Evaluation of the Copan Myco-TB kit for the decontamination of respiratory samples for the detection of mycobacteria

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BACKGROUND AND AIMS

Respiratory samples submitted for mycobacterial culture contain normal flora, contaminants and large amounts of mucus. Since mycobacterial culture requires long incubation times, laboratory detection and identification pose challenges. Decontamination procedures are used to deal with this problem. Copan (Brescia, Italy) developed the Myco-TB procedure, a ready to use decontamination and fluidization kit for the detection of mycobacteria. The goal of this study was to compare it with the decontamination procedure in use in our hospital: the Zephiran method. Since the latter is incompatible with the BACTEC™ MGIT™ 960 system for mycobacterial detection (BD, New Jersey, USA), it was only used with the Myco-TB kit while both methods were followed by culture on solid media.

Figure 4A

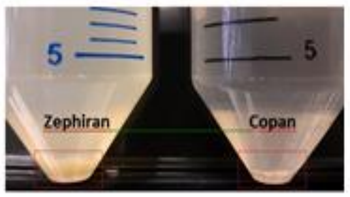
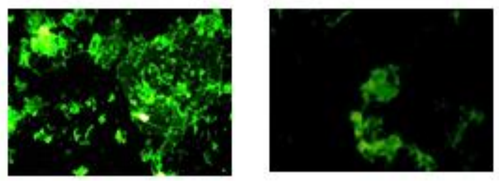


Figure 4B



MATERIAL AND METHODS

Respiratory specimens (n=295: 78 bronchial aspirates, 152 bronchoalveolar lavages, 55 expectorated sputa, 10 endotracheal aspirates) submitted to the UZ Brussel between January-July 2016 were included and divided into two aliquots. One was subjected to the Myco-TB method and one to the Zephiran technique. After decontamination, the samples were processed for auramine staining and culture by inoculating one Löwenstein-Jensen (LW) and one Ogawa (OG) slant. For the Myco-TB method, samples were also cultured in the MGIT system. **Figure 1** gives an overview of the Myco-TB procedure. **Figure 2** demonstrates the contents of the Myco-TB kit.

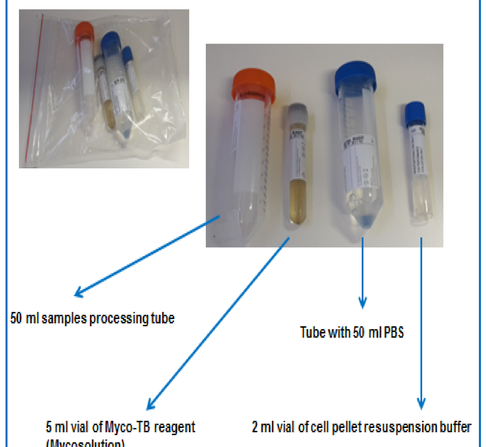
Figure 1:

1. Remove a tube of MYCO-TB from the plastic pack
2. Label a centrifuge tube
3. Transfer the sample inside the tube with sample ID
4. Add 5 ml of Myco-TB reagent

Sample volume	Mycosolution
5 ml	5 ml

5. Vortex sample for 30 seconds
6. Let it seat at room temperature for 3 minutes
7. Open tube and add 30 ml of PBS for large tube s, 10 ml for 15 ml tubes
8. Close tube, mix a few times by inverting the tube up and down
9. Centrifuge sample for 5 minutes at 3000 G
10. Discard the supernatant by using 10 ml pipets
11. Use one drop of cell suspension to prepare microscopy
12. Add 2 ml of PBS to re-suspend cell pellet
13. Use 500 µl to inoculate the MGIT vial
14. Use 250 µl to inoculate solid Ogawa culture medium
15. Use 250 µl solid Löwenstein culture medium

Figure 2:



RESULTS

A total of **25 Mycobacteria** (13 *M. tuberculosis complex*, 5 *M. avium*, 1 *M. chimaera intracellulare, 1 M. gordonae, 3 M. xenopi, 1 M. marseillense, 1 M. peregrinum)* were recovered. Eighteen of them were cultured with the Zephiran method on solid media **(sensitivity 72%),** 22 with the Copan method on solid media **(sensitivity 88%)** and 23 with the Copan method on the MGIT system **(sensitivity 92%).** On direct microscopy, 8 of the smears were positive with the Zephiran method **(sensitivity 32%)** and 5 of them **(sensitivity 20%)** with the Copan method. Twenty-six % of the samples were contaminated with the Zephiran method on solid media, 22% with the Copan method on solid media and 2% with the Copan method on the MGIT system. The specificities for culture were all 100% **(Figure 3)**.

Figure 3

		Zephiran	Copan	
Direct microscopy				
	Sens (%)	32%	20%	
	Spec (%)	88%	100%	
		Zephiran (LW + OG)	Copan (LW + OG)	Copan + MGIT
Culture				
	Sens (%)	72	88	92
	Spec (%)	100	100	100
Contamination rate (%)		26	22	2

DISCUSSION AND CONCLUSION

The Myco-TB decontamination method is easy and rapid to perform (Zephiran 95 min versus Copan 10 min). It can be used for one specimen at a time so it can eliminate the possibility of cross contamination. It is more sensitive with respect to culture compared to the Zephiran method and gives lower contamination levels, especially when combined with the MGIT technique. However, our results suggest that the Copan method is less sensitive in direct microscopy, probably due to lower pellet volume (**Figure 4A**: cell pellet volume, **Figure 4B (left sight of the poster)**: microscopic image of a negative sample after auramine staining). Higher sensitivity can probably be obtained when PCR techniques are combined. Currently, we are testing an adapted protocol to improve the cell pellet volume.

ACKNOWLEDGEMENTS

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