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Comparison of RGM medium and MGIT for isolation of mycobacteria from sputum of cystic fibrosis patients - preliminary results

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Background: Non-tuberculous mycobacteria (NTM) pulmonary infections are an emerging issue in the cystic fibrosis (CF) population. In Europe, *Mycobacterium abscessus* complex (MABSC), which may cause accelerated lung function decline, is the predominant species of NTM in patients with CF. In our clinical practice, isolation of mycobacteria consists of culture of decontaminated sputum on solid media (Lowenstein medium) and automated liquid broth method (MGIT™, Becton Dickinson®). Unfortunately, due to bacteria and fungi overgrowth, isolation of mycobacteria from sputum of these patients remains challenging. Indeed, pulmonary tract of patients with cystic fibrosis is known to be frequently colonised by microorganisms which grow faster and resist to decontamination. RGM medium is a novel agar-based culture medium which contains growth factors and selective agents to allow isolation of rapidly-growing mycobacteria.

Material/methods: We evaluated RGM medium on 102 sputa of patients with CF. Samples were obtained from consecutive CF outpatients able to expectorate at least 2 ml, spontaneously or after complete session of physiotherapy. We inoculated 100 µl of diluted sputum (1:1 with sputazol) before decontamination onto RGM medium, then incubated solid media at 35°C and recorded growth once a week. In parallel, the same samples were inoculated on MGIT after 15 minutes decontamination using NALC (0.5%)-NaOH (4%). For all isolates, auramine smear microscopy was performed to differentiate

mycobacteria from contaminating microorganisms. Identification of NTM was confirmed by MALDI TOF MS and *rpoB* sequencing.

Results: 102 samples were obtained from 84 patients. After incubation for 14 days, 6 samples (5 patients, 5.95% of the study population) had a positive culture for mycobacteria. Performing systematic screening allowed to identify 3 patients with previously undocumented NTM. Whereas 42 MGIT had to be discontinued due to contaminants, only 6 RGM were also contaminated (41.18% vs 5.88%, respectively). Moreover, these 6 contaminated RGM cultures were still interpretable due to weak contaminants overgrowth and the contaminants all were relevant, yet infrequent, pathogens in the context of CF. Both RGM and MGIT enabled the isolation of 5 NTM strains, with 1 undetected NTM for each. All strains were identified as *Mycobacterium abscessus*. Subspecies identification is ongoing.

Conclusions: RGM cultures are far less contaminated than MGIT. Considering many cultures have to be abandoned due to contaminants overgrowth, RGM medium may improve the detection of NTM. Moreover, inoculation of RGM is very simple compared to conventional mycobacterial cultures. Together, these two advantages make the RGM medium a very promising tool for NTM detection among CF patients.

Results at 14 days		RGM medium		
		Positive	**Negative**	**Contaminated**
		(n=5 ; 5%)	(n=91 ; 89%)	(n=6 ; 6%)
MGIT medium	**Positive** (n=5 ; 5%)	4	1	0
	Negative (n=55 ; 54%)	0	54	1
	Contaminated (n=42 ; 41%)	1	36	5