.

Summary and Explanation

Tuberculosis (TB) is a bacterial infectious disease passed on by droplet infection. In 2013, there were an estimated 9.0 million incident cases of TB globally, and an estimated 1.5 million deaths occurred [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, including the so-called nontuberculous mycobacteria and the TB-causing MTBC with the species *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti*, and *M. canettii*. The species *M. bovis* comprises the pyrazinamide-resistant subspecies *M. bovis* subsp. *bovis*, the pyrazinamide-susceptible subspecies *M. bovis* subsp. *caprae*, and the nontuberculous vaccine strain *M. bovis* BCG. The **GenoType MTBC** permits the rapid and reliable differentiation of the members of the *M. tuberculosis* complex and therefore the fast application of specific treatment measures.

Principles of the Procedure

The **GenoType MTBC** test 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.

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 2-8°C. Store all constituents from Kit Component 2 at –20°C and keep strictly separated from contaminating DNA. Avoid repeated freezing and thawing of AM-A and AM-B, when processing only small sample numbers per run, aliquot AM-A and AM-B. 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

Quality Control

In order to control the correct performance of the test and the proper functioning of kit constituents, each strip includes 2 control zones:
- a Conjugate Control zone (CC) to check the binding of the conjugate on the strip and a correct chromogenic reaction
- a Universal Control zone (UC) which detects, as far as is known, all mycobacteria and members of the group of gram-positive bacteria with a high G+C content

Observe the usual precautions for amplification setup. It is essential that all materials (such as pipette tips) coming in contact with the reagents are free from DNases.
Do not interchange or pool Amplification Mixes or membrane strips from different kits unless the lots are identical.
A negative control sample for detection of possible contamination events containing water (molecular biology grade) instead of DNA should be part of each test run; the respective test strip should show the CC band only.

Specimen Requirements

Bacteria grown on solid medium or in liquid medium may be used as starting material for DNA extraction. The test must not be used for detection directly from patient specimens.

Precautions for handling specimens

Patient specimens and cultures made from patient specimens must always be considered as potentially infectious and must be handled accordingly (e.g. see [2] or [3]). Always wear suitable protective clothing and gloves. Samples from patients at risk (infected by pathogenic microorganisms 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.
Handling of potentially infectious specimens must be carried out in a class II safety cabinet. Potentially infectious samples 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" [4], the "Clinical Microbiology Procedures Handbook" [5], 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 2-8°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. The specimens used for decontamination must not be older than 4 days.

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" [4]. After decontamination, the cell pellet should be resuspended in a maximum of 1 to 1.5 ml of phosphate buffer. Cultivation can be performed either on solid medium (e.g. Loewenstein-Jensen, Middlebrook) or in liquid medium (e.g. MGIT (BD Diagnostics, Franklin Lakes, USA)). Handling of potentially infectious specimens must be carried out in a class II safety cabinet.

DNA Extraction

Bacteria grown on solid medium (e.g. Loewenstein-Jensen, Middlebrook) or in liquid medium (e.g. MGIT (BD Diagnostics, Franklin Lakes, USA)) may be used as starting material for DNA extraction. The working area must be free from contaminating DNA.
The following quick protocol can be used for DNA extraction from cultured material:

1a. When using bacteria grown on solid medium, collect bacteria with an inoculation loop and suspend in approximately 300 µl of water (molecular biology grade).
1b. When using bacteria grown in liquid medium, directly apply 1 ml. Pellet bacteria by spinning for 15 min in a standard table top centrifuge with an aerosol-tight rotor or in a class II safety cabinet at approximately 10,000 x g. Discard supernatant and resuspend bacteria in 100-300 µl of water (molecular biology grade) by vortexing.
2. Incubate bacteria from 1a or 1b for 20 min at 95°C in a water bath.
3. Incubate for 15 min in an ultrasonic bath.
4. Spin down for 5 min at full speed and directly use 5 µl of the supernatant for PCR. In case DNA solution is to be stored for an extended time period, transfer supernatant to a new tube.

The assay on hand was also validated with the **GenoLyse®** kit (see chapter Ordering Information) which can alternatively be used for DNA extraction. For handling instructions, please refer to the instructions for use of the **GenoLyse®** kit.

The methods described above were used for performance evaluation of the **GenoType MTBC**. 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.

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. After thawing, spin down AM-A and AM-B briefly and mix carefully by pipetting up and down. Pipette AM-A and AM-B only in a room free from contaminating DNA. The DNA solution should 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 0.68 ml master mix for 12 amplification reactions (12 tests kit) or, respectively, 4x 1.35 ml for 4x 24 amplification reactions (96 tests kit). Please note that the master mix needs to be prepared freshly each time. Aliquot 45 µl into each of the prepared PCR tubes and add 5 µl water (molecular biology grade) to one aliquot (negative control). In a separate working area, add 5 µl DNA solution to each aliquot (except for negative control).

Amplification profile:
When using a thermal cycler from Hain Lifescience with the respective preinstallation, select protocol "HOT 30".

15 min	95°C	1 cycle
30 sec	95°C	10 cycles
2 min	58°C	
25 sec	95°C	20 cycles
40 sec	53°C	
40 sec	70°C	
8 min	70°C	1 cycle
Heating rate		≤2.2°C/sec

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

Hybridization

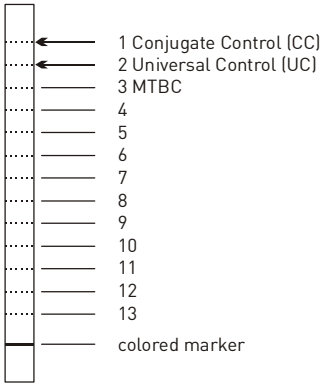
When using a hybridization instrument from Hain Lifescience, please refer to the document "Overview equipment programs" available on www.hain-lifescience.com for the name of the hybridization protocol to be used.
The following protocol describes the manual hybridization using a water bath or a **TwinCubator**.

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 37-45°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 UC 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 UC of the strip as well. Each strip has a total of 13 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.

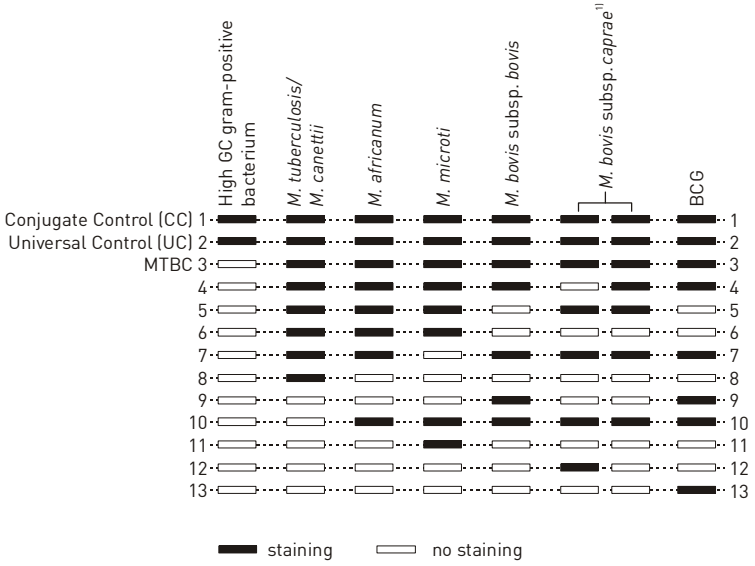
Universal Control (UC)
This zone detects, as far as is known, all mycobacteria and members of the group of gram-positive bacteria with a high G+C content. If this zone and the Conjugate Control zone stain positive but the remaining banding pattern cannot be assigned to a specific mycobacterium, additional methods have to be applied to identify the respective bacterial species.

MTBC
This zone hybridizes, as far as is known, with amplicons generated from all members of the *Mycobacterium tuberculosis* complex.

Other bands
Specific probes, for evaluation see interpretation chart.

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

Interpretation Chart



1) Approximately 5% of the subspecies *M. bovis caprae* exhibit a banding pattern according to the right column.

Limitations

Strictly adhere to the established protocols and procedures in order to obtain correct test results and to avoid contaminations.
Use of this assay is limited to qualified personnel well trained in the test procedure and familiar with molecular biological methods.

The test only works within the limits of the genomic regions the primers and probes were chosen from.
The presence of multiple bacterial species in the sample to be analyzed might hamper the interpretation of the test.
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. The test reflects the current state of knowledge of Hain Lifescience.

Performance evaluation of this assay was carried out using the methods for DNA extraction from cultured material which are described in chapter DNA Extraction. Until the present edition of the instructions on hand, the performance of the test has not been validated with other DNA extraction methods or sample materials.

The results of this test may only be interpreted in combination with additional laboratory and clinical data available to the responsible physician.

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) not mixed properly, interchanged, or added in wrong amounts. Prepare a new master mix and repeat amplification.
- Incubation temperature too high. Repeat reverse hybridization.
- The extracted bacterial species cannot be detected by the Universal Control. Use alternative identification method.

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. 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.

Material Required but not Included in the Kit

- Absorbent paper
- Adjustable pipettes for 10, 20, 200, and 1,000 µl
- Class II safety cabinet
- Disposable gloves
- Disposable sterile pipette tips with filter
- DNA extraction reagents for amplification use 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
- Table top centrifuge, if applicable with aerosol-tight rotor
- Thermal cycler
- Timer
- Tweezers
- Ultrasonic bath
- Water (distilled)
- Water (molecular biology grade)
- Water bath

Kit Contents

Order no. **Tests**	**301** **12**	**30196** **96**
Kit Component 1 of 2 (store at 2-8°C)		
Membrane strips coated with specific probes (MTBC STRIPS)	12	2x 48
Denaturation Solution (DEN) contains <2% NaOH, dye	0.3 ml	2x 1.2 ml
Hybridization Buffer (HYB) contains <10% anionic tenside, dye	20 ml	120 ml
Stringent Wash Solution (STR) contains >25% of a quaternary ammonium compound, <1% anionic tenside, dye	20 ml	120 ml
Rinse Solution (RIN) contains buffer, <1% NaCl, <1% anionic tenside	50 ml	3x 120 ml
Conjugate Concentrate (CON-C) contains streptavidin-conjugated alkaline phosphatase, dye	0.2 ml	1.2 ml
Conjugate Buffer (CON-D) contains buffer, 1% blocking reagent, <1% NaCl	20 ml	120 ml
Substrate Concentrate (SUB-C) contains <70% dimethyl sulfoxide, <10% 4-nitro blue tetrazolium chloride, <10% 5-bromo-4-chloro-3-indolyl phosphate	0.2 ml	1.2 ml
Substrate Buffer (SUB-D) contains buffer, <1% $MgCl_2$, <1% NaCl	20 ml	120 ml
Tray, evaluation sheet	1 of each	4 of each
Instructions for use, template	1 of each	1 of each
Kit Component 2 of 2 (store at –20°C)		
Amplification Mix A (AM-A GT MTBC) contains buffer, specific primers, nucleotides, Taq polymerase	0.15 ml	4x 0.3 ml
Amplification Mix B (AM-B GT MTBC) contains buffer, salts, dye	0.53 ml	4x 1.05 ml

Ordering Information

	Order no.
GenoType MTBC (kit for analysis of 12 samples)	301
GenoType MTBC (kit for analysis of 96 samples)	30196
GenoLyse® (kit for manual DNA extraction of 12 samples)	51612
GenoLyse® (kit for manual DNA extraction of 96 samples)	51610

Performance Characteristics

Diagnostic performance

DNA Extraction with quick method

Diagnostic performance characteristics were determined in a study with 82 clinical isolates grown on solid medium [6] and 77 clinical isolates grown in liquid medium [7]. DNA extraction was performed using the quick method described in chapter DNA Extraction. The **GenoType MTBC** was compared to various biochemical and molecular differentiation methods (e.g. determination of colony morphology, nitrate reduction on modified Dubos broth, niacin accumulation test, growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH), spoligotyping, and RD1-PCR).

In the first study, 82 clinical isolates grown on **solid medium** were analyzed [6]. For all samples, the **GenoType MTBC** results were in agreement with the results of the biochemical or molecular differentiation (see table 1).

Table 1: Results of the *M. tuberculosis* complex differentiation (bacteria grown on solid medium; DNA extraction with quick method)

Result **GenoType MTBC**	Number of isolates		Result conventional methods
M. tuberculosis	31	31	*M. tuberculosis*
M. africanum	4	4	*M. africanum*
M. bovis subsp. *bovis*	17	17	*M. bovis* subsp. *bovis*
M. bovis subsp. *caprae*	17	17	*M. bovis* subsp. *caprae*
M. bovis BCG	4	4	*M. bovis* BCG
M. microti	9	9	*M. microti*
Total	82	82	

Diagnostic sensitivity: 100%
Diagnostic specificity: 100%
Positive predictive value: 100%
Negative predictive value: 100%

In the second study, 77 clinical isolates grown in **liquid medium** were analyzed [7]. For all samples, the **GenoType MTBC** results were in agreement with the results of the biochemical or molecular differentiation (see table 2).

Table 2: Results of the *M. tuberculosis* complex differentiation (bacteria grown in liquid medium; DNA extraction with quick method)

Result **GenoType MTBC**	Number of isolates		Result conventional methods
M. tuberculosis	71	71	*M. tuberculosis*
M. africanum	1	1	*M. africanum*
M. bovis subsp. *bovis*	5	5	*M. bovis* subsp. *bovis*
Total	77	77	

Diagnostic sensitivity: 100%
Diagnostic specificity: 100%
Positive predictive value: 100%
Negative predictive value: 100%

The Primer Nucleotide Mix (PNM) has been replaced by new kit constituents, namely **Amplification Mixes** A and B (AM-A and AM-B). In order to check if the change of kit constituents impacts test results, 72 liquid and solid cultures were tested with both kit variants. DNA was extracted using the quick method described in chapter DNA Extraction. In addition, 2 negative controls (water instead of DNA added to the aliquoted master mix) were tested. The results were identical for all samples (see table 3).

Table 3: Results of the *M. tuberculosis* complex differentiation (Primer Nucleotide Mix compared to Amplification Mixes; DNA extraction with quick method)

Result **GenoType MTBC** with Primer Nucleotide Mix	Number of isolates		Result **GenoType MTBC** with Amplification Mixes
M. tuberculosis	60	60	*M. tuberculosis*
M. africanum	4	4	*M. africanum*
M. bovis subsp. *bovis*	2	2	*M. bovis* subsp. *bovis*
M. bovis subsp. *caprae*	1	1	*M. bovis* subsp. *caprae*
M. bovis BCG	2	2	*M. bovis* BCG
High GC gram-positive bacterium	3	3	High GC gram-positive bacterium
Negative	2	2	Negative
Total	74	74	

DNA Extraction with **GenoLyse®**

In a study comprising 52 *Mycobacterium*-positive cultures (growth on Loewenstein-Jensen medium or in MGIT medium), DNA was extracted with the **GenoLyse®** kit and then tested with the **GenoType MTBC**. For comparison, DNA was isolated with the quick method in parallel and then tested with the **GenoType MTBC**.

With both extraction methods, identical results were obtained (see table 4).

Table 4: Results of the *M. tuberculosis* complex differentiation (DNA extraction with quick method compared to DNA extraction with **GenoLyse®**)

Result after DNA extraction with quick method	Number of isolates		Result after DNA extraction with **GenoLyse®**
M. tuberculosis	47	47	*M. tuberculosis*
M. bovis BCG	2	2	*M. bovis* BCG
High GC gram-positive bacterium	3	3	High GC gram-positive bacterium
Total	52	52	

The Primer Nucleotide Mix (PNM) has been replaced by new kit constituents, namely **Amplification Mixes** A and B (AM-A and AM-B). In order to check if the change of kit constituents impacts test results, 92 solid cultures were tested with both kit variants. DNA was extracted using the **GenoLyse®** kit. In addition, 2 negative controls (water instead of DNA added to the aliquoted master mix) were tested. The results were identical for all samples (see table 5).

Table 5: Results of the *M. tuberculosis* complex differentiation (Primer Nucleotide Mix compared to Amplification Mixes; DNA extraction with **GenoLyse®**)

Result **GenoType MTBC** with Primer Nucleotide Mix	Number of isolates		Result **GenoType MTBC** with Amplification Mixes
M. tuberculosis	48	48	*M. tuberculosis*
M. bovis subsp. *bovis*	2	2	*M. bovis* subsp. *bovis*
M. bovis BCG	2	2	*M. bovis* BCG
High GC gram-positive bacterium	40	40	High GC gram-positive bacterium
Negative	2	2	Negative
Total	94	94	

Further performance studies of the **GenoType MTBC** were published [8,9,10].

Analytical performance

Analytical specificity

The specificity of the **GenoType MTBC** test is ensured by the accurate design of specific primers and probes which considers, among others, homology comparisons of the sequences published in gene databases, and by stringent reaction conditions.

The analytical specificity of the **GenoType MTBC** (with Primer Nucleotide Mix) was determined with 98 strains of 75 different species and subspecies. The study included the *Mycobacterium tuberculosis* complex strains *M. africanum*, *M. bovis* BCG (5x), *M. bovis* subsp. *bovis* (7x), *M. bovis* subsp. *caprae, M. microti,* and *M. tuberculosis* (2x) as well as the following strains not detectable with the test system: *Actinomyces naeslundii*, *Aggregatibacter actinomycetemcomitans, Bacillus cereus*, *Bacteroides forsythus*, *Corynebacterium ammoniagenes*, *C. bovis*, *C. durum*, *Escherichia coli*, *Enterococcus faecalis, Gordona rubropertinctus*, *Klebsiella oxytoca*, *M. abscessus*, *M. alvei* (2x), *M. asiaticum*, *M. avium, M. celatum, M. chelonae, M. duvalii, M. feldmannii, M. fortuitum* (2 sequevars), *M. gastri, M. genavense*, *M. goodii*, *M. gordonae, M. haemophilum*, *M. heckeshornense*, *M. immunogenum* (2x), *M. interjectum* (2x), *M. intermedium* (2x), *M. intracellulare* (2x), *M. kansasii, M. lentiflavum, M. mageritense*, *M. malmoense, M. marinum*, *M. mucogenicum*, *M. nonchromogenicum, M. palustre* (2x), *M. peregrinum, M. phlei, M. ratisbonense, M. scrofulaceum, M. shimoidei*, *M. simiae, M. smegmatis, M. szulgai, M. terrae, M. triplex* (2x), *M. ulcerans*, *M. vaccae, M. xenopi, MRSA*, *Nocardia abscessus*, *N. africana*, *N. amarae*, *N. asteroides*, *N. farcinica*, *Porphyromonas gingivalis, Prevotella intermedia, Rhodococcus equi* (3x), *R. erythropolis*, *Saccharomonospora glauca*, *Staphylococcus aureus, S. epidermidis, S. intermedius, Tannerella forsythia*, *Treponema denticola, Tsukamurella inchonensis*, *T. paurometabola* (3x).

The 17 *M. tuberculosis* complex strains were correctly identified. The further 81 isolates displayed no MTBC band and no species-specific banding pattern. Hence, the analytical specificity was 100%.

Reproducibility

Intra-assay precision

In order to determine the intra-assay precision of the **GenoType MTBC** (with Primer Nucleotide Mix), one *M. microti* strain, one *M. bovis* BCG strain, one *M. gordonae* strain, and one negative control were set up in five parallels and tested under identical conditions. DNA extraction was performed using the quick method described in chapter DNA Extraction. All strains showed the expected signals in all five parallels and the negative controls were negative. Hence, the intra-assay precision was 100%.

In order to determine the intra-assay precision of the **GenoType MTBC** (with Amplification Mixes), two *M. bovis* BCG strains, one *M. gordonae* strain, and one negative control were set up in four parallels and tested under identical conditions. DNA extraction was performed using the **GenoLyse®** kit. All strains showed the expected signals in all four parallels and the negative controls were negative. Hence, the intra-assay precision was 100%.

Inter-assay precision

In order to determine the inter-assay precision of the **GenoType MTBC** (with Primer Nucleotide Mix), one *M. microti* strain, one *M. bovis* BCG strain, one *M. gordonae* strain, and one negative control were set up in five parallels and tested at three different points in time. The other experimental conditions (instruments, lot numbers, operator, etc.) were identical. DNA extraction was performed using the quick method described in chapter DNA Extraction. All strains showed the expected signals in all five parallels and the negative controls were negative. Hence, the inter-assay precision was 100%.

In order to determine the inter-assay precision of the **GenoType MTBC** (with Amplification Mixes), two *M. bovis* BCG strains, one *M. gordonae* strain, and one negative control were tested at three different points in time. The other experimental conditions (instruments, lot numbers, operator, etc.) were identical. DNA extraction was performed using the **GenoLyse®** kit. All strains showed the expected signals and the negative controls were negative. Hence, the inter-assay precision was 100%.

Interfering substances

There are substances that may inhibit PCR reactions. Such inhibitors may, for example, originate from the culture medium. In order to assess if the medium influences the **GenoType MTBC**, 5 different *M. tuberculosis* samples and 5 different *M. bovis* subsp. *bovis* samples were cultured in 4 different media (solid media: Loewenstein-Jensen, Stonebrink, and Middlebrook-7H10, liquid medium: MGIT (BD Diagnostics, Franklin Lakes, USA)). Then the culture samples and 5 non-inoculated media samples were tested with the **GenoType MTBC** (with Primer Nucleotide Mix).

All *M. tuberculosis* samples and all *M. bovis* subsp. *bovis* samples showed the correct results and the non-inoculated controls were negative. Hence, it can be excluded that the tested media import inhibitors into the **GenoType MTBC** test.

Other inhibitors may be imported by reagents used for DNA extraction. When using the recommended DNA extraction methods (see chapter DNA Extraction) according to the respective instructions for use, however, the DNA solution does not contain inhibiting substances in relevant concentrations.

Stability

Shelf life of the **GenoType MTBC** 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 2014. WHO/HTM/TB/2014.08. World Health Organization, Geneva, Switzerland 2014.
2. 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.
3. 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).
4. 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.
5. Isenberg HD. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C., USA 1992.
6. Richter E, Weizenegger M, Rüsch-Gerdes S, Niemann S. Evaluation of GenoType MTBC assay for differentiation of clinical *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol* 2003; 41:2672-2675.
7. Richter E, Weizenegger M, Fahr AM, Rüsch-Gerdes S. Usefulness of the GenoType MTBC assay for differentiating species of the *Mycobacterium tuberculosis* complex in cultures obtained from clinical specimens. *J Clin Microbiol* 2004; 42:4303-4306.
8. Gomez MP, Herrera-León L, Jiménez MS, Rodríguez JG. Comparison of GenoType MTBC with RFLP-PCR and multiplex PCR to identify *Mycobacterium tuberculosis* complex species. *Eur J Clin Microbiol Infect Dis* 2007; 26:63-66.
9. Neonakis IK, Gitti Z, Petinaki E, Maraki S, Spandidos DA. Evaluation of the GenoType MTBC assay for differentiating 120 clinical *Mycobacterium tuberculosis* complex isolates. *Eur J Clin Microbiol Infect Dis* 2007; 26:151-152.
10. Somoskovi A, Dormandy J, Rivenburg J, Pedrosa M, McBride M, Salfinger M. Direct comparison of the GenoType MTBC and genomic deletion assays in terms of ability to distinguish between members of the *Mycobacterium tuberculosis* complex in clinical isolates and in clinical specimens. *J Clin Microbiol* 2008; 46:1854-1857.

Important Changes in IFU-301-10

Chapter	Change
Storage and Disposal of Kit Constituents	**New:** "Avoid repeated freezing and thawing of AM-A and AM-B; when processing only small sample numbers per run, aliquot AM-A and AM-B."
Precautions for Handling Kit Constituents	Classification of the kit reagents according to GHS criteria (Regulation (EC) No. 1272/2008)
Quality Control	**New:** "Do not interchange or pool Amplification Mixes or membrane strips from different kits unless the lots are identical."
Amplification	**New:** Heating rate for thermal cyclers ≤2.2°C/sec

CE IVD

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