Revolutionizing Decontamination and Concentration Method for the Diagnosis of Tuberculosis

ASM General Meeting, June 16-19, San Francisco

Tanıl Kocagöz[1], Işın Akyar[1], Sedat Altın[2], Pınar Başören[3], Perihan Karaduman[4], Elif Yeşilyurt[4], Sinem Öktem Okullu[1], Nihan Aytekin[1], Sesin Kocagöz[1], Thomas Silier[3]

1- Acıbadem Univ., Sch. of Med., Istanbul, TURKEY, 2- T.R. Ministry of Health, Yedikule Chest Dis. Chest Surgery Ed. and Res. Hosp., Istanbul, TURKEY,
3- Salubris Inc., Woburn, MA, U.S.A., 4- Trends in Innovative Biotechnology Organization (TIBO), Istanbul, TURKEY (tanilkocagoz@gmail.com)

Introduction

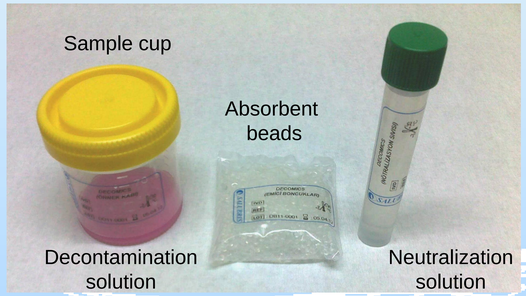

Figure 1. Decomics: Decontamination and concentration kit with absorbent beads

Culture is the gold standard method for the diagnosis of tuberculosis. Since sputum contains several rapid growing microorganisms, it requires decontamination and concentration before being inoculated to culture media, for isolation of mycobacteria. The decontamination and concentration methods used commonly worldwide are Petroff and Kubica methods.

Petroff method was developed in 1915. It uses 4% NaOH for decontamination and HCl for neutralization of pH. The preparation of its solutions is fairly simple. However, since the method does not use a buffer solution, it is hard to adjust the final pH of the processed specimen.

Kubica method, which was developed almost 50 years later (in 1963), included N-acetyl-L-cysteine (NALC) in decontamination solution, which helps the liquefaction of sputum samples, enabled lowering the concentration of NaOH to 2%. The method also uses phosphate buffer instead of HCl, which enabled better pH adjustment of processed specimen, and thus improved the recovery rate of mycobacteria and the time required for growth detection. A major drawback of Kubica method is the instability of NALC in NaOH solution and high contamination rate by phosphate buffer if stored in large containers after sterilization.

All current decontamination and concentration methods require centrifugation. This limits the application of decontamination and concentration and thus mycobacterial culture to tuberculosis laboratories with elaborate capabilities.

We have developed and tested a decontamination and concentration method and kit called “Decomics”, based on absorbent beads, which does not require centrifugation (figure 1).

Materials and Methods

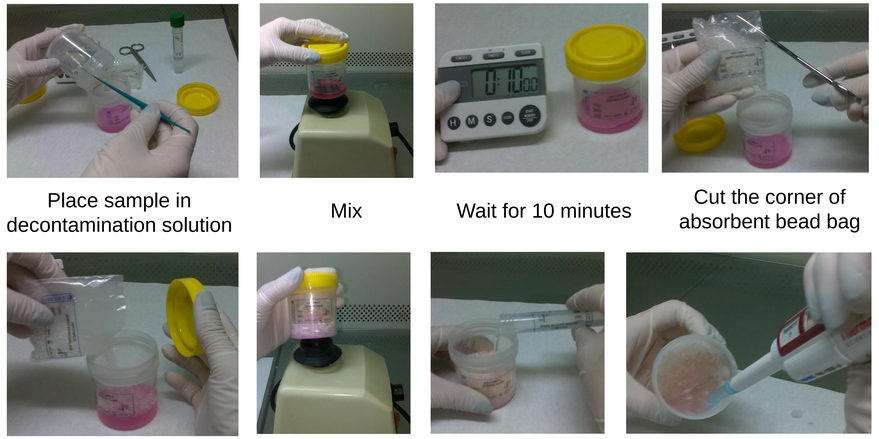

Figure 2. Decontamination and concentration procedure of Decomics

For evaluation of Decomics, 270 sputum samples of at least 2ml, were split into two equal parts and processed by Kubica method using a ready to use kit, Mycoprosafe (Salubris, Inc.) and Decomics.

Decomics consists of three components: 1- Sample cup containing decontamination solution with pH indicator; 2- Absorbent beads; 3- Neutralization solution (figure 1). Samples of 0.5 to 5.0ml are put into the decontamination solution and kept for 10 minutes. Then, absorbent beads are added into the sample and incubated for 5 additional minutes, making total decontamination duration 15 minutes. Neutralization solution is added and incubated for 5 minutes. Absorbent beads, which have pores smaller than bacteria, absorb most of the solutions and leave 2-3ml concentrated sample behind (figure 2).

Samples processed by Kubica method and Decomics, were inoculated to Löwenstein Jensen (LJ) and TK SLC (rapid mycobacterial culture medium). LJ tubes were followed visually and TK SLC tubes by Mycolor TK (the automated incubator reader) which automatically indicates duration of time to growth detection.

Results

Among 270 samples, 70 (25.9%) were AFB positive by both decontamination and concentration methods. The number of mycobacterial isolates obtained from samples processed by Decomics and Kubica methods were 95 (35.2%) and 88 (32.6%), respectively (table 1). Decomics enabled isolation of maycobacteria 7.4% more than Kubica method. The difference was statistically significant as evaluated by McNemar test (p=0.0156). Median time to growth detection among isolates which were obtained both by Decomics and Kubica methods were 14 and 16 days in TK SLC and 25 and 28 days in LJ, respectively. There was only one contaminated LJ culture among samples processed by Kubica method and there was no contamination among samples processed by Decomics. The total processing time with Decomics for one sample was approximately 20 minutes compared to 45 minutes with Kubica method. Sample processing was safer and easier with Decomics since the whole procedure was completed in the same cup.

Table 1. Comparative results of Decomics and Kubica methods

n=**270**	**Decomics**		**Mycoprosafe** (Kubica)	
Total Mycobacterial Isolates	**95** (35.2%)		**88** (32.6%)	
Culture Medium	LJ	TK SLC	LJ	TK SLC
Mycobacterial Isolates	93	92	87	87
Time to Growth Detection (Median: days)	**25**	**14**	**28**	**16**
Microscopy AFB (+)	70 (25.9%)		70 (25.9%)	

Discussion

The efficiency of Decomics in isolation of mycobacteria from clinical samples was better than classical NaOH-NALC (Kubica method). Elimination of need for centrifugation, decreased processing time by half and higher isolation rate of mycobacteria are important advantages of Decomics.
Decomics may find wide application in both elaborate and also in rural area tuberculosis laboratories with limited resources, enabling them to do mycobacterial culture.