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FULL PAPER

Comparison of MycoPrep and the new MYCO-TB kit: rapid and efficient digestion and decontamination of respiratory specimens for the detection of Mycobacteria

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SUMMARY

The long incubation time required for Mycobacteria detection may allow cultures to become overgrown by contaminating organisms. Therefore, samples need to be decontaminated before solid and liquid culture. MYCO-TB is a ready-to-use digestion and decontamination kit with single-sample formulation developed by Copan. Sample processing time (3 minutes) is shorter than that of other commercial kits. The aim of this study was to compare the performance of MYCO-TB with MycoPrep, both based on N-acetyl-L-cysteine and sodium hydroxide solution, in terms of culture contamination and Mycobacterial detection by culture.

We tested 162 respiratory samples: the overall proportions of contamination of both liquid and solid media were 1.8% for MYCO-TB and 1.8% for MycoPrep. Mycobacterial growth was detected without significant differences in times to positivity (TTP) in liquid culture: 10.5 days for MYCO-TB and 11.1 days for MycoPrep.

Samples decontaminated with MYCO-TB were suitable for molecular assays such as Xpert MTB/RIF Ultra and GenoType CMdirect.

Extending decontamination times (up to 10 minutes) with MYCO-TB of 20 Mycobacteria-positive specimens did not produce any difference in TTP in liquid culture or in Ultra *IS1081/IS6110* probe Ct values.

In conclusion, the MYCO-TB kit proved to be effective for the rapid digestion and decontamination of respiratory materials for the detection of Mycobacteria, making it possible to reduce the manual skills required and lower the risk of contamination. Longer decontamination time could be used for samples with a high level of contamination, such as those from cystic fibrosis patients.

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INTRODUCTION

Mycobacteria infections in humans can be caused by *Mycobacterium tuberculosis* complex (MTBc) and Non-tuberculous Mycobacteria (NTM). MTBc is responsible for Tuberculosis (TB), a major air-borne infectious disease with human-to-human transmission, while NTM are environmental and opportunistic pathogens, most frequently associated with pulmonary disease (NTM-PD).

The gold standard for diagnosing both pulmonary TB (PTB) and NTM-PD is culture (CLSI, 2018), which typically requires a long incubation period. Respiratory samples (e.g., sputum, bronchial aspirates and bronchoalveolar lavages) are contaminated by rapidly growing normal flora, which in culture can overgrow and prevent the detection of Mycobacteria. Therefore, the decontamination of clinical samples is a crucial step to maximizing mycobacterial yield (Steingart *et al*., 2006). The standard protocol for gentle but effective digestion and decontamination usually recommends using a solution based on N-acetyl-L-cysteine (NALC) and sodium hydroxide (NaOH) (Kent and Kubica, 1985).

The process of digesting and decontaminating clinical samples requires a high level of manual skills and the preparation of reagents which are not available in bulk, often using procedures that are not well standardized.

MYCO-TB is a new digestion and decontamination kit containing NALC-NaOH, developed by Copan (Italy). It is based on ready-to-use reagents formulated for single sample processing and requires a shorter sample processing time (3 minutes) than that of other commercial kits.

The aims of this study were:

1) to compare the performance of MYCO-TB with that of MycoPrep (MycoPrep, Becton Dickinson, USA), the NALC-NaOH solution currently used in our laboratory, in terms of culture contamination and Mycobacteria detection in liquid and solid culture;
2) to verify the suitability of samples decontaminated with MYCO-TB for molecular assays;
3) to evaluate Mycobacteria detection with extended time of decontamination with MYCO-TB (up to 10 minutes).

Key words:
Respiratory samples, decontamination, Mycobacteria, MYCO-TB, MycoPrep, liquid culture, solid culture.

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MATERIALS AND METHODS

Samples

This study was conducted at the Microbiology Laboratory of S. Orsola-Malpighi University Hospital, referral centre for the diagnosis of TB and NTM infections for the metropolitan area of Bologna (Italy). One hundred and sixty-two respiratory samples of at least 10 ml from suspected cases of PTB or NTM-PD were randomly selected and anonymized to assess the decontamination performance of MYCO-TB compared to MycoPrep. In addition, 20 smear-positive sputum samples were selected and anonymized to evaluate Mycobacterial detection with extended time of decontamination with MYCO-TB.

All specimens were stained for acid-fast microscopic examination using Ziehl-Neelsen stain before sample decontamination. The degree of acid-fast bacilli positivity was assigned to one of four categories (1+, 2+, 3+, 4+) as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (Weitzman et al., 2010). Smear-positive samples were analysed by Xpert MTB/RIF Ultra (Ultra, Cepheid, USA) to identify MTBc and Rif susceptibility and by GenoType CMdirect (Hain Lifescience, Germany) in the case of Ultra-negative results.

Before decontamination, samples for the comparative study were divided into two 5 ml aliquots which were decontaminated with MYCO-TB and MycoPrep in parallel.

Samples for analysis of extended time of decontamination were divided into 3 aliquots and decontaminated with MYCO-TB for 3, 5 and 10 minutes.

Decontamination with MycoPrep (Becton Dickinson)

Since NALC loses its mucolytic activity on standing, the NALC component of the MycoPrep kit is contained in a sealed glass ampule in the NaOH solution (Kent and Kubica, 1985).

After reconstitution, 5 ml of NALC-NaOH solution were added to an equivalent amount of sample; then, after vortexing for 15 seconds or until the specimen had liquefied, decontamination continued at room temperature for 23 minutes. Freshly prepared 0.067 M, pH 6.8 phosphate buffer saline (PBS) was added to bring the volume up to 50 ml, and mixed by inversion to neutralize the decontamination reaction.

After centrifugation at 3000 g for 15 minutes, the supernatant was discarded and the bacterial pellet resuspended in 2 ml of PBS.

Decontamination with MYCO-TB (Copan)

The MYCO-TB kit consists of 4 ready-to-use tubes:

1) a reaction tube suitable for centrifugation;
2) a MYCO-TB solution vial containing digesting and decontaminating solution (NALC-NaOH);
3) a neutralizing tube containing PBS;
4) a resuspension vial with PBS.

The specimen (5 ml) was transferred to the reaction tube, added with 5 ml of MYCO-TB solution and vortexed for 10-30 seconds. The mixture was left at room temperature for 3 minutes. Neutralizing PBS was added to the reaction tube and mixed by inversion. Samples were centrifuged at 3300 g for 5 minutes, then the supernatant was discarded and the sediment resuspended with 2 ml of resuspension solution.

Aliquots of each decontaminated sample were stored at -20°C for Xpert MTB/RIF Ultra assay.

Mycobacteria culture and identification

Two types of solid media, Lowenstein-Jensen (LJ) and LJ plus PACT antibiotic mix (polymyxin B, amphotericin B, carbenicillin, and trimethoprim lactate) (LJ PACT, Heipha Diagnostika Biotest, Germany) were inoculated with 0.2 ml of each specimen previously decontaminated with MYCO-TB or Myco-Prep. Similarly, two 0.5 ml aliquots of decontaminated specimen (one for each decontamination method) were inoculated into liquid media (MGIT tubes, Becton Dickinson, USA). LJ agar slants were incubated at 37°C; MGIT tubes were loaded into the BACTEC MGIT 960 system.

Solid and liquid cultures were considered negative if no Mycobacteria were isolated after 42 days of incubation. Specimens were considered positive if Mycobacterial growth was detected in at least one of the three different culture media (LJ, LJ PACT, MGIT).

Positive liquid culture were identified as MTBc by SD TB Ag MPT64 Rapid test (SD Biosensor, Korea), or as Non-Tuberculous Mycobacteria (NTM) by GenoType CM (Hain Lifescience, Germany).

Mycobacteria time to positivity (TTP) in liquid culture was defined as the number of days from MGIT inoculation to positive culture result, using Epicenter software (Becton Dickinson).

Culture contamination was defined as bacterial or yeast growth preventing Mycobacteria isolation; rates of both solid and liquid culture contamination were calculated. Furthermore, collapsed solid media were defined as Lowenstein Jensen medium melting associated with the presence of Gram-negative bacteria in the biological specimens (*P. aeruginosa, K. pneumoniae, P. mirabilis, A. baumannii*).

Molecular assays

Xpert MTB/RIF Ultra (Ultra): 500 µl of decontaminated sample were mixed with Sample Reagent at a 1:3 ratio for 15 minutes at room temperature and poured into a single-use disposable cartridge of the GeneXpert module, according to the manufacturer's instructions (Chakravorty *et al.*, 2017).

GenoType CMdirect: DNA was extracted from 500 µl of decontaminated sample with the GenoLyse kit (Hain Lifescience, Germany); and multiplex amplification with biotinylated primers and reverse hybridization were performed with GenoType CMdirect kit, according to the manufacturer's instructions (Hain Lifescience, Germany).

Statistical analysis

Student's t-test was used to compare TTP in liquid culture and Ultra *IS1081/IS6110* probe Ct values of MYCO-TB- and MycoPrep-treated MTBc-positive specimens. Culture contamination of MYCO-TB- and MycoPrep-treated samples was compared with the chi-square test. Cohen's κ statistics were calculated for the assessment of agreement between the two decontamination methods.

Statistical analysis was performed using GraphPad Prism version 8.0.1 (San Diego, CA, USA). Statistical significance was set at $p<0.05$.

RESULTS

Mycobacteria detection

One hundred and sixty-two respiratory samples were selected: 81 (50%) bronchoalveolar lavages, 42 (26%) bron-

Table 1 - *Agreement between MYCO-TB and MycoPrep methods for culture contamination.*

	Contaminated Culture		*Not contaminated Culture*			
Decontamination method	*MYCO-TB and MycoPrep*	*MYCO-TB only*	*MycoPrep only*	*MYCO-TB and MycoPrep*	*Agreement*	*k*
MGIT	7	9	3	143	92.6%	0.50
LJ	16	6	8	132	91.4%	0.65
LJ PACT	4	3	6	149	94.4%	0.44
Overall (MGIT+LJ+LJ PACT)	1	2	2	157	97.5%	0.32

chial aspirates, and 39 (24%) sputum. Eighteen samples (11.1%) were positive for acid-fast microscopic examination.

Twenty out of 162 samples were positive for Mycobacteria growth (18 MTBc, 2 NTM) with both decontamination systems. No significant difference was observed between average TTP of MTBc in liquid culture with MYCO-TB (10.5±5.0 days) and MycoPrep (11.1±6.5 days).

Culture contamination

The rate of contamination of liquid cultures was 9.9% for MYCO-TB and 6.2% for MycoPrep with no significant difference. The rate of contamination of solid media was similar for both systems: 13.6% for MYCO-TB and 14.8% for MycoPrep on LJ, 4.3% for MYCO-TB and 6.2% for MycoPrep on LJ PACT. Furthermore, the proportion of collapsed solid media was similar for specimens processed with MYCO-TB (4.6%) and MycoPrep (5.3%). No mould contamination was detected after decontamination with MYCO-TB compared to 1.2% with MycoPrep.

Finally, the overall rate of contamination, defined as the total failure to isolate Mycobacteria in liquid and both solid media, was 1.8% for MYCO-TB and 1.8% for MycoPrep. *Table 1* shows the agreement between MYCO-TB and MycoPrep methods for culture contamination.

Molecular assays on samples decontaminated with MYCO-TB

In order to verify the suitability of MYCO-TB treated samples for molecular tests, Xpert MTB/RIF Ultra was performed on 30 samples previously stored at -20°C after decontamination. Ultra was valid in all tested samples: 18 MTBc-positive culture samples were Ultra-positive, 2 NTM-positive and 10 culture-negative samples were Ultra-negative.

Furthermore, Genotype CMdirect was performed successfully on a smear-positive *Mycobacterium intracellulare*-sample decontaminated with MYCO-TB.

Extended time of decontamination with MYCO-TB

Decontamination of 20 MTBc-positive specimens with MYCO-TB for 5 and 10 minutes did not produce statistically significant differences in MTBc detection time in MGIT compared to the standard protocol of 3 minutes. Mycobacteria times to positivity were 14.8±6.7 days, 15.4±8.8 days, 15.3±7.2 days for 3, 5, and 10 minutes of decontamination, respectively.

In addition, 10 samples, each decontaminated for 3, 5, and 10 minutes, were tested with Ultra. Extended times of decontamination did not affect Ultra performance: no significant difference in Ct values of *IS1081/IS6110* probe were observed (16.7±1.2 for 3 minutes, 16.6±1.0 for 5 minutes, 16.6±0.7 for 10 minutes).

DISCUSSION

MTBc isolation by culture is considered the gold standard for microbiological diagnosis of pulmonary TB; unfortunately, culture contamination can limit the diagnostic yield of Mycobacteria and the type of decontamination system influences detection (Bisognin *et al*., 2019; Genc *et al*., 2018; Someshwaran *et al*., 2016).

This is the first study to compare a new digestion and decontamination kit developed by Copan, MYCO-TB, with the system currently used in our laboratory, MycoPrep (by Becton Dickinson), both based on NALC-NaOH solution. The main findings were:

1) there was no difference in Mycobacteria detection in terms of number of positive cultures and time to positivity (TTP) in liquid culture;
2) liquid culture contamination was higher for MYCO-TB compared to MycoPrep (9.9% vs. 6.2%), however, in our laboratory we used a modified protocol of decontamination with Mycoprep solution (23 minutes instead of 15 suggested by the manufacturer), which could account for this difference. The overall rate of contamination (defined as the total failure of both liquid and solid media) was similar for both systems and fell within the Good Laboratory Practice range;
3) samples decontaminated with MYCO-TB were suitable for molecular assays, i.e., Xpert MTB/RIF Ultra assay and Genotype CMdirect;
4) extended time of decontamination (5 and 10 minutes) with MYCO-TB did not affect MTBc detection in terms of TTP in liquid culture and Ultra performance.

In literature there is only one previous study analysing MYCO-TB performance. However, it compares MYCO-TB to the Zephiran method, based on trisodium phosphate-benzalkonium, which is limited by incompatibility with MGIT, the system used worldwide for Mycobacteria detection in liquid culture (De Geyter *et al*., 2017).

In conclusion, MYCO-TB was effective in the digestion and decontamination of respiratory materials for the detection of Mycobacteria, particularly in reducing contamination by Gram-negative bacteria and mould. Ready-to-use reagents and formulation for single-samples reduce both the level of manual skills required and the risk of contamination during sample processing for Mycobacteria detection.

The rapid protocol (3 minutes) reduces the turnaround time compared to MycoPrep; however, longer time of decontamination (up to 10 minutes) with the MYCO-TB solution did not affect MTBc detection. The extended protocol could be applied to samples with a purulent aspect and high levels of contamination, such as those from patients with cystic fibrosis.

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