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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 NaOH are used to decontaminate the specimens of normal flora.

Material/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 (10E08 CFU/ml) of *Streptococcus oralis*, *Neisseria* sp., *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Candida albicans* and *Actinomyces odontolyticus* 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: Treatment of oral bacteria with MYCO-TB produced no recovery in subsequent cultures, while using NALC-NaOH in-house decontamination solution yielded 0.6E03 cell/ml of *C. albicans* and 0.7E02 cells/ml of *A. odontolyticus*. Equal killing efficiency of MYCO-TB in comparison to NALC-NaOH

decontamination method was shown for *M. africanum*, *M. bovis* and *M. kansasii*. While 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. 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.