



Comparison of the RGM medium and the mycobacterial growth indicator tube automated system for isolation of non-tuberculous mycobacteria from sputum samples of cystic fibrosis patients in Belgium

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ABSTRACT

Purpose: Pulmonary infections due to non-tuberculous mycobacteria (NTM) are an emerging issue in the cystic fibrosis (CF) population. Due to bacterial and fungal overgrowth, isolation of mycobacteria from the sputum samples of these patients remains challenging. RGM medium, a novel agar-based culture medium was evaluated for the isolation of NTM from sputum samples of CF patients.

Methodology: Sputum samples were inoculated onto RGM medium and conventional Mycobacterial Growth Indicator Tube (MGIT™, Becton Dickinson, USA). Agar plates were incubated at 35 °C and growth was recorded once a week during 42 days. We compared the yield of the two media.

Results: 217 samples were obtained from 124 CF patients. 20 samples (13 patients) had a positive culture for NTM. 79/217 (36.4%) MGIT had to be discontinued due to contamination compared to 18/217 (8.3%) for RGM. We reported equivalent NTM detection performances for RGM and MGIT ($P = 0.579$): these media enabled the isolation of 15 and 12 NTM strains respectively.

Conclusion: RGM medium increases the proportion of interpretable results and the number of NTM cultured. Taking into account the non-inferiority compared to conventional methods and ease of use of RGM medium, we estimate that this test can replace current approaches for the screening of NTM among people with CF. Additionally, RGM provides semi-quantitative results (number of colonies) and information on the morphology of colonies, which may be clinically relevant information.

1. Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental organisms widely distributed in soil and water. Over the past decades, NTM have emerged as important pathogens, particularly in cystic fibrosis (CF) patients [1–4]. The prevalence of NTM among these patients is rising and many possible reasons are highlighted to explain this trend: an increased survival of these patients and thus an increased

environmental exposure to NTM, an increased use of antibiotics or medication that might impair host immunity, an increased awareness among clinicians about NTM disease and improved laboratory methods to detect NTM [5, 6]. Although there are large variations in prevalence rates between different CF patient cohorts, the overall prevalence is estimated 6% to 13% [5]. *Mycobacterium abscessus* complex (MABSC) and *Mycobacterium avium* complex (MAC) represent almost 95% of cases of NTM lung disease in CF patients [7]. Due to their hydrophobic

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cell wall, NTM are more readily aerosolized and the primary transmission route of NTM causing pulmonary disease seems to be these aerosols [8]. While MAC is the most prevalent in North America, MABSC is the most prevalent in Europe [3, 9–12]. These species not only differ by their geographical distribution but also by the patients they affect: MABSC is more often associated with younger children with a more severe form of CF whereas MAC is frequently seen in older patients [3, 5, 7, 12]. Because NTM are ubiquitous in the environment, distinguishing laboratory contamination, transient colonization and lung disease is challenging. Therefore, diagnostic criteria for the diagnosis of pulmonary disease caused by NTM have been established by the American Thoracic Society and the Infectious Disease Society of America [14].

Current guidelines recommended culture on both solid media and automated broth-based methods following decontamination of samples [15]. However, due to the overgrowth of bacteria and fungi frequently colonizing the lungs of CF patients, many cultures need to be abandoned. In addition to sometimes being unsuccessful, decontamination procedures are labour intensive and may reduce the viability of NTM and therefore the detection of these species.

RGM agar-based medium was specifically developed to target rapidly-growing mycobacteria. In two different studies, Preece et al. demonstrated that 10-day incubation RGM medium had a superior sensitivity to 10-day incubation *Burkholderia cepacia* selective agar (BCSA) for the isolation of rapidly-growing mycobacteria from sputum samples of CF patients [16, 17]. Another study confirmed these results and demonstrated higher sensitivity of the RGM medium than standard AFB culture using a combination of MGIT and Lowenstein-Jensen medium ($P < 0.0001$) [18]. Eltringham et al. compared RGM medium incubated for 10 days and 6-week MGIT cultures and reported equivalent performance for the detection of mycobacteria in sputum samples of CF patients ($P = 1.00$) [19].

The aim of this study was to compare 42-day RGM medium and automated liquid culture using the Mycobacterial Growth Indicator Tube (MGIT™, Becton Dickinson, USA) for the detection of non-tuberculous mycobacteria from sputum of CF patients.

2. Methods

2.1. Patient samples

The study took place in Saint-Luc University Hospital in Brussels, Belgium. This tertiary hospital hosts a CF centre. Respiratory samples were obtained from adults and children with cystic fibrosis who were able to expectorate at least 2 ml of sputum sample, spontaneously or after a complete session of physiotherapy, regardless of clinical or radiological suspicion of NTM infection. Patients in whom NTM was found by conventional routine culture or with RGM medium were asked to provide repeated samples as per recommended guidelines. These samples were collected from September 2016 to March 2017 and were submitted to the Clinical Microbiology laboratory of the hospital. The ethics committee of this hospital approved this study.

2.2. Specimen processing and culture method

RGM medium was prepared at Freeman Hospital Microbiology Department (Newcastle upon Tyne, UK) as previously described by Preece et al. and transported to Saint-Luc University Hospital. Without concentration or prior decontamination step, 100 µl of diluted sputum sample (1:1 with Sputasol, Oxoid Microbiological Products) was inoculated onto RGM medium, and incubated at 35 °C in air. Growth was recorded once a week during 42 days. In parallel, these samples were inoculated into a Mycobacterial Growth Indicator Tube (MGIT™, Becton Dickinson, USA) after in-house 15 min decontamination protocol using N-acetyl-L-cysteine-sodium hydroxide solution (NALC(0.5%)-NaOH (4%)). Auramine O smear was performed for all specimens and on all

positive cultures. After inactivation and extraction steps, identification of NTM was confirmed using the Microflex LT MALDI-TOF MS and MBT Compass software using the standard reference library-Mycobacterium (Bruker Daltonics, Bremen, Germany). MALDI-TOF MS identifications were performed in accordance with the manufacturer's recommendations. *RpoB* sequencing was also performed as previously described by Andre et al. [20]. Identifications were accepted if these two methods provided concordant results. Subspecies identification of *Mycobacterium abscessus* was done using Geno-Type NTM-DR test (Hain, Lifescience). This line probe assay enables *Mycobacterium abscessus* subspecies identification but also the determination of resistance to macrolides and aminoglycosides. Whole genome sequencing was also performed to confirm subspecies identification and to investigate patient-to-patient transmission.

2.3. Statistical analysis

Differences in the performance of the two media were investigated using McNemar's test with the continuity correction. Statistical significance was taken as P -value < 0.05 .

3. Results

3.1. Recovery of mycobacteria

217 samples were obtained from 124 patients. Among these patients, 2 underwent pulmonary graft. The median age of the study population was 24.5 years (6–68), and 53% were male. A total of 20 isolates of NTM were recovered using a combination of two methods (MGIT and RGM medium). These isolates were recovered from 13 patients, giving a prevalence of 10.5% of the study population. RGM and MGIT enabled the detection of 15 and 12 NTM strains respectively, with 5 undetected NTM for RGM medium and 8 for MGIT. The majority of mycobacterial isolates using both methods were *Mycobacterium abscessus* complex (11/20, 55%). Each medium, RGM and MGIT, enabled the recovery of 9 out of 11 and 8 out of 11 strains of *Mycobacterium abscessus* (sensitivity 81.8% and 72.7%, respectively). Other rapidly-growing mycobacteria were *Mycobacterium chelonae* (3/20). As a result of extended incubation time to 42 days, this study enabled the recovery of slowly-growing mycobacteria such as *Mycobacterium avium* complex (4/20), *Mycobacterium kansasii* (1/20) and *Mycobacterium gordonae* (1/20). Every strain was identified by MALDI-TOF MS. Subspecies identification of *Mycobacterium abscessus* by Geno-Type NTM DR line probe assay allowed the identification of 4 *Mycobacterium abscessus* subsp. *abscessus*, 4 *Mycobacterium abscessus* subsp. *massiliense* and 3 *Mycobacterium abscessus* subsp. *bolletii* (Table 1). Whole genome sequencing confirmed the identification but also suggested patient-to-patient transmission. Indeed, according to MLST typing, the two patients with *M. abscessus* subsp. *massiliense* seemed to be linked and one of the patients with *M. abscessus* subsp. *bolletii* seemed to be linked to another patient of our institution, not included in this study. Only 1 of the 20 sputum samples was AFB positive using the auramine staining method. For this sample, *Mycobacterium intracellulare* was recovered on both media.

3.2. Time to results

Mean time to detection was 10.6 days (range: 4–32) using MGIT medium, and 18.3 days (range: 7–42) using RGM medium. Rapidly-growing mycobacteria were recovered with a mean time to detection of 12.3 days on RGM medium and 6.5 days on MGIT medium. For slowly-growing mycobacteria, mean times to detection were 30.3 days and 18.8 days using RGM medium and MGIT medium, respectively. All *Mycobacterium abscessus* except 2 were detected within 7 days on both culture media (range on RGM: 7–28; range on MGIT: 4–12).

Table 1

Detection of non-tuberculous mycobacteria on RGM medium and MGIT (Mycobacterial Growth Indicator Tube (MGIT™, Becton Dickinson, USA)). Sensitivity (Se) is for comparative purposes and assumes that all mycobacteria were recovered using a combination of the two culture media.

	Total either medium n	RGM medium		MGIT	
		n	Se (%)	n	Se (%)
<i>Mycobacterium abscessus</i>	11	9	81,8	8	72,7
<i>subsp abscessus</i>	4	4	100	3	75
<i>subsp massiliense</i>	4	3	75	2	50
<i>subsp bolletii</i>	3	2	66,7	3	100
<i>Mycobacterium avium</i> complex	4	2	50	3	75
<i>Mycobacterium avium</i>	2	1	50	1	50
<i>Mycobacterium intracellulare/</i> <i>chimaera</i>	2	1	50	2	100
<i>Mycobacterium chelonae</i>	3	3	100	0	0
<i>Mycobacterium kansasii</i>	1	1	100	0	0
<i>Mycobacterium goodii</i>	1	0	0	1	100
Total NTM	20	15	75	12	60

3.3. Recovery of non-mycobacterial species

Whereas 79 MGIT cultures had to be discontinued following overgrowth of contaminants, only 18 RGM agar plates showed colonies which were not identified as mycobacteria (36.4% vs. 8.3%, respectively). When overgrowth was present, the colonies found on RGM medium did not invalidate the mycobacterial culture because the plates only presented with a few contaminating colonies. Furthermore, the species identification of these contaminants revealed clinically relevant bacterial and fungal pathogens including *Achromobacter*, fungal isolates and *Burkholderia*. Contrary to RGM, many contaminants found on MGIT medium were not clinically relevant (e.g. coagulase-negative staphylococci).

4. Discussion

In the setting of CF, NTM have emerged as important respiratory pathogens. Prevalence within this population is rising and patients with RGM infection have been shown to have greater declines in lung function [21]. Detection of RGM is important not only for medical management of these patients but also for infection control purposes. Actually, direct person-to-person transmission of NTM was recently suspected to be a novel route of infection, although indirect routes via fomites or aerosols (produced during physiotherapy and spirometry testing for example) have been suggested [13]. Therefore, systematic screening and early detection of NTM in this population has become a cornerstone of bacteriological follow-up, as it allows improving the medical management and potentially preventing transmission. In this context, a previous study demonstrated greater sensitivity of RGM medium than BCSA for the recovery of rapidly-growing mycobacteria ($P = 0.023$) [17]. Another study reported by Plongla et al. confirmed these results and demonstrated higher sensitivity of RGM medium than standard AFB culture combining MGIT and Lowenstein-Jensen medium ($P < 0.0001$) and superiority for the recovery of rapidly-growing mycobacteria compared with BCSA ($P < 0.0001$) [18]. Conversely, we reported equivalent performances for RGM and MGIT ($P = 0.579$). These results are in accordance with another similar study suggesting that RGM medium may potentially replace, or at least augment, automated liquid culture for the detection of NTM in CF patients [19].

As demonstrated by Plongla et al., we showed that extended incubation time significantly improved the recovery of both slowly-growing and rapidly-growing mycobacteria [18]. It is important to point out the fact that cultures on RGM medium were read only once a week and incubation was extended to 42 days, which can distort the mean time to detection for RGM medium. Indeed, contrary to other

similar studies, average time to detection on RGM medium is longer for both rapidly-growing and slowly-growing mycobacteria. However, as non-tuberculous mycobacterial infections are generally considered as chronic disease for which repeated sampling is required, an increased delay of up to two weeks for diagnosis would probably not have major consequences.

Because growth of mycobacteria is slower than growth of other bacterial and fungal species commonly found in the respiratory tract of patients with CF, detection of mycobacteria may be compromised. Despite prior decontamination, many cultures of sputum samples have to be abandoned due to bacterial and fungal overgrowth. Therefore, high selectivity is mandatory to inhibit or restrict this overgrowth. In the present study, contamination rates were significantly lower for RGM medium compared with MGIT ($P < 0.0001$), which is in accordance with previous studies and leading to a significantly higher rate of interpretable results on RGM medium.

RGM medium not only gives qualitative but also semi-quantitative information. Furthermore, *M. abscessus* can exhibit rough and smooth phenotypes (apparent on RGM medium), that are associated with different pathogenicity and virulence levels [22].

There are three shortcomings to this study. First, we didn't centrifuge sputum samples before inoculation on RGM medium, which could potentially enhance the detection rate. Second, we incubated RGM agar plates at 35 °C while rapidly-growing mycobacteria may grow better at 30 °C. Third, NALC–NaOH decontamination was performed prior to inoculation of MGIT. Even if this decontamination method is the primary recommendation of the most recent guidelines, the chlorhexidine decontamination method is superior and yields more non-tuberculous mycobacteria [15, 23]. This method also showed better performances than NALC–NaOH-oxalic acid method. Actually, Ferroni et al. demonstrated that sputum samples of cystic fibrosis patients treated with chlorhexidine yielded twice as many NTM positive cultures as those treated by the NALC–NaOH-oxalic acid method ($P < 0.0001$). NTM may be sensitive to oxalic acid decontamination method, reducing yield on culture [24]. For this reason, oxalic acid is reserved for specimen overgrown by bacteria in a two-step decontamination approach [14, 25].

In conclusion, RGM medium is a simple and effective culture medium allowing the isolation of NTM from sputum samples of patients with CF. Even if we could not statistically demonstrate the superiority of RGM medium compared to MGIT ($P = 0.579$), the low contamination rate despite the fact that no decontamination is required constitutes the major advantage of RGM compared to MGIT. Furthermore, it provides important qualitative and quantitative information for the interpretation of a positive culture. Both the number of colonies and their morphology may provide useful information and impact the treatment decision. Taking into account the non-inferiority compared to conventional methods and the ease of use of the new RGM medium, we estimate that this test can replace current approaches as a first-line screening test for routine sputum samples of CF patients.

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References

- [1] Bar-On O, Mussaffi H, Mei-Zahav M, Prais D, Steuer G, Stafler P, et al. Increasing nontuberculous mycobacteria infection in cystic fibrosis. *J Cyst Fibros* 2015;14(1):53–62.
- [2] Roux AL, Catherinot E, Ripoll F, Soismier N, Macheras E, Ravilly S, et al.

- Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in France. *J Clin Microbiol* 2009;47(12). 4124-8.
- [3] Qvist T, Gilljam M, Jonsson B, Taylor-Robinson D, Jensen-Fangel S, Wang M, et al. Epidemiology of nontuberculous mycobacteria among patients with cystic fibrosis in Scandinavia. *J Cyst Fibros* 2015;14(1):46–52.
- [4] Hill UG, Floto RA, Haworth CS. Non-tuberculous mycobacteria in cystic fibrosis. *J R Soc Med* 2012;105(Suppl 2). S14-8.
- [5] Martiniano SL, Nick JA. Nontuberculous mycobacterial infections in cystic fibrosis. *Clin Chest Med* 2015;36(1). 101-15.
- [6] Leung JM, Olivier KN. Nontuberculous mycobacteria in patients with cystic fibrosis. *Semin Respir Crit Care Med* 2013;34(1). 124-34.
- [7] Catherinot E, Roux AL, Vibet MA, Bellis G, Ravilly S, Lemonnier L, et al. Mycobacterium avium and Mycobacterium abscessus complex target distinct cystic fibrosis patient subpopulations. *J Cyst Fibros* 2013;12(1):74–80.
- [8] Halstrom S, Price P, Thomson R. Review: environmental mycobacteria as a cause of human infection. *Int J Mycobacteriol* 2015;4(2):81–91.
- [9] Qvist T, Pressler T, Hoiby N, Katzenstein TL. Shifting paradigms of nontuberculous mycobacteria in cystic fibrosis. *Respir Res* 2014;15:41.
- [10] Sermet-Gaudelus I, Le Bourgeois M, Pierre-Audigier C, Offredo C, Guillemot D, Halley S, et al. Mycobacterium abscessus and children with cystic fibrosis. *Emerg Infect Dis* 2003;9(12). 1587-91.
- [11] Seddon P, Fidler K, Raman S, Wyatt H, Ruiz G, Elston C, et al. Prevalence of nontuberculous mycobacteria in cystic fibrosis clinics, United Kingdom, 2009. *Emerg Infect Dis* 2013;19(7). 1128-30.
- [12] Pierre-Audigier C, Ferroni A, Sermet-Gaudelus I, Le Bourgeois M, Offredo C, Vu-Thien H, et al. Age-related prevalence and distribution of nontuberculous mycobacterial species among patients with cystic fibrosis. *J Clin Microbiol* 2005;43(7). 3467-70.
- [13] Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P, et al. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. *Science* 2016;354(6313):751–7.
- [14] Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;175(4):367–416.
- [15] Floto RA, Olivier KN, Saiman L, Daley CL, Herrmann JL, Nick JA, et al. US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis. *Thorax* 2016;71(Suppl 1):i1–22.
- [16] Preece CL, Perry A, Gray B, Kenna DT, Jones AL, Cummings SP, et al. A novel culture medium for isolation of rapidly-growing mycobacteria from the sputum of patients with cystic fibrosis. *J Cyst Fibros* 2016;15(2). 186-91.
- [17] Preece CL, Wichelhaus TA, Perry A, Jones AL, Cummings SP, Perry JD, et al. Evaluation of various culture media for detection of rapidly growing mycobacteria from patients with cystic fibrosis. *J Clin Microbiol* 2016;54(7). 1797-803.
- [18] Plongla R, Preece CL, Perry JD, Gilligan PH. Evaluation of RGM medium for isolation of nontuberculous mycobacteria from respiratory samples from patients with cystic fibrosis in the United States. *J Clin Microbiol* 2017;55(5). 1469-77.
- [19] Eltringham I, Pickering J, Gough H, Preece CL, Perry JD. Comparison of mycobacterial growth indicator tube with culture on RGM selective agar for detection of mycobacteria in sputum samples from patients with cystic fibrosis. *J Clin Microbiol* 2016;54(8). 2047-50.
- [20] Andre E, Belkhir L, Goeminne L, Colmant A, Sottiaux JY, et al. Immune reconstitution inflammatory syndrome due to Mycobacterium genavense in an HIV-infected patient: impact of rapid species identification by rpoB sequencing on clinical management. *J Med Microbiol Case Rep* 2015:2.
- [21] Esther Jr. CR, Esserman DA, Gilligan P, Kerr A, Noone PG. Chronic Mycobacterium abscessus infection and lung function decline in cystic fibrosis. *J Cyst Fibros* 2010;9(2). 117-23.
- [22] Jonsson B, Ridell M, Wold AE. Phagocytosis and cytokine response to rough and smooth colony variants of Mycobacterium abscessus by human peripheral blood mononuclear cells. *APMIS* 2013;121(1):45–55.
- [23] De Bel A, De Geyter D, De Schutter I, Mouton C, Wellemans I, Hanssens L, et al. Sampling and decontamination method for culture of nontuberculous mycobacteria in respiratory samples of cystic fibrosis patients. *J Clin Microbiol* 2013;51(12). 4204-6.
- [24] Ferroni A, Vu-Thien H, Lanotte P, Le Bourgeois M, Sermet-Gaudelus I, Fauroux B, et al. Value of the chlorhexidine decontamination method for recovery of nontuberculous mycobacteria from sputum samples of patients with cystic fibrosis. *J Clin Microbiol* 2006;44(6). 2237-9.
- [25] Bange FC, Bottger EC. Improved decontamination method for recovering mycobacteria from patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2002;21(7). 546-8.