Evaluation of MYCO-TB decontamination kit for successful detection of mycobacteria

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Background

Clinical specimens from patients infected by slow-growing mycobacteria usually contain other commensal fast-growing microorganisms, rending difficult isolation and identification of mycobacterial pathogens. Therefore, reagents such as NALC-NaOH are used to decontaminate the specimens of normal flora.

Methods

In the present study, two different methods of decontamination (N-Acetyl-L-cysteine-NAOH [NALC-NaOH] and MYCO-TB decontamination solutions) were tested to evaluate decontamination efficiency.

Part I. Standardized amounts (10^8 to 10^4 CFU/ml) of *Streptococcus oralis*, *Neisseria sp.*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Actinomyces odontolyticus,* and *Candida albicans* were spiked into artificial saliva. Bacterial cells were subjected to NALC-NaOH and MYCO-TB decontamination. Resulting culture suspensions were plated on blood agar and Middlebrook 7H10 agar plates and incubated at 37 ° C for two weeks.

Part II. *Mycobacterium africanum*, *M. tuberculosis* (*Mtb*), *M. bovis*, *M. avium*, *M. kansasii*, *M. fortuitum*, and *M. peregrinum* were spiked into artificial saliva. Bacterial cells were subjected to NALC-NaOH and MYCO-TB decontamination. Bacteria were plated onto 7H10 agar plates and incubated at 37 ° C for 7 weeks. Additionally, bacteria were inoculated into MGIT growth medium.

Part III. Clinical respiratory samples were subjected to NALC-NaOH and MYCO-TB treatment followed by plating onto 7H11 agar plates, inoculation into MGIT medium, staining by auramine/Ziehl-Neelsen dye, and DNA extraction for *Mtb* real-time PCR.

Results

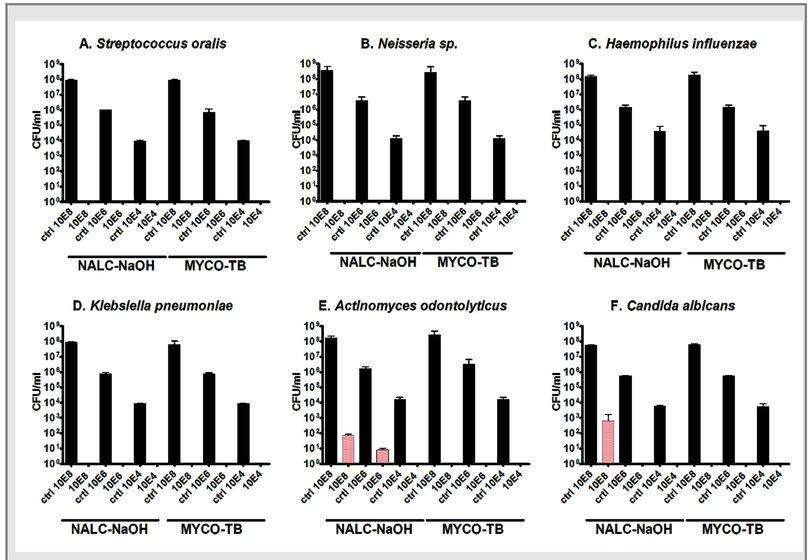

Figure 1. Assessment of the efficacy of NALC-NaOH in-house decontamination protocol compared to MYCO-TB protocol. Number of cell/ml of oral commensals after treatment with NALC-NaOH and MYCO-TB decontamination protocols, respectively. 10^8, 10^6 and 10^4 (3 technical replicates) CFU of *S. oralis* (A), *Neisseria* sp. (B), *H. influenzae* (C), *K. pneumoniae* (D), *A. odontolyticus* (E), and *C. albicans* (F) were suspended in PBS + 0.1% BSA (artificial saliva). The cells were subjected to NALC- NaOH or MYCO-TB decontamination kit according manufacturing instructions. Then, the bacteria were plated onto blood agar plates and 7H11 agar plates, used as control, and incubated at 37 ° C for 48 hours. Untreated bacterial cells were used as the control (crtl). Each bar displays the mean and the standard deviation (whiskers) of three technical replicates.

Part I. Treatment of oral bacteria with MYCO-TB produced no recovery in subsequent cultures, while using NALC-NaOH in-house decontamination solution yielded 0.6 x 10^3 cell/ml of *C. albicans* and 0.7 x 10^2 cells/ml of *A. odontolyticus*.

Results - continued

Table 1. CFU of *M. tuberculosis* complex and non-tuberculous mycobacteria after decontamination.

Species	CFU/ml following decontamination in				
	Ctrl	NALC-NaOH		MYCO-TB kit	
	-	+	C	+	C
M. africanum	0.3 x10^6	0.3 x10^3	-	0.2 x10^3	-
M. tuberculosis	0.8 x10^6	2.7 x10^3	-	7.2 x10^3	-
M. bovis	0.6 x10^6	0.5 x10^3	-	0.4 x10^3	-
M. avium	1 x10^6	0.8 x10^6	-	0.2 x10^4	-
M. peregrinum	1 x10^3	0.5 x10^2	-	0	-
M. fortuitum	1 x10^5	0.7 x10^2	-	0.1 x10^2	-
M. kansasii	1 x10^5	2.0x10^3	-	2.1 x10^3	-

+, growth of mycobacterial cells per ml of artificial saliva upon treatment; - (Ctrl), growth of mycobacterial cells per ml of artificial saliva without any treatment;
C, possible contaminants grown together with mycobacterial cells verified by Ziehl-Neelsen staining.

Parts II & III. Equal killing efficiency of MYCO-TB in comparison to NALC-NaOH decontamination method was shown for *M. africanum*, *M. bovis*, and *M. kansasii*. Lower CFU/ ml were recovered when samples with *M. avium*, *M. peregrinum* and *M. fortuitum* were treated with MYCO-TB compared to NALC-NaOH solution. *Mtb* was resistant to MYCO-TB decontamination solutions (Table 1). There was no difference in the analyses results in clinical samples after either MYCO-TB or NALC-NaOH treatment regarding the growth of *Mtb* in MGIT and in microscopy results.

CONCLUSIONS

MYCO-TB possesses higher decontamination efficacy against oral bacteria in comparison to an NALC-NaOH in-house decontamination protocol. Moreover, MYCO-TB has equally low killing effects on *M. africanum*, *M. bovis* and *M. kansasii* as NALC-NaOH. Moreover, the MYCO-TB protocol can be performed faster than the in-house protocol. In fact, it takes only 3 mins for decontamination compared to the 15 mins needed for the in-house protocol. The MYCO-TB kit has moderate killing effects on nontuberculous mycobacteria *M. avium*, *M. peregrinum* and *M. fortuitum*. Importantly, it displays weaker killing effects on *Mtb* than NALC-NaOH.

These data indicate that MYCO-TB could be a valid alternative to in-house decontamination protocols. Downstream diagnostic protocols, such as culture of Mtb and smear microscopy, applied to clinical samples upon the treatment with MYCO-TB were not affected by its usage.

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