



Fast detection of non-tuberculous mycobacteria in medical device-water samples: evaluation of the STANDARD™ M10 MTB/NTM cartridge

V. Ferraro^{a,b}, P. Caiazza^b, F. Sorella^{a,b}, F. Bisognin^{a,b,*}, C.M. Crovara Pesce^{a,b}, P. Dal Monte^{a,b}

^a Department of Medical and Surgical Sciences, Alma Mater Studiorum – University of Bologna, Bologna, Italy

^b Microbiology Unit, IRCCS Azienda Ospedaliero – Universitaria of Bologna, Bologna, Italy

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SUMMARY

Introduction: Non-tuberculous mycobacteria (NTM) are environmental pathogens increasingly being recognized as a source of healthcare-associated infections, particularly through contaminated medical water systems such as heater–cooler units and haemodialysis machines. The rising prevalence of NTM-related diseases underlines the urgent need for enhanced surveillance with more effective and rapid diagnostic tools. This study aimed to compare the performance of the STANDARD™ M10 MTB/NTM, a novel cartridge-based real-time PCR assay for detecting NTM in environmental water samples, with conventional mycobacteria growth indicator tube (MGIT) liquid culture.

Methods: In accordance with the Emilia-Romagna surveillance programme, water samples from medical devices were processed using standard culture methods for NTM detection. The remaining decontaminated samples were stored at -20 °C. For this study, 150 stored aliquots were selected and tested with STANDARD™ M10 MTB/NTM assay.

Findings: NTM were detected by culture in 37/150 (24.7%) samples, with *Mycobacterium chimaera* and *Mycobacterium saskatchewanense* being the most frequently isolated species. The STANDARD™ M10 MTB/NTM assay detected 32/37 culture-positive samples, and tested negative in all ($N = 113$) culture-negative samples, corresponding to a sensitivity of 86.5% (95% CI: 0.72–0.94) and a specificity of 100% (95% CI: 0.97–1.00), with a strong concordance ($\kappa = 0.90$).

Conclusions: Compared with culture, STANDARD™ M10 MTB/NTM assay proved to be a reliable and rapid tool for detecting NTM in environmental water samples, demonstrating high specificity and predictive value. Its implementation could support more timely and comprehensive environmental monitoring, helping to prevent the spread of these pathogens and reducing the risk of nosocomial infections.

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* Corresponding author. Address: Microbiology Unit, Department of Medical and Surgical Sciences, Alma Mater Studiorum – University of Bologna, Via Massarenti n. 9, 40138, Bologna, Italy.

E-mail address: francesco.bisognin@studio.unibo.it (F. Bisognin).

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Introduction

Non-tuberculous mycobacteria (NTM) are a large and varied group of acid-fast environmental bacteria, widely distributed in soil, dust, water and on inert surfaces. The lipid-enriched cell wall combined with the ability to form biofilms enhances their survival both in natural environments and in the presence of chemical disinfectants commonly used in water systems. Due to their resistance to disinfection processes, NTM have emerged as significant agents of waterborne opportunistic infections in healthcare facilities [1].

Several studies have identified NTM as a significant risk in clinical environments, particularly in the colonization and infection of medical devices such as central venous catheters, haemodialysis machines, heater-cooler unit (HCU) equipment, and hospital ice machines [2,3]. The most prevalent mycobacterial species involved are *Mycobacterium avium* complex (MAC), *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium fortuitum*.

Mycobacterium chimaera, a member of the MAC, has been associated with infections linked to HCUs used during cardiac surgery. These devices have been implicated in the aerosol transmission of *M. chimaera*, leading to postoperative infections in cardiac surgery patients [4,5]. *Mycobacterium saskatchewanense* was recently isolated from ultrapure dialysis fluid, in haemodialysis equipment [6,7].

Although traditionally considered opportunistic pathogens, the increasing incidence of NTM infections in healthcare facilities is particularly concerning for immune-compromised individuals, patients with underlying pulmonary conditions, and those undergoing haemodialysis or post-surgical recovery [8]. This trend highlights the urgent need for improved surveillance, more efficient diagnostic techniques, and the development of better therapeutic strategies.

In Italy, there are laws and regulations concerning the monitoring and safety of water in healthcare environments, although there is currently no specific regulation that exclusively governs the monitoring of water within medical devices. Legislative Decree 18/2023, which transposes EU Directive 2020/2184 on the quality of water intended for human consumption, establishes the microbiological quality standards for potable water. In addition, the technical document “Rapporti ISTISAN 22/32” highlights the importance of monitoring water distribution systems in healthcare settings by including supplementary parameters, based on site-specific risk assessment (e.g., *Pseudomonas aeruginosa* and *Legionella* spp.). Following the *M. chimaera* outbreaks that emerged globally from 2011 in patients undergoing cardiac surgery with exposure to contaminated HCUs, in 2015 the ECDC issued recommendations and technical protocols for the laboratory diagnosis and environmental investigation of *M. chimaera* [9], which were later adopted by the Italian Ministry of Health [10].

To date, the most widely used method to detect NTM from clinical and environmental samples is liquid culture, using BD BACTEC™ MGIT™ mycobacteria growth indicator tubes (MGIT tubes, Becton, Dickinson and Company, USA), preceded by

decontamination with N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) solution [11,12]. Time to culture positivity is highly variable and species-dependent, as NTM can be either rapid or slow-growing. For negative samples, incubation may last up to 42 days. Given the prolonged turnaround time of conventional culture methods, there is a pressing need for faster and more accurate diagnostic tools to improve NTM-detection.

STANDARD™ M10 MTB/NTM (SD Biosensor, Korea) is an in-vitro molecular diagnostic test that simultaneously detects the DNA from *Mycobacterium tuberculosis* complex (MTB, targeting the IS6110 region) and NTM (targeting the 16S–23S rRNA internal transcribed spacer region). The test uses real-time polymerase chain reaction (PCR) nucleic acid amplification technology and comes as a fully integrated cartridge containing all the reagents necessary for both nucleic acid extraction and amplification. This simplified, automated system provides results within 77 min [13].

This retrospective study aimed to assess the sensitivity and specificity of the STANDARD™ M10 MTB/NTM assay for detecting NTM in environmental water samples, using MGIT liquid culture as the gold standard reference method.

Methods

Study design

This retrospective study was conducted at the Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Italy, within the Emilia-Romagna surveillance programme for the detection of mycobacteria in environmental samples. The study focused on water samples collected from HCUs and haemodialysis devices. Active surveillance and microbiological investigations are ongoing, in accordance with the guidelines established by the Italian National Health Authorities [10]. Between November 2018 and December 2023 a total of 1369 water samples were collected at monthly intervals from 92 HCUs installed in the cardiac surgery departments of five hospitals; 747 water samples were also collected from 489 haemodialysis instruments (dialysis) distributed across 26 hospitals throughout the Emilia-Romagna region.

According to European Centre for Disease Prevention and Control (ECDC) guidelines, each sample (1 L of water) was concentrated using filtration, decontaminated and then inoculated into liquid (MGIT, BD) and solid (LJ, Bionef) media [9]. The remaining concentrated water samples were stored at –20 °C. For this study 150 water samples were selected, 95 collected from HCUs and 55 from haemodialysis machines.

The sensitivity and specificity of the STANDARD™ M10 MTB/NTM assay was compared with that of conventional culture, which is considered the gold standard.

STANDARD™ M10 MTB/NTM

The STANDARD™ M10 is a fully automated platform that integrates sample preparation, nucleic acid extraction,

Table I

Comparison between culture and molecular method results for different non-tuberculous mycobacteria species isolated from environmental water samples

Mycobacterium strain	Mean TtP (\pm SD) days	LJ positive (N)	STANDARD™ M10 MTB/NTM (positive results)	Mean Ct (\pm SD)
<i>M. chimaera</i> (N = 19)	14.5 \pm 6.6	14/19	14/19	35.1 \pm 1.9
<i>M. saskatchewanense</i> (N = 7)	16.9 \pm 6.7	4/7	7/7	35.7 \pm 1.7
<i>M. abscessus</i> (N = 3)	4.3 \pm 0.3	3/3	3/3	34.1 \pm 1.9
<i>M. paragordoniae</i> (N = 3)	7.5 \pm 0.9	3/3	3/3	35 \pm 1.3
<i>M. gordonae</i> (N = 2)	15.8 \pm 8.3	1/2	2/2	33.3 \pm 0.1
<i>M. mucogenicum</i> (N = 1)	7.8	1/1	1/1	36.2
<i>M. lentiflavum</i> (N = 1)	22.5	0/1	1/1	36.1
<i>M. chelonae</i> (N = 1)	4.6	1/1	1/1	33.3

LJ, Löwenstein–Jensen; SD, standard deviation; TtP, mean time to positivity.

amplification, and real-time PCR detection of target sequences in various specimen types. The system consists of the STANDARD™ M10 Module and the STANDARD™ M10 Console, which includes preloaded software for test execution and result visualization.

The system uses single-use disposable cartridges that contain all necessary reagents for nucleic acid extraction and amplification. Because the cartridges are self-contained, cross-contamination between samples is minimized. The STANDARD™ M10 MTB/NTM cartridge is provided with an internal control, and we analysed each sample singly.

The STANDARD™ M10 MTB/NTM assay utilizes multiple fluorescent detection channels to differentiate between MTB, targeting the IS6110 region and NTM, targeting the 16S–23S rRNA internal transcribed spacer region. An additional fluorescence channel is dedicated to an internal control to verify the integrity and performance of the PCR reaction [13].

The STANDARD™ M10 MTB/NTM assay has been validated for clinical use, particularly for detecting mycobacteria in respiratory specimens. Due to the viscous nature of these samples, the kit is supplied with a Pretreatment Kit, containing phosphate-buffered saline, DL-dithiothreitol, and isopropanol. Incubation with this solution promotes sample liquefaction and homogenization prior to nucleic acid extraction, thereby enhancing PCR efficiency and reducing the presence of potential inhibitors. This kit also includes a single-use graduated pipette and syringe, for transferring the pre-treated sample into the cartridge.

Sample processing

One litre of water was collected from each HCU and haemodialysis device (N = 150) in sterile plastic bottles containing sodium thiosulphate and processed within 24 h. Samples were concentrated 100-fold by filtration through nitrocellulose membranes (0.45 μ m diameter) using the Microsart filtration system (Sartorius, Germany); the membranes were transferred into a Falcon tube containing 10 mL of Ringer's solution, and vortexed for 30 s to resuspend the residue. The concentrated samples were then decontaminated (1:1 ratio) using the N-Acetyl-L-Cysteine Sodium Hydroxide Mycoprep solution (Becton, Dickinson and Company, USA). After centrifugation (15 min at 2500 \times g), the sediment was resuspended in 2 mL of Ringer's solution.

Aliquots of 500 μ L of this suspension were inoculated into MGIT liquid culture, the gold standard for mycobacterial

detection, and 250 μ L into Löwenstein–Jensen (LJ) solid culture medium, at 37 °C. The remaining decontaminated sample was stored at -20 °C.

Cultures were considered negative after 42 days of incubation without mycobacterial growth. Positive cultures were confirmed by Ziehl–Neelsen staining and further identified as NTM using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker, Germany). For culture-positive samples, the time to positivity (TtP), defined as the number of days from MGIT inoculation to detection, was recorded using Epicenter software (Becton Dickinson, USA).

For this study, stored decontaminated aliquots were thawed and tested using the STANDARD™ M10 MTB/NTM cartridge. According to the manufacturer's protocol, 500 μ L of each sample were mixed with 1 mL of the pretreatment solution and incubated at room temperature for 15 min.

Following cartridge activation, 1.4 mL of the pretreated sample were transferred into a syringe and loaded into the cartridge. Analysis was performed using the dedicated console, which provides qualitative results (positive/negative). For positive samples, the cycle threshold (Ct) value was recorded.

Statistical analysis

Sensitivity, specificity, positive predictive value, negative predictive value, and 95% confidence intervals (CIs) were calculated using 2 \times 2 contingency tables to assess the performance of the STANDARD™ M10 MTB/NTM cartridge compared with culture. Concordance between the two methods was evaluated using Cohen's kappa (κ) statistic.

Statistical significance of differences was assessed using the chi-square (χ^2) test, and comparison of TtP between groups was performed using the Mann–Whitney U test. All analyses were conducted using GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA). A P-value of <0.05 was considered statistically significant.

Results

Culture results

Of the 150 water samples, 37 were positive for NTM by culture: 29 of which were isolated from HCUs, and eight from haemodialysis devices. All NTM isolates grew in liquid culture (MGIT), and 27 also tested positive in solid culture

(Löwenstein–Jensen). The mean TtP in MGIT culture was 13.4 ± 6.7 days.

Mycobacterial species were identified by MALDI-TOF analysis as follows: 19 (50%) *M. chimaera*, seven (20%) *M. saskatchewanense*, three (8%) *Mycobacterium paragordoniae*, three (8%) *M. abscessus*, two (5%) *Mycobacterium gordonae*, one (3%) *Mycobacterium mucogenicum*, one (3%) *Mycobacterium lentiflavum*, and one (3%) *M. chelonae*.

STANDARD™ M10 MTB/NTM results

Compared with positive liquid culture, STANDARD™ M10 MTB/NTM detected NTM genome in 32/37 water samples: 14 (44%) *M. chimaera*, seven (22%) *M. saskatchewanense*, three (10%) *M. paragordoniae*, three (10%) *M. abscessus*, two (5%) *M. gordonae*, one (3%) *M. mucogenicum*, one (3%) *M. lentiflavum* and one (3%) *M. chelonae*. The mean Ct value was 35 ± 1.9 ; the mean TtP in MGIT culture was 12.4 ± 6.7 days.

In contrast, the mean TtP of the five *M. chimaera* strains that were MGIT positive and STANDARD™ M10 MTB/NTM negative was 19 ± 6.7 days, and this difference was statistically significant by Mann–Whitney test ($P < 0.04$).

All 113 samples that tested negative by culture were also negative by STANDARD™ M10 MTB/NTM. No invalid results were observed. Based on these findings, the sensitivity and specificity of the STANDARD™ M10 MTB/NTM assay were 86.5% (95% CI 0.72–0.94) and 100% (95% CI 0.97–100), respectively, with a positive predictive value of 100% and a negative predictive value of 95.8%. Table I shows the comparison between culture and molecular method.

The overall concordance between culture and STANDARD™ M10 MTB/NTM was 95.9%, with a Cohen's kappa coefficient of 0.90, indicating excellent agreement.

Discussion

Over the last few decades, increasing evidence has shown that NTM are present in hospital water systems, making them a reservoir of risk for healthcare-associated infections [3,7,14–16]. Mycobacteria exhibit a strong tendency to form biofilms, resist disinfection, and are commonly found in hospital water networks. Outbreaks in healthcare settings have been linked to cardiac surgery due to colonized humidifiers and heater–cooler devices, suboptimal disinfection protocols, and contaminated hospital water sources [17]. NTMs can cause a wide range of diseases, particularly in immunocompromised individuals [1,18].

Consequently, the spread of NTM represents a public health concern that requires specific surveillance programme aimed at reducing healthcare-facility-associated infections [19]. According to the ECDC guidelines [9] and Italian National Health Authorities recommendations [10], from November 2018, the Emilia-Romagna Region launched an active surveillance programme for the detection of NTM in environmental samples and designated our laboratory as the regional referral centre.

Culture remains the gold standard for detecting NTM; however, due to their slow growth rate, molecular assays can significantly accelerate diagnosis. The STANDARD™ M10 MTB/NTM assay is a novel cartridge-based, real-time PCR test designed for the simultaneous detection and differentiation of

MTB and NTM from clinical specimens. This fully automated, sample-to-result system offers rapid turnaround times and may support early clinical decision-making [11]. Moreover, the effectiveness of the methodology in detecting NTM is particularly relevant because these bacteria are often overlooked in traditional diagnostic tests but are increasingly recognized as a cause of chronic infections, especially in immunocompromised patients or those with pre-existing lung conditions [8].

This is the first study to evaluate the performance of STANDARD™ M10 MTB/NTM assay on environmental water samples collected from medical devices such as HCUs and haemodialysis systems. These devices are recognized reservoirs for NTM, frequently exhibiting intermittent contamination due to biofilm formation and fluctuating microbial loads, which complicates detection and risk management.

Our results showed that STANDARD™ M10 MTB/NTM assay has high specificity (100%) compared with culture, the gold standard, even in complex matrices such as sanitary water. No false-positive results were observed; this is an important finding because molecular methods could amplify non-viable mycobacteria DNA. The assay also exhibited a high positive predictive value, as all PCR-positive samples were confirmed by culture. This indicates that a positive molecular result is highly reliable for identifying contaminated devices.

The assay's sensitivity was 86.5%. A small number of culture-positive samples ($N = 5$) yielded negative molecular results. This probably reflects the known limitations of PCR-based methods in detecting low bacterial loads. In these cases, a low bacterial burden was suggested by a longer time to positivity in liquid culture, compared with culture-positive/PCR-positive samples. Therefore, a negative result cannot fully exclude the presence of NTM, and repeated or complementary testing may still be warranted in high-risk settings. We cannot exclude the possibility that sample freezing, combined with the low bacterial load, may have further contributed to the PCR negativity.

In our previous paper we evaluated the accuracy and the reproducibility of MGIT system for NTM culture on environmental matrix, and we showed that the MGIT system was highly sensitive in detecting NTM from sanitary water. The lowest concentration detected by the MGIT system was 4 colony-forming units/mL both for *M. chimaera* and *M. saskatchewanense*. [12]. Therefore, the MGIT system remains today the most sensitive method and is the gold standard for the detection of mycobacteria even in environmental matrices.

Thanks to the sensitivity and specificity of the methodology, the STANDARD™ M10 MTB/NTM assay enables rapid identification of mycobacteria, even in challenging conditions such as contaminated water or biofilms within medical devices [20].

Compared with in-house PCR assays performed on InGenius platform (ELITechGroup, Italy), which target species-specific genes [21], the STANDARD™ M10 MTB/NTM targets conserved genomic regions (16S–23S rRNA internal transcribed spacer) shared among most clinically relevant NTMs. This broader target range improves the assay's ability to detect a wide spectrum of NTM species, including those that might be missed by more narrowly focused tests. None the less, a known limitation of the STANDARD™ M10 MTB/NTM assay is its inability to detect *Mycobacterium celatum* [20].

In conclusion, this study demonstrates that detecting NTM using the STANDARD™ M10 MTB/NTM cartridge on concentrated and decontaminated water samples can complement

traditional culture methods, reducing the time to perform preventive isolation of a contaminated device.

Overall, the STANDARD™ M10 MTB/NTM offers a rapid, cartridge-based molecular approach suitable for environmental surveillance, even in settings with limited laboratory infrastructure. Its high specificity and positive predictive value make it a promising tool for early identification of contaminated medical devices, contributing to the timely implementation of corrective action and the prevention of healthcare-associated infections.

Author contributions

V. Ferraro: Writing – original draft, Methodology, Data curation. **P. Caiazza:** Data curation. **F. Sorella:** Methodology, Data curation. **F. Bisognin:** Writing – review & editing, Supervision, Formal analysis, Data curation. **C.M. Crovara Pesce:** Methodology. **P.D. Monte:** Writing – review & editing, Supervision, Data curation, Conceptualization.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Ethics statement

This study did not require ethical approval.

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