Comparison of four decontamination methods for cultivation of mycobacteria from sputum samples

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Background: Mycobacteria growth is considerably slower than other bacteria. Therefore, processing of respiratory samples for mycobacterial culture requires decontamination. The aim of decontamination is to reduce the microbial contamination and to interfere as little as possible with mycobacterial growth. Decontamination procedures may affect the viability of mycobacteria. There is a fine line between over- and underdecontamination. This study aimed to compare four different decontamination methods with regards to contamination rates and yield of mycobacterial recovery.

Methods: Patients treated for drug susceptible or resistant tuberculosis submitted sputum samples on four consecutive days per week. The decontamination procedures were assigned by block randomization with the unit of randomization being the day of the week. In addition, artificial sputum samples were spiked with seven different species of mycobacteria of known concentrations. Colony forming units (CFU) of mycobacteria on 7H10 agar plates were compared between samples with and without prior decontamination to determine recovery rates (Figure 1, *M. abscessus* - left plate prior decontamination, further sequence as listed below). The following methods or CE-marked test kits were compared: MycoDDR™ (IMMY), modified Petroff NALC-NaOH (self-made reagents), OMNIgene® SPUTUM reagent (DNA Genotek) and NAC-PAC™ (AlphaTec™). All decontaminated samples were inoculated on Löwenstein-Jensen (LJ), Stonebrink and mycobacteria growth indicator tubes (MGIT). Solid media was reviewed for growth of mycobacteria weekly for 8 weeks. For all MGIT cultures Kinyoun staining and blood plates were performed to assess contamination.

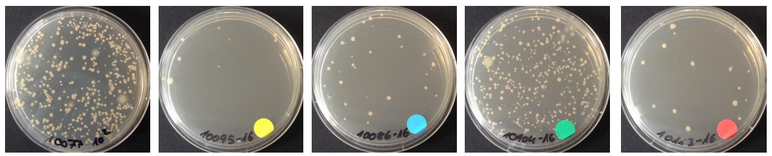


Figure 1: 7H10 agar plates for CFU

Results:

Patient sputum samples

A total of 335 clinical sputum samples were included in the study. Overall contamination rates were lowest in samples decontaminated using Myco DDR™ (table 1). Medium pH results (n=30) were closest to 8 with NALC-NaOH and OMNIgene and closest to 7 with Myco DDR™. OMNIgene® SPUTUM reagent showed the highest contamination rates (30%) on solid media, but low rates were observed in liquid media.

Table 1: Proportion contaminated

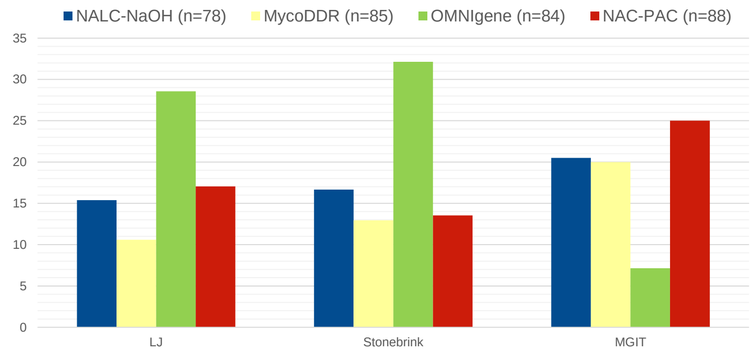
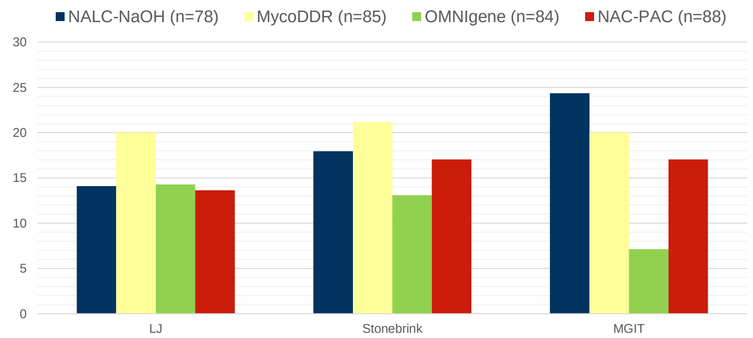


Table 2: Proportion recovery



Artificial sputum samples:

Time to positivity (table 4) in the spiked samples with decontamination was 2-3 days delayed compared to the control for all methods except OMNIgene® SPUTUM reagent which showed no growth in liquid media except with *M. kansasii*. Recovery rates (table 3) on 7H10 were 20-40% for slow growing mycobacteria, but much lower for fast growing mycobacteria without significant differences between the methods.

Table 3: Proportion recovery

Strains/ Methods	NALC-NaOH	MycoDDR	OMNIgene	NAC-PAC
M. tub (n=3)	39.5%	54.6%	13.4%	50.9%
M. kansasii (n=3)	16.6%	21.7%	17.0%	18.8%
M. xenopi (n=4)	29.8%	41.2%	16.0%	37.8%
M. intracellulare (n=4)	22.9%	17.6%	28.6%	18.6%
M. gordonae (n=4)	15.7%	15.8%	25.0%	17.0%
M. abscessus (n=4)	0.4%	0.2%	5.8%	0.3%
M. marinum (n=4, 30°C)	0.6%	0.4%	15.5%	0.8%

Table 4: Time to positivity

Strains/ Methods	Growth Control	NALC-NaOH	MycoDDR	OMNIgene	NAC-PAC
M. tub (n=4)	7;21	11;22	10;15	neg.	12;06
M. kansasii (n=6)	5;23	7;05	7;07	7;03	7;00
M. xenopi (n=4)	15;07	18;00	20;13	neg.	19;20
M. intracellulare (n=4)	6;07	4;02	4;04	neg.	4;03
M. gordonae (n=4)	6;02	7;00	13;5	neg.	7;17
M. abscessus (n=4)	2;15	4;20	6;06	neg.	5;08

Conclusion: Performance of Myco DDR™ and NAC-PAC™ was comparable to the NALC-NaOH method with Myco DDR™ performing superior. OMNIgene® SPUTUM reagent showed the highest contamination rates. No growth of mycobacteria was observed in samples decontaminated with OMNIgene® SPUTUM reagent in the liquid system, which might explain the falsely low contamination rates.