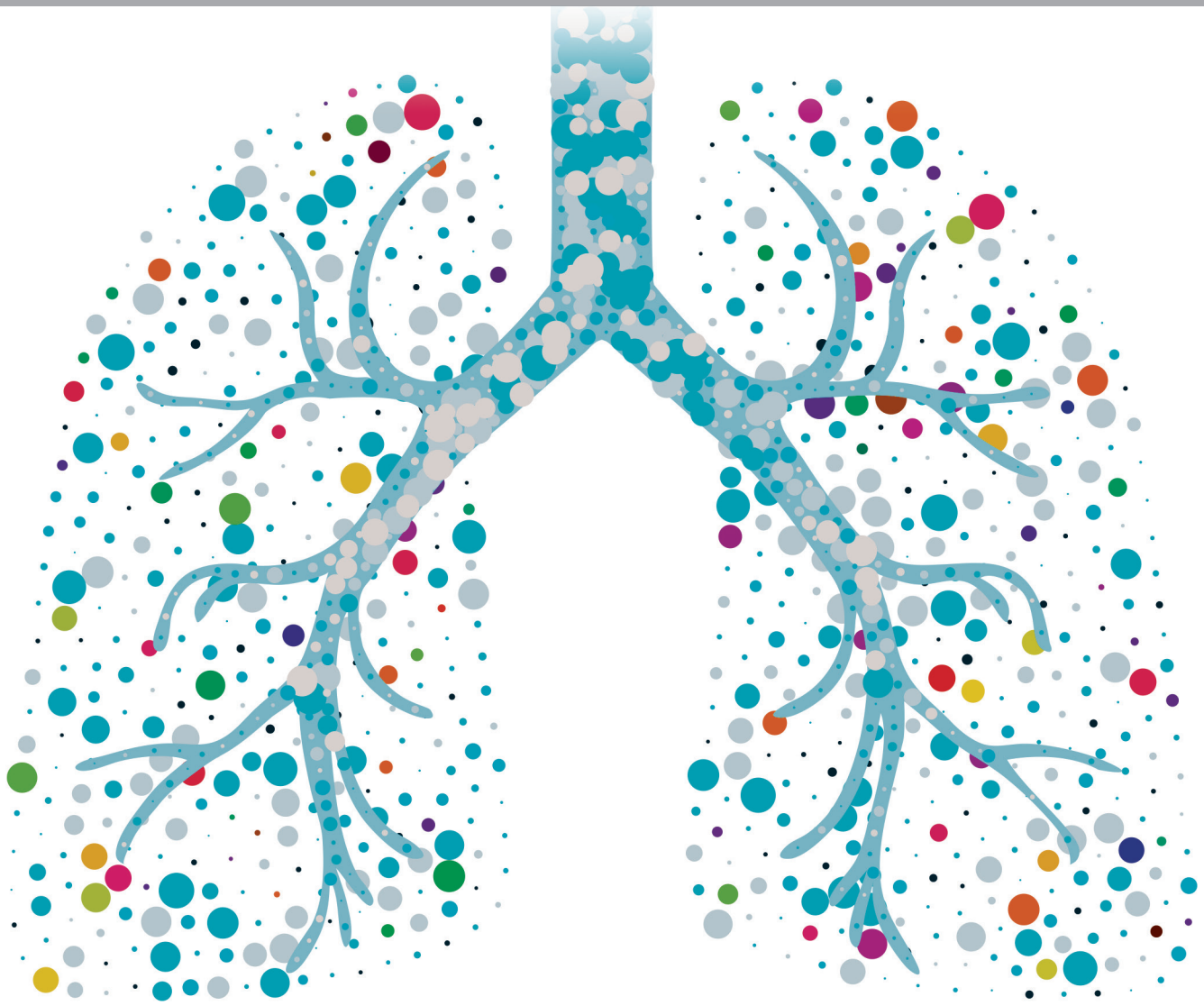
BIOMÉRIEUX

DIAGNOSING NONTUBERCULOUS MYCOBACTERIAL INFECTIONS

Selection of publications

2020 EDITION



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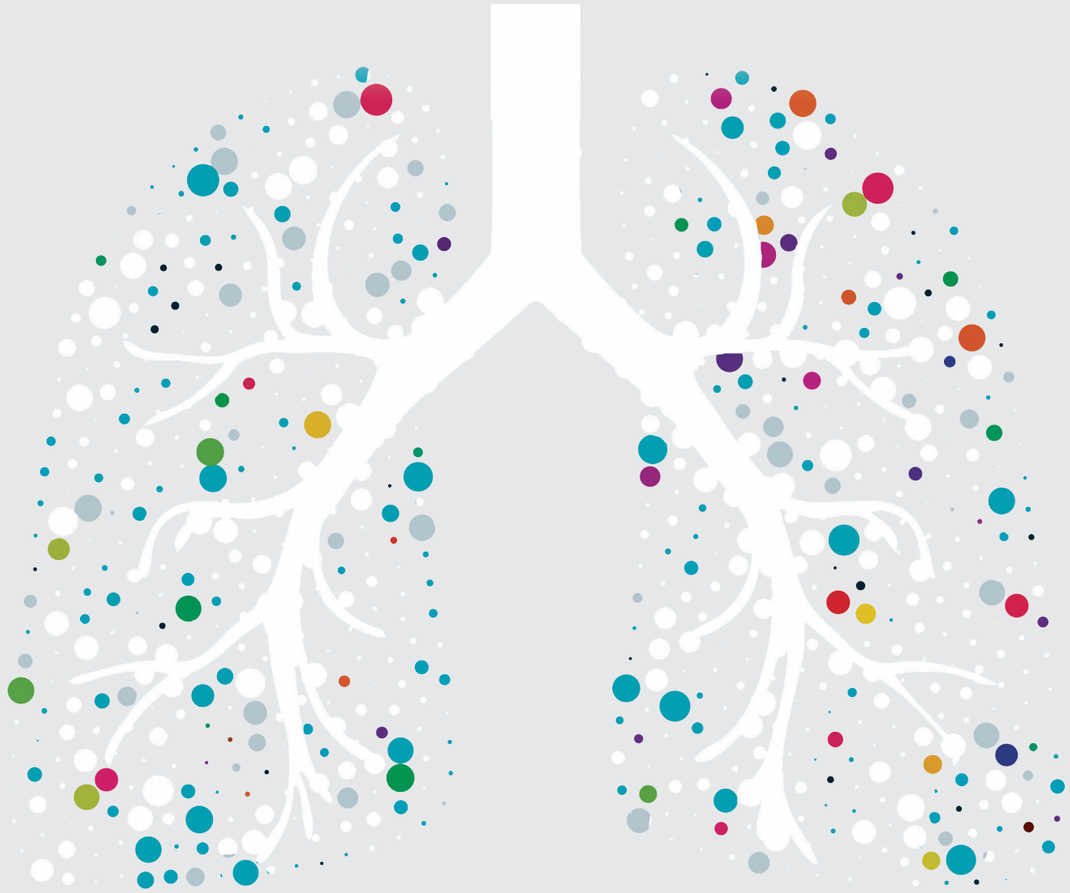

NTM Elite Agar

A NOVEL, HIGHLY SELECTIVE CULTURE MEDIUM FOR EFFECTIVE ISOLATION OF NONTUBERCULOUS MYCOBACTERIA

Nontuberculous mycobacteria (NTM) are recognized as significant respiratory pathogens in patients with cystic fibrosis (CF)[1]. However, recovery of mycobacteria from the sputum of patients with CF is challenging due to the overgrowth of cultures by other bacteria and fungi[1]. A novel culture medium ("RGM medium") has been developed that allows isolation of NTM without chemical decontamination of samples[2]. Large studies in Europe[3] and the United States[4] have shown that significantly more NTM can be recovered using RGM medium when compared with standard Acid-Fast Bacilli (AFB) culture methods. RGM medium is particularly effective for isolation of *Mycobacterium abscessus* complex.

For patients with CF, international guidelines state that culture for NTM should be performed annually in individuals with a stable clinical course who are able to produce sputum[1]. Despite this, an analysis in our own hospital revealed that only 1 in 3 patients with CF had specimens referred for AFB culture over a 15-month period. Furthermore, around 16% of AFB cultures were contaminated and NTM culture had to be abandoned.

These limitations prompted us to change our approach, and culture all respiratory specimens from patients with CF onto RGM medium. This has allowed us to integrate culture for NTM alongside culture for other CF pathogens. A further benefit is that RGM medium can be used to culture cough swabs, although the yield of NTM (1.9%) is lower than that found in sputum samples (8.5%). Nevertheless, we have been able to diagnose NTM infection in 15 children with CF by culture of cough swabs. Finally, we have never had to abandon cultures on RGM medium due to excessive contamination.

In conclusion, the routine use of RGM medium has allowed us to perform enhanced surveillance of our CF population and provide a significantly improved service to our respiratory physicians and our patients.

RGM medium is commercialized by bioMérieux as NTM Elite agar, which shows an equivalent performance to RGM medium.

John D. Perry, Clinical Scientist, Freeman Hospital, Newcastle upon Tyne, UK

The articles summarized in this Selection of Publications provide scientific support for the performance of NTM Elite agar, as well as other culture media provided by bioMérieux, for the detection of NTM in cystic fibrosis and non-cystic fibrosis patient samples.

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CHRONIC PULMONARY DISEASE AND CYSTIC FIBROSIS

Nontuberculous mycobacteria (NTM) are ubiquitous environmental mycobacteria responsible for causing chronic pulmonary infections. NTM are associated with preexisting pulmonary diseases such as Chronic Obstructive Pulmonary Disease (COPD), bronchiectasis and cystic fibrosis (CF), prior tuberculosis (TB) or immunological defects, either acquired (HIV, iatrogenic) or genetic.

Chronic pulmonary disease is the most common clinical manifestation (90%) of NTM.
Symptoms are non-specific (cough, sputum production, dyspnea, fever, hemoptysis, chest pain, weight loss ...). Other manifestations are skin and soft tissue infections, septic arthritis and disseminated infections. Diagnosis is often complicated by co-existing lung disease symptoms.

Among NTM, there are:

- **Slow Growing Mycobacteria** (> 7 days) including *M. avium* complex (80% of all NTM in USA)[1] and *M. kansasii;*
- **Rapidly Growing Mycobacteria** (RGM) (< 7 days), including *M. abscessus* complex (10-20% of RGM isolates worldwide), *M. fortuitum* and *M. chelonae.*

Bronchiectasis is a rare condition that affects the lungs by widening and stretching the airways. They become so stretched that small pockets are created in some places where germs, dust and mucus accumulate. There are various causes of bronchiectasis such as CF, previous infections (pneumonia, tuberculosis) and ciliary alteration. Nodular-bronchiectatic disease represents the association of nodules with bronchiectasis and is commonly associated with NTM and more specifically with *M. abscessus* complex[2].

Chronic Obstructive Pulmonary disease (COPD) is characterized by an obstructive ventilatory disorder and an accelerated decline in maximum expiratory volume per second, usually associated with chronic bronchitis or emphysema[3].

Cystic fibrosis is a chronic genetic disease affecting the lungs and the digestive system (pancreas). Pulmonary CF is characterized by abnormal airway secretions and chronic endobronchial infections. *M. abscessus* complex is clinically more important than other NTM because it is associated with more severe CF forms[4].

EPIDEMIOLOGY:

Prevalence of NTM has significantly increased among patients with CF. NTM has increased almost 3-fold in Israel (from 5% in 2003 to 14.5% in 2011)[4] as well in other countries. Worldwide, the prevalence is estimated to be between 6 to 24% in CF patients according to several studies[5,6]. It is not clear if this represents increasing rates of infection or improved surveillance. This change in epidemiology has led to changes in international infection control guidelines (i.e.: negative pressure rooms to reduce the risk of airborne contamination).
NTM can be found in 30% of patients with non-cystic fibrosis bronchiectasis[7].

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DIAGNOSTIC APPROACH:

Detection of NTM generally takes place at the time of diagnosis of infection, when clinical, radiologic and epidemiology elements are observed, leading to a suspicion of mycobacteria (TB or NTM).

The isolation of NTM from respiratory specimens is complicated due to potential overgrowth of coexisting bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Staphylococcus aureus*, and fungi such as *Aspergillus fumigatus*.

For this reason, a decontamination step is generally performed on respiratory specimens prior to plating on culture media. This may, however, lead to suboptimal recovery of NTM, especially rapidly growing mycobacteria (RGM) because the decontamination may have a deleterious effect by decreasing or completely nullifying the recovery of NTM.

The Standard Procedures guidelines for the isolation of mycobacteria from clinical specimens for CF and non-CF patients include the combination of solid and liquid media in order to optimize the recovery of NTM[8].

According to current guidelines, the detection of NTM in clinical samples from CF patients involves[9] :

- Sputum decontamination
- Staining of sputum smears for acid fast bacilli
- Culture on both solid (Lowenstein-Jensen medium) and liquid (Middlebrook broth base) media to increase sensitivity.

SURVEILLANCE:

The Cystic Fibrosis Foundation and the European Cystic Fibrosis Society (ECFS) for the management of NTM in CF patients recommend an annual screening by culture in case of CF on spontaneous sputum in a stable clinical situation.

Surveillance for NTM is also recommended in case of exacerbated bronchiectasis or in case of administration of TNF (Tumor Necrosis Factor) alpha blockers or certain other immune-suppressive therapies[10].

TREATMENT:

Treatment initiation is recommended on the basis of 3 criteria[11]:

- Positive AFB cultures on at least 2 separate occasions
- Clinical symptoms consistent with NTM infection
- Exclusion of other comorbidities common in CF (including adequate treatment of coinfections...)

The management of underlying diseases such as bronchiectasis and COPD is essential to effectively treat other airway infections including *P. aeruginosa* or *S. aureus* infections.

Treatment of *M. avium* complex relies on macrolide (azithromycin or clarithromycin), rifampicin and ethambutol for 18-24 months (until sputum is negative by culture for 12 months)[2].

Treatment of *M. abscessus* complex is more challenging and relies on an initial phase of 4 weeks with intravenous amikacin, imipenem, tigecycline and an additional oral macrolide (azithromycin or clarithromycin) and a continuation phase with nebulised amikacin plus an oral macrolide in combination with 1-3 drugs selected on the susceptibility and the tolerance of the drug (clofazimine, linezolide...) until sputum is negative by culture for 12 months.

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ABBREVIATIONS & ACRONYMS

AFB	Acid-fast bacillus
AFBC	Acid-fast bacillus culture
BCC	*Burkholderia cepacia* complex
BCSA	*Burkholderia cepacia* selective agar
CAN	Columbia blood agar + CNA agar medium
CF	Cystic fibrosis
COL	Columbia agar
COPD	Chronic obstructive pulmonary disease
HAEM	*Haemophilus* agar
MABSC	*Mycobacterium abscessus* complex
MAC	MacConkey agar
MALDI-TOF MS	Matrix-assisted laser desorption ionization-time of flight mass spectrometry
MAN	Mannitol Salt agar
MAVC	*Mycobacterium avium* complex
MGIT	Mycobacterial growth indicator tube
MS	Mass spectrometry
NTM	Nontuberculous mycobacteria
PAID	PA chromID (CHROMID® *P. aeruginosa*)
PA	*Pseudomonas aeruginosa* (*P. aeruginosa*)
PCR	Polymerase chain reaction
POST	12 months after first isolation
PRE	12 months prior to first isolation
RGM	Rapidly growing mycobacteria
RGM30	RGM medium incubated at 30°C
RGM35	RGM medium incubated at 35°C
SA	*Staphylococcus aureus (S. aureus)*
SAID	CHROMID® *S. aureus*
SAIDE	CHROMID® *S. aureus* ELITE (SAIDE) agar medium
S. aureus	*Staphylococcus aureus*
SCV	Small colony variants
TB	Tuberculosis

NTM Elite Agar

NTM Elite Agar

NTM Elite agar* is an innovative selective agar plate for screening and an aid to diagnosis by isolation of colonies of nontuberculous mycobacteria, such as *Mycobacterieum avium* complex, *Mycobacteroides abscessus***, *Mycobacteroides chelonae* complex**, and *Mycobacterium gordonae*, from sputum samples from cystic fibrosis patients and from respiratory specimens from non-cystic fibrosis patients.

NTM Elite agar is intended for use without prior decontamination of specimens.

The innovative selective and nutrient cocktail of **NTM Elite agar** allows good performance with higher sensitivity and specificity compared to usual culture media used for mycobacteria culture.

The incubation temperature of 30°C promotes growth of nontuberculous mycobacteria on **NTM Elite agar** while preventing development of *Mycobacterium tuberculosis* complex. Consequently, it can be used in routine microbiology labs, depending on local regulations.

*For more information, go to: www.mybiomerieux.com

**The nomenclature for *Mycobacterium abscessus* and *Mycobacterium chelonae* complex was updated in February 2018: Oren A., Garrity G. List of new names and new combinations previously effectively, but not validly, published. VALIDATION LIST NO. 181 *International Journal of Systematic and Evolutionary Microbiology.* 2018;68(5):1411–1417

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JOURNAL OF CYSTIC FIBROSIS
2016;15(2):186-91

A novel culture medium for isolation of rapidly-growing mycobacteria from the sputum of patients with cystic fibrosis.

Preece CL, Perry A, Gray B, Kenna DT, Jones AL, Cummings SP, Robb A, Thomas MF, Brodlie M, O'Brien CJ, Bourke SJ, Perry JD.

OBJECTIVE

In this study, a novel agar-based selective culture medium, RGM medium, commercialized by bioMérieux as NTM Elite agar, was evaluated with pure cultures of mycobacteria and other bacteria and fungi. The performance of the RGM medium was then compared with a selective growth agar, *Burkholderia cepacia* selective agar (BCSA), to assess the ability to isolate nontuberculous mycobacteria (NTM) from patients with cystic fibrosis (CF).

STUDY DESIGN

In the preliminary arm of this study, a collection of rapidly-growing mycobacteria previously-isolated from CF sputum samples (n=118) and other bacteria and fungi (n=98) were inoculated onto RGM medium. These pure cultures were assessed for growth (30°C) over 7 days. Then, in the direct comparison study arm, 502 clinical sputum samples were collected from 210 patients with CF. Each sample was homogenized and cultured onto BCSA medium and RGM medium, and incubated for 10 days (30°C) and growth was recorded after 4, 7, and 10 days.

RESULTS

For the pure cultures tested, all but one mycobacteria sample (117/118) grew well on the RGM medium, with 94% of other bacteria and fungi inhibited.

In the direct comparison arm of the study, a total of 55 of the 502 sputum samples collected yielded NTM isolates (using both methods). NTM sensitivity was significantly better using RGM medium over BCSA, 98% vs. 31%, respectively. Specific isolates identified and culture-medium sensitivities are listed in **Table 1**.

Regarding selectivity, shown in **Table 2**, out of the total samples collected, 419 isolates of non-mycobacteria were recovered on BCSA medium vs. only 46 on RGM medium. Additionally, the RGM medium prevented the growth of any fungi or Gram-positive bacteria.

CONCLUSIONS

RGM medium provides an effective culture method for isolating RGM from sputum samples from patients with CF. Not requiring a decontamination step, RGM medium makes it possible to ensure simple, systematic culture screening of all CF-patient sputum samples submitted for analysis.

"RGM medium allows for the systematic screening of all sputum samples routinely referred for culture from patients with CF."

KEY FINDINGS

- The comparison between BCSA and RGM medium for isolation of mycobacteria from sputum of patients with CF showed a better recovery of NTM with RGM medium, with a sensitivity of 98% vs. 31% with BCSA.
- The selectivity of RGM medium showed better performance with occurrence of only 46 isolates of non-mycobacteria vs. 419 isolates with BCSA.
- RGM medium offers a simple and effective culture method for the isolation of RGM from sputum samples from patients with CF without the need for decontamination of samples.

Table 1. Mycobacteria recovered from culture of 502 sputum samples on *Burkholderia cepacia* selective agar (BCSA) and RGM medium

Adapted from Preece CL., et al. *J Cyst Fibros.* 2016;15:186-191

SPECIES	TOTAL (either medium)	BCSA		RGM	
	n	n	Sensitivity (%)	n	Sensitivity (%)
M. abscessus subsp. *abscessus*	31	13	42	31	100
M. abscessus subsp. *massiliense*	11	3	33	11	100
M. chelonae	6	1	17	5	83
M. avium	2	0	0	2	100
M. llatzerense	2	0	0	2	100
M. salmoniphilum	2	0	0	2	100
M. mucogenicum	1	0	0	1	100
Total mycobacteria	**55**	**17**	**31**	**54**	**98**

Table 2. Other species recovered from culture of 502 sputum samples on *Burkholderia cepacia* selective agar (BCSA) and RGM medium

Adapted from Preece CL., et al. *J Cyst Fibros.* 2016;15:186-191

	NUMBER OF ISOLATES (N)	
	BCSA	RGM
Fungi and Yeasts	226	0
Arthrographis kalrae	2	0
Aspergillus fumigatus	69	0
Aspergillus terreus	6	0
Candida spp.	121	0
Exophiala dermatitidis	23	0
Scedosporium apiospermum	5	0
Gram-negative bacteria	136	46
Pseudomonas spp.	32	2
Burkholderia cepacia complex	30	18
Stenotrophomonas maltophilia	24	0
Achromobacter spp.	21	18
Enterobacteriaceae	14	2
Inquilinus limosus	4	2
Ochrobactrum spp.	4	0
Pandoraea spp.	3	3
Acinetobacter lwoffii	1	0
Methylobacterium radiotolerans	1	0
Rhizobium radiobacter	1	0
Sphingomonas sp.	1	0
Delftia acidovorans	0	1
Gram-positive bacteria	57	0
Staphylococcus spp.	29	0
Enterococcus spp.	14	0
Streptococcus spp.	8	0
Granulicatella adiacens	2	0
Lactobacillus paracasei	2	0
Micrococcus luteus	1	0
Nocardia cyriacigeorgica	1	0
Total non-mycobacteria	**419**	**46**

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JOURNAL OF CLINICAL MICROBIOLOGY
2016;54(7):1797-1803

Evaluation of various culture media for detection of rapidly growing mycobacteria from patients with cystic fibrosis.

Preece CL, Wichelhaus TA, Perry A, Jones AL, Cummings SP, Perry JD, Hogardt M.

OBJECTIVE

The purpose of this study was to assess 7 selective media for their ability to detect nontuberculous mycobacteria (NTM) isolates in patients with cystic fibrosis (CF). Five of the media tested, all commercially available, were *Burkholderia cepacia* selective agars (BCSA), designed for the isolation of *Burkholderia cepacia* complex (BCC). The other 2 selective media were designed to isolate NTM: a novel selective agar, RGM medium, commercialized by bioMérieux as NTM Elite agar, and a commercially-available medium, Middlebrook 7H11 agar.

STUDY DESIGN

All 7 media were challenged with 147 isolates of rapidly growing mycobacteria (RGM) as well as 185 isolates of other species. The RGM isolates included 9 mycobacteria species or subspecies; the non-mycobacterial strains included those frequently recovered from the sputum of patients with CF. The media plates were incubated for 10 days and read at days 4, 7, and 10. Colonies were identified using MALDI-TOF mass spectrometry.

Once identified, the most NTM-selective brand of BCSA medium was compared to RGM medium in order to assess the ability to isolate NTM, based on 224 sputum samples from 133 patients with CF (adults and children) collected over a 1-year period.

RESULTS

Sensitivity: substantial differences were demonstrated as to the ability of the 5 brands of BCSA media to support the growth of mycobacteria (ranging from 40.1% to 95.9% at 4 days). There were also substantial differences in incubation times required for growth of the RGM. By comparison, the two NTM-selective agars recovered from 97.3 to 100% of the RGM at timepoints ranging from 4 to 10 days.

Selectivity: the ability of the BCSA media and Middlebrook 7H11 agar to inhibit non-mycobacterial growth also varied, with most of them showing poor inhibition of multiple non-mycobacterial organisms. RGM medium demonstrated excellent selectivity, with the ability to inhibit growth of 90% of non-mycobacteria, compared with only 40.3 % for the Middlebrook 7H11 agar.

In the follow-up comparison of RGM medium with the most selective brand of BCSA (bioMérieux product no. 33631), 17 isolates of mycobacteria were recovered from 224 sputum samples. All 17 isolates of mycobacteria (100%) were recovered on RGM medium vs. 7 (41%) recovered on the BCSA medium. The BCSA medium failed to detect mycobacteria from 7 of 12 patient samples. The average time to detection for mycobacteria was 7.9 days for RGM medium and 7 days for the BCSA. In addition to demonstrating superior sensitivity, RGM medium was also shown to be much more selective than BCSA for the inhibition of non-mycobacteria: only 17 non-mycobacterial isolates were recovered on the RGM medium vs. 59 on BCSA. These data are presented in **Table 1**.

CONCLUSIONS

Study results confirmed that the NTM sensitivity of RGM medium was superior to that of the 5 commercially available BCSA media. The selectivity of RGM medium was also shown to be superior to the BCSA media and to the other NTM-selective medium (Middlebrook 7H11). RGM medium was by far the most selective of all the agars tested.

In conclusion, RGM medium provides a convenient and simple method for the culture of mycobacteria. The authors suggest the RGM medium technology could be embedded within existing diagnostic methods for routine culturing of all submitted sputum samples from patients with CF.

Table 1. Numbers of isolates of mycobacteria and other species recovered from cultures of 224 sputum samples on BSCA and RGM medium

Adapted from Preece CL., et *al. JCM* 2016;54(7):1797-1803

SPECIES	NO. OF ISOLATES RECOVERED	
	RGM medium	BCSA
Total mycobacteria[a]	17	7
M. abscessus complex[b]	9	6
Mycobacterium avium complex	1	0
M. chelonae	1	0
M. mucogenicum	2	0
Mycobacterium simiae	3	1
Mycobacterium sp.	1	0
Total nonmycobacteria	17	59
Achromobacter spp.	6	13
Burkholderia multivorans	5	7
Chryseobacterium sp.	0	1
Cupriavidus sp.	1	1
Proteus mirabilis	0	4
Pseudomonas aeruginosa	0	7
Serratia marcescens	0	2
Sphingobacterium spiritivorum	0	1
Stenotrophomonas maltophilia	0	2
Aspergillus fumigatus	2	9
Aspergillus terreus	0	1
Candida spp.	1	7
Exophiala dermatitidis	0	1
Geotrichum sp.	1	1
Trichosporon mycotoxinivorans	1	1
Unidentified fungus	0	1
No growth	190	160

"We conclude that RGM medium offers a superior option, compared with the other selective agars, for screening and monitoring of rapidly growing mycobacteria from the sputum of patients with CF."

KEY FINDINGS

- RGM medium supported the growth of 100% of mycobacterial isolates and was more selective than any other medium included in this study (including Middlebrook 7H11).
- RGM medium demonstrated superior sensitivity and selectivity compared to 5 different BCSA media.
- When the best-performing brand of BCSA was compared to RGM medium, the BCSA medium failed to detect mycobacteria from 7 of 12 patient samples.
- The convenience of using RGM medium could enable routine screening for NTM in sputum samples from CF patients.

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JOURNAL OF CLINICAL MICROBIOLOGY
2016;54(8):2047-50

Comparison of mycobacterial growth indicator tube with culture on RGM selective agar for detection of mycobacteria in sputum samples from patients with cystic fibrosis.

Eltringham I, Pickering J, Gough H, Preece CL, Perry JD.

OBJECTIVE

This study compared 2 methods for the recovery of nontuberculous mycobacteria (NTM) in sputum samples from patients with cystic fibrosis (CF): a standard automated liquid culture method with a mycobacterial growth indicator tube (MGIT) and a new selective agar, rapidly growing mycobacteria (RGM) medium, commercialized by bioMérieux as NTM Elite agar.

STUDY DESIGN

Sputum samples were routinely collected from 187 CF patients, both children and adults, between August and December 2015, for culture of NTM using the acid-fast bacillus (AFB) technique. Each sample was decontaminated and inoculated in an MGIT tube according to standard methods and cultured for 42 days. Each sample was also cultured onto RGM medium, without a decontamination step, and incubated at 30°C for 10 days. RGM medium cultures were examined for growth at 4, 7 and 10 days.

RESULTS

Concerning isolation of mycobacteria, based on a combination of both methods, 28 isolates were recovered from the sputum samples of 28 distinct patients. *Mycobacterium abscessus* complex accounted for over 90% of all the isolates, as shown in **Table 1**.

Concerning sensitivity, there was no statistical difference for the recovery of mycobacteria between the MGIT method and the RGM medium (24/28 samples or 86% vs. 23/28 samples or 82%, respectively). The appearance of *M. abscessus* complex on RGM medium is shown in **Figure 1**.

Concerning time to results, the average time for positive results was similar (6 days using MGIT and 5.7 days on RGM medium). For negative results, the average was 44 days with the liquid culture method and 10 days using RGM medium.

Concerning selectivity, 34% of the MGIT cultures required further decontamination steps and ultimately no results were available for 6.4% of MGIT samples due to successive contamination events; these samples had to be abandoned. For RGM medium cultures, non-mycobacterial species were recovered from 31 specimens (16.6% of total), mostly Gram-negative bacilli.

CONCLUSIONS

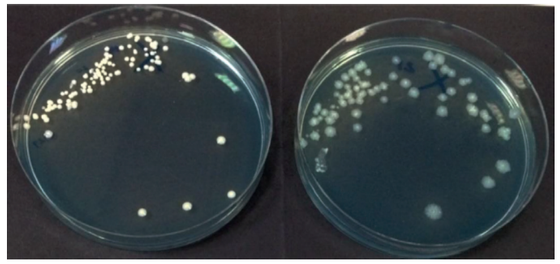
Consensus guidelines by international experts recommend using automated liquid culture as the principal method for the detection of mycobacteria in samples from CF patients. However, the automated liquid culture method is labor-intensive, time-consuming and expensive. For sputum samples from patients with CF, RGM medium offers a simple, convenient alternative method to the standard liquid culture method. With RGM medium, no decontamination step is required and sample processing is straightforward. RGM medium is therefore easy to integrate into routine culture methods.

In this study, the sensitivity of RGM medium was equivalent to that of the MGIT method for the recovery of NTM, and the authors suggest that for routine surveillance of patients with CF, RGM medium could potentially replace automated liquid culture methods.

Table 1. Numbers of mycobacteria recovered from 187 sputum samples using the MGIT and culture on RGM medium
Adapted from Eltringham I., et al. *JCM* 2016;54(8):2047-2050

SPECIES	NO. OF MYCOBACTERIA RECOVERED		
	Total	MGIT	RGM
M. abscessus complex	21	19	19
M.avium complex	1	1	1
M. chelonae	3	2	2
M. mucogenicum	3	2	1
Total	**28**	**24**	**23**

Figure 1. Appearance of *Mycobacterium abscessus* subsp. *abscessus* on RGM medium showing smooth (left) and rough (right) colony types after 7 days of incubation at 30°C
Adapted from Eltringham I., et al. *JCM* 2016;54(8):2047-2050



"The use of RGM medium is an attractive alternative method for culture of sputum samples for isolation of NTM."

KEY FINDINGS

- RGM medium offers a simple, inexpensive method for recovery of NTM in sputum samples from CF patients, and could potentially replace liquid culture methods.
- This comparative study found no statistical difference between RGM medium and the standard liquid culturing method for the recovery of NTM, and time-to-results was roughly the same for both methods.
- The RGM medium method requires no decontamination step, and can easily be embedded into routine culture methods, allowing all samples referred for routine culture to be screened for NTM.

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JOURNAL OF CLINICAL MICROBIOLOGY
2017;55(5):1469-1477

Evaluation of RGM Medium for isolation of non tuberculous mycobacteria from respiratory samples from patients with cystic fibrosis in the United States.

Plongla R, Preece CL, Perry JD, Gilligan PH.

OBJECTIVE

In order to assess methods for the isolation of NTM in sputum samples from cystic fibrosis (CF) patients, this study compared the performance of a novel selective agar, RGM (rapidly growing mycobacteria) medium, which is commercialized by bioMérieux as NTM Elite agar, with *Burkholderia cepacia* selective agar (BCSA) and a standard acid-fast bacillus culture (AFBC) method.

Additionally, the study examined whether an extended incubation time, from 10 to 28 days, enhances the isolation of certain slow-growing and clinically important NTM species. Finally, the study assessed the use of MALDI-TOF mass spectrometry to identify known species of mycobacteria cultured on RGM medium.

STUDY DESIGN

Comparison between RGA medium and BCSA

Eight hundred and sixty-nine (869) respiratory samples were collected from 487 CF patients and inoculated directly onto RGM and BCSA media. The RGM plates were incubated at 30°C in air for 28 days. The BCSA plates were initially incubated at 35°C in air for 4 days and then incubated at 30°C for 28 days. Plates were examined at 4, 7, 10, 14, 21, and 28 days.

Comparison between RGM medium and AFBC media

To assess comparative recovery rates of NTM using RGM medium vs. AFBC media, a subset of 212 samples from 172 CF patients was tested simultaneously on mycobacterial growth indicator tube (MGIT) and Lowenstein Jensen (LJ) medium after a standard decontamination process. RGM medium and conventional mycobacterial culture (AFBC) were then incubated and continuously monitored. RGM medium was examined at days 4, 7, 10, and 14 days and then weekly up to 28 days and for AFBC, monitoring was extended up to 56 days.

The performance of MALDI-TOF mass spectrometry for the identification of rapidly growing mycobacteria on BCSA medium vs. on RGM medium was evaluated using 41 archived isolates previously identified by MALDI-TOF MS. MALDI-TOF MS was performed on the isolates after 72 to 96 hours of incubation at 30°C.

RESULTS

A total of 98 NTM were isolated from 869 (11.3%) respiratory samples provided by 487 CF patients. The sensitivity of the RGM medium for the recovery of NTM was determined to be significantly higher than that of BCSA (96.9% vs. 35.7%, respectively).

The study found that extending incubation time to 28 days helped to detect an additional 35 (out of 98) NTM isolates on the RGM medium, including both rapid- and slow-growing species, (**Figure 1**).

The selectivity of RGM medium was found to be higher than that of BCSA, since it detected fewer nonmycobacterial organisms. Nonmycobacterial species were recovered from 243 BCSA cultures (28.1%) compared to 95 from RGM medium cultures (10.9%).

In the subset of samples studied to compare RGM medium with the AFBC culturing method, the sensitivity of the RGM medium in isolating NTM was significantly higher than that of AFBC (92.2% vs. 47.1%, respectively). Regarding the selectivity of the RGM medium, nonmycobacterial species were recovered from 28 (13.2%) RGM plates. A total of 84 AFBC samples were discarded because of overgrowth that prevented the recovery of NTM.

The study found that extending incubation time to 28 days helped to detect an additional 11 (out of 51) NTM isolates on the RGM medium, including 2 *M. abscessus* complex (MABSC) and 2 *M. avium* complex (MAVC) isolates. The cumulative time to positivity is shown in **Figure 2**.

Using archived NTM isolates, MALDI-TOF mass spectrometry was found to correctly identify all mycobacteria grown on RGM medium.

CONCLUSIONS

RGM medium was shown to be highly effective for the recovery of NTM from patients with CF. Extending incubation of RGM medium cultures to 28 days facilitated the isolation of slow-growing species, including some MAVC organisms.

NTM Elite Agar

Figure 1. Cumulative time to detection of NTM recovered on RGM medium and BCSA from 869 respiratory samples of 487 CF patients

Adapted from Plongla R., et al. *JCM* 2017;55(5):1469-1477

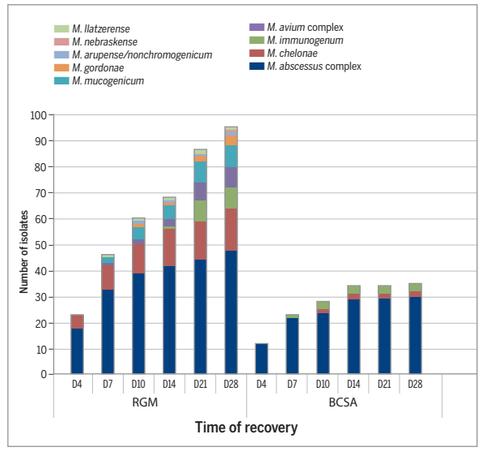
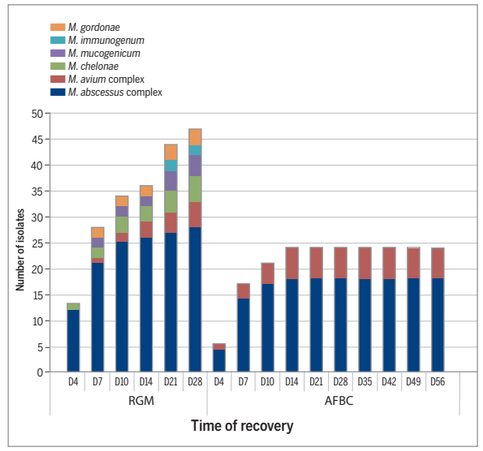


Figure 2. Cumulative time to detection of NTM recovered from 212 respiratory samples of 172 CF patients using RGM medium and conventional mycobacterial culture (AFBC) with MGIT and solid medium

Adapted from Plongla R., et al. *JCM* 2017;55(5):1469-1477



"RGM medium offers a simple and highly effective tool for the isolation of NTM from patients with CF."

KEY FINDINGS

- RGM medium had a higher rate of recovery of NTM (96.9% sensitivity vs. 35.7%) and was more selective than BCSA. The sensitivity of RGM medium for the recovery of NTM was also significantly higher than that of AFBC (92.2% vs. 47.1%, respectively).
- Extending incubation time on RGM medium to 28 days enhances isolation of both rapidly growing mycobacteria and such slow-growing species as *Mycobacterium avium* complex (MAVC) organisms.
- MALDI-TOF mass spectrometry is an effective method for the identification of mycobacteria growing on RGM medium.
- The use of RGM medium is simple and enhances the recovery of NTM, thus potentially replacing mycobacterial culturing of CF respiratory samples.

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JOURNAL OF CLINICAL TUBERCULOSIS AND OTHER MYCOBACTERIAL DISEASES
2018;13:1-4

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 in Belgium.

Scohy A, Gohy S, Mathys V, Sapriel G, Toussaint L, Bressant F, Zitouni A, Teylaert MN, Vander Meeren MC, Colmant A, Simon A, Perry JD, Lebecque P, André E.

OBJECTIVE

This study aimed to evaluate 2 methods for the isolation of nontuberculous mycobacteria (NTM), a cause of pulmonary infection in patients with cystic fibrosis (CF). It compared rapidly growing mycobacteria (RGM) medium, a selective novel agar-based culture medium commercialized by bioMérieux as NTM Elite agar, and an automated liquid culture technique using the Mycobacterial Growth Indicator Tube (MGIT) for the detection of NTM in sputum samples.

STUDY DESIGN

Sputum samples from patients with CF were plated onto RGM medium and then incubated at 35°C. Growth was recorded once weekly over 42 days. The samples were also inoculated into conventional MGIT medium after undergoing a decontamination step. The yields of the two media were then compared.

RESULTS

A total of 217 respiratory samples were collected from 124 CF patients, both adults and children, from September 2016 to March 2017. Using a combination of both the RGM medium and MGIT methods, 20 isolates of NTM were recovered. RGM medium enabled the isolation of 15 NTM strains vs. 12 strains with MGIT.

Mycobacterium abscessus complex represented the majority of mycobacterial isolates (11/20, or 55%) recovered. RGM medium demonstrated a sensitivity of 81.8% for the recovery of *M. abscessus,* compared to 72.7% for MGIT. The extended incubation time of this study (42 days) also enabled the recovery *Mycobacterium avium* complex (4/20) and other slow-growing mycobacteria. These results are summarized in **Table 1**.

Concerning the recovery of non-mycobacterial species, 18/217 (8.3%) of RGM cultures had nonmycobacteria contaminants compared to 79/217 (36.4%) of MGIT cultures, which were discontinued due to overgrowth of contaminants. The contaminating colonies observed on RGM medium did not invalidate these plates because of their relatively small number and, following identification, the clinical relevance of these bacterial and fungal pathogens. In contrast, many contaminants found on MGIT medium, such as coagulase-negative staphylococci, were not clinically relevant.

CONCLUSIONS

In this comparative study of culture methods for the isolation of NTM, RGM medium and conventional MGIT delivered similar performances of sensitivity, however, RGM medium demonstrated greater ease of use because it did not require a decontamination step.

The authors concluded that its low contamination rate (despite the lack of a decontamination step) constitutes a major advantage for RGM medium, leading to a significantly higher rate of interpretable results on RGM medium compared to MGIT.

Additionally, RGM medium provides both semi-quantitative results (number of colonies) and qualitative information (colony morphologies), that are associated with different pathogenicity and virulence especially with *M. abscessus.*

NTM Elite Agar

Table 1. Detection of nontuberculous mycobacteria on RGM medium and MGIT
Adapted from Scohy A., et al. *J Clin Tuberc Other Mycobact Dis*. 2018;13:1-4

	TOTAL either medium	RGM medium		MGIT	
	n	n	Se (%)	n	Se (%)
Mycobacterium abscessus	11	9	81.8	8	72.7
subsp *abscessus*	4	4	100	3	75
subsp *massiliense*	4	3	75	2	50
subsp *bolletii*	3	2	66.7	3	100
Mycobacterium avium complex	4	2	50	3	75
Mycobacterium avium	2	1	50	1	50
Mycobacterium intracellulare/chimaera	2	1	50	2	100
Mycobacterium chelonae	3	3	100	0	0
Mycobacterium kansasii	1	1	100	0	0
Mycobacterium gordonae	1	0	0	1	100
Total NTM	**20**	**15**	**75**	**12**	**60**

Sensitivity (Se) is for comparative purposes and assumes that all mycobacteria were recovered using a combination of the two culture media..

"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."for identification of most common clinical isolates of filamentous fungi."

KEY FINDINGS

- RGM medium enabled the isolation of 15 NTM strains vs. 12 strains with MGIT.
- While 79/217 MGIT cultures (36.4%) had to be discontinued due to contaminant overgrowth, contamination overgrowth on the RGM medium plates (18/217, or 8.3%) did not require invalidation of those cultures.
- In addition to semi-quantitative information, RGM medium also provides for the interpretation of a positive culture (such as the number of colonies and their morphology), which may be clinically relevant and impact treatment decisions.
- Given the ease of use of RGM medium and its equivalent performance when compared to conventional culture methods, RGM medium could potentially replace current techniques for first-line routine screening of sputum samples from CF patients.

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BMC PULMONARY MEDICINE
2019;19(1):19

An evaluation of methods for the isolation of nontuberculous mycobacteria from patients with cystic fibrosis, bronchiectasis and patients assessed for lung transplantation.

Stephenson D, Perry A, Appleby MR, Lee D, Davison J, Johnston A, Jones AL, Nelson A, Bourke SJ, Thomas MF, De Soyza A, Lordan JL, Lumb J, Robb AE, Samuel JR, Walton KE, Perry JD.

OBJECTIVE

Rapidly growing mycobacteria (RGM) medium, a selective agar culture medium commercialized by bioMérieux as NTM Elite agar, has been developed for the isolation of nontuberculous mycobacteria (NTM) from the sputum of patients with cystic fibrosis (CF). This study evaluated this novel RGM medium for its ability to isolate several NTM species in patients with CF, as well as patients with bronchiectasis or other lung diseases that may require lung transplantation.

STUDY DESIGN

A total of 1,002 respiratory specimens were collected from 676 patients between April 2017 and July 2018. The 1,002 specimens were comprised of 405 samples from CF patients, 323 samples from patients with bronchiectasis and 274 samples from patients with other lung diseases requiring lung transplantation. Specimens were cultured directly onto two sets of RGM medium and then incubated (one set at 30°C, one set at 37°C). These were compared with samples cultured using a conventional acid-fast bacilli (AFB) culture technique: the samples were decontaminated and then both plated on solid medium (Löwenstein-Jensen medium) and inoculated into a liquid broth mycobacterial growth indicator tube (MGIT).

RESULTS

The optimal incubation temperature for RGM was observed to be 30°C, Then a comparison of RGM medium incubated at 30°C versus AFB culture was performed. RGM medium recovered significantly more total NTM isolates than the AFB culture for all three patient groups, yielding a sensitivity of 94.6% vs. 22.4% for conventional AFB culture. Similarly, among specific NTM species, RGM at 30°C recovered significantly more *Mycobacterium abscessus* complex isolates than AFB culture, with a sensitivity of 96.1% vs. 58.8%, respectively. Lastly, the sensitivity of RGM medium at 30°C for isolating *Mycobacterium avium* complex was numerically (but not significantly) higher compared with AFB culture (83% vs. 70.2%, respectively).

CONCLUSIONS

This study confirmed the utility of, and indication for, RGM medium for the isolation and identification of NTM pathogens from multiple patient types (those with CF, bronchiectasis and other lung diseases), with significantly higher sensitivity than the recommended AFB culture.

"In the largest study of RGM medium to date, we reaffirm its utility for isolation of NTM from patients with CF. Furthermore, we show that it also provides an effective tool for culture of respiratory samples from patients with bronchiectasis and other lung diseases."

KEY FINDINGS

- This largest-study-to-date of RGM medium confirmed its utility for the isolation of NTM, demonstrating better NTM recovery than conventional AFB culture from patients with CF.
- In addition to CF patients, the study also showed that RGM medium is an effective tool for culture of respiratory samples from patients with bronchiectasis and other lung diseases.

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JOURNAL OF CLINICAL MICROBIOLOGY
2019;57(2):e01519-18

Performance of RGM medium for isolation of non tuberculous mycobacteria from respiratory specimens from non-cystic fibrosis patients.

Rotcheewaphan S, Odusanya OE, Henderson CM, Stephenson D, Olivier KN, Perry JD, Zelazny AM.

OBJECTIVE

To test the ability to isolate rapid- and slow-growing NTM, this study evaluated a new selective medium for RGM, commercialized by bioMérieux as NTM Elite agar. The study is one of the first to evaluate the performance of RGM medium for the recovery of NTM in specimens from non-CF patients with underlying lung conditions such as COPD and bronchiectasis.

STUDY DESIGN

Cultured at 2 incubation temperatures (RGM30 [30ºC] and RGM35 [35ºC]), RGM medium was compared to both the mycobacterial growth indicator tube (MGIT) system and Middlebrook 7H11 agar medium for the isolation of NTM, as well as to the MGIT and Middlebrook 7H11 used in combination (NIH's acid-fast bacillus culture, or AFBC, protocol).

A total of 203 respiratory specimens from 163 non-CF patients obtained between October 2017 and March 2018 were inoculated on RGM medium and incubated for 28 days at both incubation temperatures. Comparator specimens were decontaminated using N-Acetyl-L-cysteine–sodium hydroxide (NALC-NaOH) and neutralized, then inoculated into MGIT and incubated for 42 days, and into Middlebrook 7H11 agar and incubated for 21 and 42 days at 35°C. NTM were identified by either MALDI-TOF MS or gene sequencing.

RESULTS

Samples from 85 patients (52.1%) provided a total of 133 NTM isolates, which were recovered using one or more of the three culture methods.

The sensitivity of RGM30 for the recovery of NTM was significantly higher than either the MGIT (76.7% vs. 59.4%, respectively) or Middlebrook 7H11 agar (76.7% vs. 47.4%, respectively) alone, but was not significantly different from MGIT and Middlebrook 7H11 used in combination (i.e., the AFBC protocol). The RGM35 plates demonstrated significantly lower sensitivity than either the MGIT system or AFBC (49.6% vs. 59.4% and 49.6% vs. 63.9%, respectively), similar to that of Middlebrook 7H11 alone.

RGM medium was highly effective at inhibiting nonmycobacterial growth, with a rate of breakthrough contamination of 5.4% and 4.4% for RGM30 and RGM35, respectively.

CONCLUSIONS

RGM medium offers a simple method of specimen processing for the isolation of NTM from respiratory specimens from non-CF patients.

The sensitivity of RGM medium was similar to that of the AFBC combination technique, but was statistically superior to either the MGIT or Middlebrook 7H11 agar alone. The authors suggest that combining the MGIT system and RGM medium could increase NTM recovery rates, for both rapid- and slow-growing NTM.

"RGM medium provides an alternative method for the recovery of NTM from respiratory specimens from non-CF patients by offering a simple and rapid method for specimen processing."

KEY FINDINGS

- RGM medium offers a simple and rapid alternative method of specimen processing for the recovery of NTM from respiratory specimens from non-CF patients.
- The combination of the MGIT system and RGM medium, with incubation at 30°C, increases the recovery rate of NTM, compared with another AFBC agar.

NTM Elite Agar

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HELIYON
2019;5(10):e02684

Evaluation of a novel rapidly-growing mycobacteria medium for isolation of *Mycobacterium abscessus* complex from respiratory specimens from patients with bronchiectasis.

Brown-Elliott BA, Molina S, Fly T, Njie O, Stribley P, Stephenson D, Wallace RJ Jr, Perry JD.

OBJECTIVE

Methods used to decontaminate respiratory specimens prior to culturing for the isolation of nontuberculous mycobacteria (NTM) may have a deleterious effect by decreasing or completely nullifying the recovery of NTM. Contamination by fungal or other bacterial species may also occur.

This study evaluated the performance of a novel solid rapidly-growing mycobacteria (RGM) medium, commercialized by bioMérieux as NTM Elite agar, which does not require a decontamination method for the recovery of NTM, compared to the routine detection method with a pre-decontamination process, using a biplate containing Middlebrook 7H11/Mitchison selective 7H11 agars and the Versatrek™ broth detection system.

In this single-center study, specimens from patients with underlying bronchiectasis were cultured and studied in particular for isolates of the *Mycobacterium abscessus* complex.

STUDY DESIGN

A total of 297 mycobacterial samples were obtained from 116 patients who had cystic fibrosis (CF) or non-CF bronchiectasis.. Specimens were divided into 2 aliquots. One was plated directly onto the novel RGM medium, as no decontamination of the specimen was necessary, and was then incubated at 30°C for 28 days. The second aliquot was first decontaminated and then plated onto an agar biplate containing Middlebrook 7H11 and Mitchison selective agar (routine acid-fast bacilli, or AFB, culture), and inoculated into VersaTrek broth media. The broth and agar plate were incubated at 35°C up to 6 weeks and 3 weeks respectively.

RESULTS

The recovery of *M. abscessus* complex increased by approximately 12% with use of the RGM medium. Only 2% of RGM medium plates were contaminated (bacterial, fungal and/or yeasts), compared with 48% - 95% contamination for the comparative methods.

CONCLUSIONS

Study findings corroborate previous studies regarding isolation of *M. abscessus* complex from patients with chronic bronchiectasis: recovery is enhanced when utilizing RGM medium, without the need for specimen decontamination.

"The RGM medium significantly enhances the recovery of M. abscessus complex while streamlining the culture process as there is no need for any kind of specimen processing, thus reducing labor time and costs."

KEY FINDINGS

- In a performance comparison of RGM medium against an agar biplate (Middlebrook 7H11/Mitchison agars) and broth media (VersaTrek), the recovery of *M. abscessus* complex isolates increased by 12% with RGM medium.
- Contamination was significantly reduced by the use of RGM medium, dropping from 48% (on biplate) and 95% (broth culture) to 2% with RGM medium.

CHROMID® S. aureus Elite
CHROMID® P. aeruginosa
BCSA

DETECTION OF PATHOGENS IN RESPIRATORY SAMPLES FROM CYSTIC FIBROSIS PATIENTS USING OTHER CULTURE MEDIA

CHROMID® S. aureus Elite
CHROMID® P. aeruginosa
BCSA

Among the range of culture media plates in the bioMérieux portfolio, 3 dedicated media are specifically designed for the detection of pathogens involved in the colonization and/or infection of cystic fibrosis patients.

CHROMID® S. aureus Elite*

CHROMID® S. aureus Elite agar is a chromogenic medium for the selective isolation and the direct identification of *Staphylococcus aureus*: in 18 to 24 up to 48 to 72 hours for respiratory samples from patients with cystic fibrosis.

CHROMID® S. aureus Elite agar is composed of a nutrient rich base which combines different peptones and a chromogenic substrate to enable the growth and direct identification of *S. aureus* through the appearance of typical colonies which spontaneously turn pink. The presence of thymidine in the agar favors the growth of certain variants of *S. aureus*. The selective mixture inhibits most yeasts and bacteria not belonging to the species *S. aureus*.

CHROMID® P. aeruginosa*

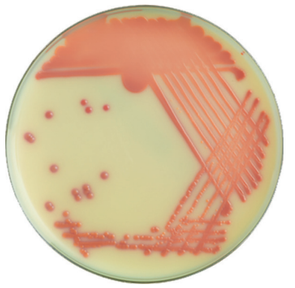
CHROMID® P. aeruginosa agar is a chromogenic medium for the direct identification of *Pseudomonas aeruginosa* in pulmonary specimens from patients with cystic fibrosis in 24 hours. The incubation can be extended to 5 days.

CHROMID® P. aeruginosa agar contains a combination of peptones and amino acids that favor the growth of *P. aeruginosa*. The specific hydrolysis of a chromogenic aminopeptidase substrate (2 bioMérieux patents) causes the *P. aeruginosa* colonies to turn a violet color, with or without a metallic gold sheen. The selective mixture inhibits most Gram-positive bacteria, yeast, molds and Enterobacterales.

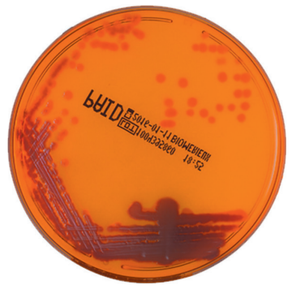
BCSA*

BCSA agar meaning *Burkholderia cepacia* Selective Agar is a selective isolation medium for the detection of the species *B. cepacia* from pulmonary specimens from patients with cystic fibrosis.

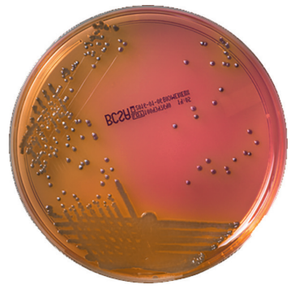
The presence of a peptone base and sugars allows optimum growth of the microorganisms. Crystal violet and the antibiotics present in the medium inhibit most microbial species other than *B. cepacia.*



CHROMID® S. aureus Elite



CHROMID® P. aeruginosa



BCSA agar

*For more information, go to: www.mybiomerieux.com

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JOURNAL OF CYSTIC FIBROSIS
2015;14(1):S73

Comparison of two chromogenic media for isolation of *Staphylococcus aureus* from respiratory samples from patients with cystic fibrosis.

Preece C, Perry A, Jones AL, Cummings SP, Bourke SJ and Perry JD.

OBJECTIVE

The study was designed to compare the ability of two chromogenic culture media, CHROMID S. aureus and CHROMID S. aureus Elite, to isolate *Staphylococcus aureus* in sputum and cough samples taken from patients with cystic fibrosis (CF).

STUDY DESIGN

A total of 123 sputum samples and 108 cough swabs were collected from 171 CF patients and cultured on the two experimental media, incubated, and read at 20, 48, and 72 hours. Colonies were identified using MALDI-TOF mass spectrometry.

RESULTS

A total of 52 samples (22%) yielded *S. aureus* isolates. At 20 hours, the sensitivity of CHROMID S. aureus was 62%, rising to 79% at 72 hours. CHROMID S. aureus Elite demonstrated higher sensitivity: at 20 hours a sensitivity of 79%, rising to 92% at 72 hours. CHROMID S. aureus Elite also showed high specificity, with fewer false positive colonies than CHROMID S. aureus (68 vs. 146, respectively).

CONCLUSIONS

This comparison demonstrated the effectiveness of CHROMID S. aureus Elite for the isolation of *S. aureus*, including in challenging clinical samples that may contain auxotrophic isolates of *S. aureus* ("small colony variants": these difficult-to-detect *S. aureus* variants require growth supplementation and/or incubation times that the wild-type *S. aureus* strain does not). CHROMID S. aureus Elite was also effective in clinical samples containing other bacterial species, including many demonstrating antibiotic resistance.

At 18 hours of incubation, CHROMID S. aureus Elite had equivalent sensitivity to CHROMID S. aureus samples incubated for 72 hours. Finally, CHROMID S. aureus Elite cultures yielded significantly fewer false positive samples, thus avoiding the need for additional investigation of isolates.

Both the sensitivity and specificity of CHROMID S. aureus Elite show superior performance to CHROMID S. aureus for isolation of *S. aureus*-containing respiratory samples from patients with CF.

"CHROMID S. aureus Elite is a superior option to CHROMID S. aureus for recovery of S. aureus from respiratory samples from patients with CF."

KEY FINDINGS

- CHROMID® S. aureus Elite was able to detect more *S. aureus* in test samples, including small colony variants, which are more difficult to detect.
- Due to its higher specificity, fewer false positives were observed with CHROMID® S. aureus Elite resulting in fewer investigations.

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PEDIATRIC PULMONOLOGY
2016;51:333-333

Clinical evaluation of a new chromogenic medium for the isolation of *Staphylococcus aureus* from cystic fibrosis patients: earlier and more sensitive!

Tafani V, Safrani-Lahyani J, Trouillet-Assant S, Chiganne M, Vincent F, Doleans-Jordheim A, Laurent F.

OBJECTIVE

This study compared the performance of a novel chromogenic medium, CHROMID S. aureus Elite (SAIDE), with 3 commercially available media for the isolation of *Staphylococcus aureus* in clinical samples from patients with cystic fibrosis (CF).

STUDY DESIGN

A total of 202 pulmonary samples were tested on the novel SAIDE media, as well as on: CHROMID S. aureus (SAID), BBL™ CHROMagar™ *S. aureus* (BBL), and Columbia blood agar + CNA (CNA). Plates were read after 24, 48, and 72 hours. The identification of chromogenic colonies was confirmed using MALDI-TOF mass spectrometry.

For each medium, 3 criteria were independently determined: sensitivity, specificity, and selectivity. Once determined, a second set of values was calculated comparing the 3 criteria for SAIDE vs. the other test media, using a stricter statistical standard (per Clinical and Laboratory Standards Institute guidelines).

RESULTS

Numerically, the sensitivity of SAIDE was consistently higher than the other 3 media at all timepoints; SAIDE sensitivity was significantly higher than BBL at all timepoints. Regarding specificity, both SAIDE and BBL were 100% at all timepoints. Finally, selectivity was significantly higher for SAIDE, vs. all 3 comparator media.

CONCLUSION

In this set of samples from CF patients, CHROMID S. aureus Elite medium demonstrated a more sensitive and selective detection of *S. aureus*, compared with the 3 standard media tested.

"In real life conditions, CHROMID S. aureus Elite demonstrates a better, earlier and easier detection of S. aureus using clinical samples from CF patients compared to the classical media used in this population."

KEY FINDINGS

- CHROMID® S. aureus Elite offers better detection of *S. aureus* for CF patients.
- The specificity of CHROMID® S. aureus Elite was 100 % in this study.

CHROMID® S. aureus / CHROMID® P. aeruginosa

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CLINICAL MICROBIOLOGY AND INFECTION
2011;17:S57-S58

Comparison of chromogenic and selective media for the detection of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in respiratory samples from cystic fibrosis patients.

Nobre Machado A., Nonhoff C, Roisin S, Vergison A, Van Bambek, F, Legros N, Hay G, Buidin P, Thiroux C and Denis O.

OBJECTIVE

Chronic respiratory infection in cystic fibrosis (CF) patients is caused primarily by *Staphylococcus aureus* (*S. aureus*, SA) and *Pseudomonas aeruginosa* (*P. aeruginosa,* PA). Small colony variants (SCV) of SA and PA complicate laboratory identification of isolates.

This study compared the performance of chromogenic media to conventional media for the detection and identification of SA and PA, including both normal and SCV phenotypes.

STUDY DESIGN

A total of 159 respiratory samples collected from 64 CF patients were plated onto 1 of 7 selective detection media; 5 were conventional, non-chromogenic and 2 were chromogenic. The 5 non-chromogenic media were: Columbia agar (COL), *Haemophilus agar* (HAEM), MacConkey agar (MAC), Mannitol Salt agar (MAN), and *Burkholderia cepacia* selective agar (BCSA); the 2 chromogenic media were CHROMID S. aureus (SAID) and CHROMID P. aeruginosa (PAID).

Culture plates were incubated for 5 days and examined daily. For SA isolates, identification was confirmed by a multiplex polymerase chain reaction (PCR) technique. PA isolates with an atypical biochemical profile were further characterized by rRNA sequencing.

RESULTS

***S. aureus* detection:** 58 normal and 21 SCV phenotype isolates of SA were identified from 48 samples. The sensitivities of SAID (89.9%) and MAN (81%) were not significantly different. The specificities of SAID and MAN were 67.2% and 77.1%, respectively. SA SCV isolate recovery was higher for SAID than MAN (21 vs. 18 isolates).

***P. aeruginosa* detection:** 133 PA isolates were identified from 72 samples. PAID demonstrated significantly higher sensitivity than MAC (88.7% vs. 75.9%). The specificities of PAID and MAC were 90.8% and 64.5%, respectively. PAID was able to recover more PA SCV isolates than MAC (44 vs. 31), although the authors found that the mucoid phenotype of PA was less expressed on chromogenic media. Mucoid colony variants frequently produced translucent colonies on PAID, however, and this may go undetected when adhering to manufacturer's recommendations.

CONCLUSIONS

Both of the chromogenic media tested, SAID and PAID, performed better than conventional media for the detection of SA and PA isolates, in particular for the isolation of atypical variants. The authors caution, however, that mucoid translucent colonies on PAID cultures should be further identified to rule out PA.

"In our CF patients centre, both SAID and PAID chromogenic media demonstrated better performances than conventional media for the detection of SA and PA isolates, especially in the recovery of atypical variants."

KEY FINDINGS

- This study demonstrated the superiority of chromogenic media compared with conventional media especially for *S. aureus* and *P. aeruginosa* detection.
- CHROMID® S. aureus and CHROMID® P. aeruginosa detect more small colony variants of *S. aureus* and more *P. aeruginosa* colonies, respectively.
- Translucent colonies isolated on CHROMID® P. aeruginosa require further identification.

BCSA

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JOURNAL OF CYSTIC FIBROSIS
2012;11(1):S42

Outbreak of *Burkholderia contaminans* infection in children with cystic fibrosis: Short term lung function and nutritional outcome.

Renteria F, Massa S, Bettiol M, Gatti B, D'Alessandro V, Prates S, Segal E and Diez G.

OBJECTIVE

Burkholderia cepacia is a significant cause of morbidity in patients with cystic fibrosis (CF). At the authors' CF center, the prevalence of *Burkholderia cepacia* increased from 2% to 20% between 2003 and 2005. This outbreak was attributed to the recently described *Burkholderia contaminans* species. No clinical data, however, have been published about its impact on patients with CF. This study aimed to assess the impact of *Burkholderia contaminans* on the pulmonary health and nutritional status of children with CF.

STUDY DESIGN

This observational retrospective study assessed CF patients with *Burkholderia contaminans*, diagnosed by having 2 positive sputum cultures using *Burkholderia cepacia* selective agar (BCSA) medium over 6 months, and confirmed by recA gen and polymerase chain reaction (PCR) analyses. Patient lung function and nutritional status were evaluated 12 months prior to first isolation (PRE) and 12 months after isolation (POST).

RESULTS

The study included 49 patients with *Burkholderia contaminans*, of whom 42% were coinfected with *Pseudomonas aeruginosa* at isolation. The *Burkholderia contaminans*-positive patients coinfected with *P. aeruginosa* had significantly lower lung function and nutritional status in the post isolation period. By contrast, *Burkholderia contaminans*-positive patients who were *Pseudomonas*-negative did not have significantly lower lung function or nutritional status.

CONCLUSION

CF patients have poorer lung function and nutritional status after *Burkholderia contaminans* infection. Those coinfected with *Pseudomonas aeruginosa* have the lowest values of lung function and nutritional status.

"CF patients have lower values of lung function and nutritional status after Burkholderia contaminans infection [as diagnosed using BCSA medium]."

KEY FINDINGS

- The authors demonstrate the impact of detection of *B. contaminans* (part of the *B. cepacia* complex) in CF patient samples.
- BCSA detects *B. contaminans*, which must then be confirmed by molecular biology (recA gen and PCR analyses).

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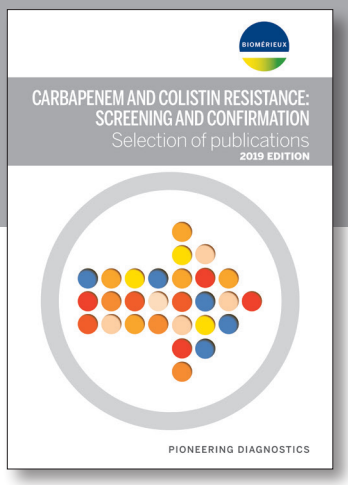

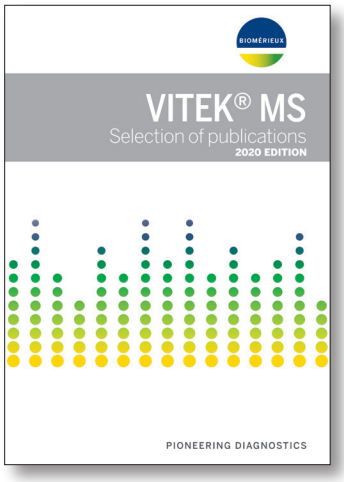

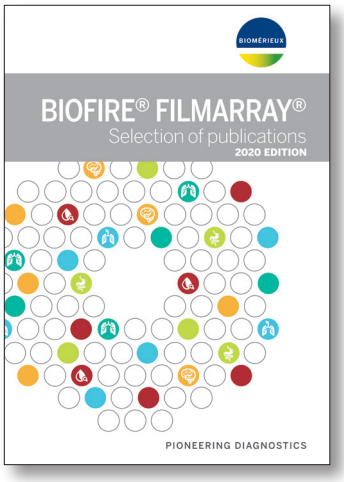

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