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TECHNICAL NOTE

Recovery of *Mycobacterium tuberculosis* from Löwenstein-Jensen media contaminated with other organisms

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SUMMARY

Growth of contaminating organisms along with *Mycobacterium tuberculosis* on Löwenstein-Jensen (LJ) medium is common. However, there is no documented evidence on the decontamination procedure adopted in mycobacteriology laboratories to recover *M. tuberculosis* from the contaminants grown on LJ medium. At the National Institute for Research in Tuberculosis, of 1048 LJ slopes with *M. tuberculosis* received from intermediate reference laboratories, 98 (9%) were contaminated. Of these, 87 (89%) *M. tuberculosis* cultures were retrieved after decontamination with 1% cetrimide. The use of cetrimide as a decontaminating agent to retrieve *M. tuberculosis* cultures grown with contaminants is documented.

KEY WORDS: *M. tuberculosis*; cetrimide; decontamination; Löwenstein-Jensen

THE INDIAN National Institute for Research in Tuberculosis (NIRT) receives Löwenstein-Jensen (LJ) cultures of *Mycobacterium tuberculosis* for second-line drug susceptibility testing (DST) as a part of drug resistance surveillance for the DOTS-Plus programme. About 9% of such cultures are found to be contaminated with other organisms. In addition, around 2% of *M. tuberculosis* cultures are found to be contaminated with other organisms in our laboratory. A simple method is needed to recover *M. tuberculosis* from contaminated LJ slopes.

We report the use of the cetrimide method as a decontaminating agent for sputum samples and detection of drug resistance.[1] Briefly, a cotton swab dipped in sputum specimen was treated with 1% cetrimide solution and inoculated onto drug-free and drug-containing LJ medium. The cetrimide swab method was reported to be useful to reduce the contamination rate and recovery of *M. tuberculosis* from sputum samples. It was shown that 1% cetrimide can kill non-acid-fast bacilli more effectively than sodium hydroxide, sulphuric acid and oxalic acid.[2] These observations led us to explore the use of the 1% cetrimide method to recover *M. tuberculosis* from contaminants on LJ slopes. The findings are presented here.

MATERIALS AND METHODS

A total of 1048 LJ slopes with *M. tuberculosis* were received by courier at the NIRT from four states (Andhra Pradesh, Gujarat, Kerala and Tamil Nadu) between July 2009 and March 2011. They were received within 3–4 days of dispatch from the intermediate reference laboratories (IRL). The LJ slopes were incubated overnight at 37°C to check for the presence of any contaminating organisms. On visual examination, 98 slopes were found to be either contaminated with other organisms or dislodged/crumbled. These slopes were subjected to a decontamination procedure by the 1% cetrimide method.

Decontamination by 1% cetrimide solution

One gram of anhydrous cetrimide powder (BD Diagnostics, Sparks, MD, USA) was dissolved in 100 ml of distilled water and sterilised by autoclaving at 121°C for 15 minutes and stored at room temperature for not more than 30 days. One loopful (3 mm, 24 standard wire gauge) of *M. tuberculosis* culture, either scraped carefully, avoiding the contaminating microbial colonies, or a full sweep of the entire surface on LJ medium, was suspended in 0.5 ml of 1% cetrimide solution with 10–12 glass beads (3 mm) and vortexed for 30–40 s. An additional 0.5 ml of cetrimide was added if the suspension was too turbid. The suspension was kept for 60 min at room temperature. Approximately 10 μl of the suspension was inoculated onto a pair of LJ slopes. The LJ slopes were incubated at 37°C and a weekly reading was taken for 8 weeks, or until growth of *M. tuberculosis* was seen. Growth was graded as 3+ (confluent), 2+ (>100 colonies),

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Table 1 Retrieval of *Mycobacterium tuberculosis* from LJ medium contaminated with other organisms using 1% cetrimide

IRL	Slopes received	Slopes contaminated	Cultures retrieved using cetrimide		
			Positive	NG	Cont
1	130	4	3	1	0
2	864	83	75	4	4
3	13	3	2	1	0
4	16	0	NA	NA	NA
5	25	8	7	1	0
Total	1048	98 (9%)	87 (89%)	7 (7%)	4 (4%)

LJ = Löwenstein-Jensen; IRL = intermediate reference laboratory; NG = no growth; Cont = contamination; NA = not applicable.

1+ (20–99 colonies) and actual number (<20 colonies). If no growth was observed even at the 8th week, the culture was designated no growth. All cultures that showed mycobacterial growth were confirmed as *M. tuberculosis* using standard phenotypic methods.[3]

RESULTS

M. tuberculosis was retrieved from 87 (89%) of 98 contaminated LJ slopes (Table 1). It was not recovered from seven slopes. Only contaminating organisms were recovered from four contaminated slopes.

The grading of *M. tuberculosis* cultures obtained after decontamination is shown in Table 2. The majority of the cultures (41/87) were graded as 2+ and 3+ (11/87); 33 cultures were graded as 1+ and only two cultures were graded as colonies. Of the 7 cultures that showed no growth after cetrimide treatment, 5 had 2+ growth, one had 1+ growth and only one had colonies. Of the four cultures that were contaminated in the cetrimide method, two cultures had 1+ initial grading, one had 2+ growth and one had 3 colonies. One culture yielded 3+ growth after treatment with cetrimide, which was originally recorded as contaminated culture.

DISCUSSION

Storage and transportation of cultures must be performed appropriately, as failure to do so can increase culture contamination rates and reduce culture yield. At the NIRT, of 5000 samples processed consecutively between January and June 2007, 36/1719 (2%) LJ slopes with *M. tuberculosis* cultures were found to be contaminated. There is therefore a need for an effective, inexpensive decontaminating substance/methodology for retrieval of *M. tuberculosis* from contaminants on LJ slopes. This report documents the efficiency of cetrimide to recover *M. tuberculosis* from predominantly contaminated LJ cultures using a modified technique previously described by others.[1,2]

As this study was conducted retrospectively, information on the contaminated microflora present in the LJ slopes and on the number of cultures for which selection/sweep of colonies was performed was not recorded. A limitation of this procedure could be selection of bacterial population as a result of subculture, which may influence the DST pattern.

In this study, a significant proportion (89%) of cultures was retrieved from contaminated LJ slopes. The efficiency of 1% cetrimide is evident from the retrieval of *M. tuberculosis* colonies (<20 colonies) contaminated with other organisms. The overall contamination rate was 4%. It is to be noted that the corresponding LJ slopes were heavily contaminated. Failure to recover seven cultures may be attributed to old cultures or prolonged and/or poor storage before transportation to the NIRT. The advantages of this method are that the reagent is inexpensive and its shelf life at room temperature is long. It is also cost-saving, as additional samples do not need to be processed and patients do not need to be called for repeat examination. The 1% cetrimide method has been practised for over a decade at the NIRT to recover *M. tuberculosis* from LJ slopes contaminated with other organisms. The method described here is easily adaptable and similar to that used to subculture *M. tuberculosis* on LJ medium.

Table 2 Grades of *Mycobacterium tuberculosis* cultures obtained after cetrimide decontamination

Initial culture grade*	Culture grade after decontamination using cetrimide*						
	3+	2+	1+	Colonies	NG	Cont	Total
3+	—	1				—	1
2+	3	25	18		5	1	52
1+	6	12	12	2	1	2	35
Colonies	1	3	3		1	1	9
Cont	1						1
Total	11	41	33	2	7	4	98

*Culture grades = 3+ (confluent growth); 2+ (>100 colonies); 1+ (20–99 colonies); countable colonies (1–19 colonies).
NG = no growth; Cont = contamination.

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RÉSUMÉ

On observe communément le développement d'organismes contaminants à côté de *Mycobacterium tuberculosis* dans le milieu de Löwenstein-Jensen (LJ). Toutefois il n'existe pas de preuves documentées concernant la procédure de décontamination adoptée par les laboratoires de mycobactériologie pour récupérer *M. tuberculosis* au sein des contaminants apparus sur le milieu de LJ. Sur les 1.048 tubes de LJ comportant *M. tuberculosis* et reçus à l'Institut National de Recherche de la Tuberculose en provenance de laboratoires de référence intermédiaires, on a trouvé 98 contaminations (9%). Sur ces 98, on a pu récupérer des cultures de *M. tuberculosis* dans 87 cultures (89%) après décontamination au moyen de cétrimide à 1%. On documente dans cet article l'utilisation de cétrimide comme agent décontaminant pour récupérer les cultures de *M. tuberculosis* qui se sont développées parallèlement avec des contaminants.

RESUMEN

El crecimiento de microrganismos contaminantes con *Mycobacterium tuberculosis* en los cultivos en medio de Löwenstein-Jensen (LJ) es frecuente. Sin embargo, no se cuenta con estudios científicos sobre el procedimiento de descontaminación que se aplica en los laboratorios de micobacteriología con el fin de recuperar *M. tuberculosis* de los cultivos contaminados. En el Instituto Nacional de Investigación de Tuberculosis se encontró contaminación en 98 de los 1048 (9%) tubos de LJ con *M. tuberculosis* que se recibieron de los laboratorios intermedios de referencia. De estos 98 cultivos de *M. tuberculosis* se recuperaron 87 (89%) tras una descontaminación con cetrimida al 1%. Se documenta el uso de cetrimida como descontaminante para recuperar las cepas de *M. tuberculosis* de los cultivos contaminados.