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Factors contribute to efficiency of specimen concentration of *Mycobacterium tuberculosis* by centrifugation and magnetic beads

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ABSTRACT

Background: A concentration of specimen is recommended for the effective recovery of *Mycobacterium tuberculosis* (MTB), but the bacteriological efficiency is not well evaluated. The present study evaluated the factors contributing to concentration efficiency of centrifugation and bead-based technique (TB-Beads; Microsens, UK) to recover MTB by using simple *in vitro* specimens.

Methods: Four specimens were prepared (6.5×10^3; 8.1×10^4; 7.9×10^5; and 6.4×10^6 cfu/mL) of different concentrations with or without 5×10^4 of THP-1 cells (RIKEN BRC, Japan). Specimens were subjected to centrifugation at 2000, 3000, and 4000g for 15 min, and to TB-Beads. The concentration and recovery rate were calculated to evaluate the efficiency of each method.

Results: The specimens containing a higher number of bacteria and THP-1 cells had a tendency to yield a higher concentration and recovery rate ($p = 0.001$–0.083). MTB was recovered more efficiently with THP-1 cells from the 6.5×10^3 cfu/mL specimen by centrifugation ($p \leqslant 0.001$) than without them; 24.7–54.4% of MTB were recovered with THP-1 cells by centrifugation at3000g for 15 min, while the recovery using TB-Beads was a maximum of 12.7%.

Conclusions: The efficiency of centrifugation depends on the bacterial density and the co-existence of THP-1 cells. The efficiency of TB-Beads was not as high as centrifugation.

Introduction

Many diagnostic tools have been developed over the years for the detection of *Mycobacterium tuberculosis* (MTB), but the diagnosis of tuberculosis (TB) still largely depends on examination of sputum smear, especially in low- and middle-income countries, although this method lacks sensitivity [1]. To increase the sensitivity of smear and subsequent culture examination, an optimal concentration of the specimen is essential. The centrifugation technique is generally recommended to collect

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Abbreviations: MTB, *Mycobacterium tuberculosis*; RCF, relative centrifugal forces

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bacteria from clinical specimens [2,3]. However, this technique requires an expensive device, not stable in settings with irregular power supply, and has biohazard risks. At the same time, the quantitative efficiencies of centrifugation still remain to be elucidated. On the other hand, several bead-based bacterial collection techniques also have been recently developed to recover MTB from clinical specimens without centrifugation. 'TB-Beads', which are ligand-coated paramagnetic beads invented by Microsens Medtech Ltd, can concentrate MTB in any sample type and overcome the above disadvantages of centrifugation [4–8]. In this study, the factors contributing to concentration efficiency of centrifugation and bead-based technique (TB-Beads; Microsens, UK) to recover MTB from simulated specimens were evaluated.

Materials and methods

Preparation of the samples

Simple *in vitro* specimens were prepared that contain four different concentrations of MTB and saline with or without THP-1 cells (RIKEN BRC, Japan), which are human monocyte cells. First, the original MTB H37Rv (ATCC 24,279) suspension ($OD_{530\ nm} = 0.18$) was serially diluted with saline to prepare four specimens of different concentrations (confirmed as 6.5×10^3; 8.1×10^4; 7.9×10^5; and 6.4×10^6 cfu/mL by culture, and named as Specimen A, B, C, and D, respectively), imitating smear scanty, 1+, 2+, and 3+, respectively. Specimens A and B were considered as paucibacillary samples, and specimens C and D as polybacillary samples. Then, at final concentration, 1×10^4 cells/mL of THP-1 cells were added to another series of specimens. These two series (bacteria with and without THP-1) of specimens were subjected to centrifugation and magnetic bead method (TB-Beads).

The centrifugation followed the conventional procedures: 5 mL of the specimen was centrifuged at three different relative centrifugal forces (RCF): 2000, 3000, and 4000*g*, for 15 min at 4 °C. The sediment was re-suspended in 0.5 mL of sterile saline after discarding the supernatant. The experiment was performed in triplicate.

The TB-Beads were used according to the manufacturer's instructions. Briefly, 5 mL of the specimen was mixed with the same volume of TB-Beads solution, and left for 2 min at room temperature to enable capturing of the mycobacteria by the beads. After collecting the bead-bacteria complex by using magnetic force, the supernatant was discarded by decantation. The bead-bacteria complex was re-suspended in 5 mL of TB-Beads wash solution, and the same process was repeated for rinsing. The bacteria were removed from the beads by adding 100 μL of elution buffer. The experiment was performed in triplicate.

100 μL of each specimen was inoculated onto Middlebrook 7H10 agar medium supplemented with OADC enrichment (Becton Dickinson, Sparks, MD) by appropriate dilutions, and the number of recovered colonies was enumerated.

The final concentration (cfu/mL) of bacteria in each treated specimen was calculated from the colony counts. The final concentration (density) was divided by the original one to calculate concentration rate. Similarly, the number of collected colonies after each treatment was divided by that of the original one to determine the recovery rate.

Data analysis

The mean values were compared by unpaired *t* tests. Analysis of variance (ANOVA) and multiple comparisons (Tukey test) were used to analyze the different concentration methods at different concentrations of the samples. All the analyses were performed using SPSS version 16 for Windows (SPSS Inc., Chicago, IL, USA).

Ethical considerations

Ethical approval was not required for this laboratory-based study.

Results

The final concentration of MTB recovered from each specimen is shown in Fig. 1. The bacterial concentration of specimen A without THP-1 cells after centrifugation was significantly lower than that of specimen A with THP-1 cells ($p \leqslant 0.001$), while the other different concentration specimen did not show any significant difference.

The concentration rate and recovery rate of centrifugation are shown in Figs. 2 and 3. The specimens containing a high number of bacteria (specimens C and D) with THP-1 cells had a tendency to yield a higher concentration rate and recovery rate than the specimens containing a low number of bacteria (specimens A and B) by centrifugation. MTB was recovered more effectively from specimen A with THP-1 cells than without THP-1 cells by using centrifugation at the same RCF. The concentration rate and recovery rate of MTB from specimen C with THP-1 cells by centrifugation at 3000*g* was lower than that obtained at 2000*g* ($p = 0.027$), otherwise, the concentration rate and recovery rate among specimens from the same bacterial suspension were not significantly different at the RCF of 2000–4000*g*.

The concentration rate and recovery rate obtained using TB-Beads decreased with an increase in the bacterial number (Figs. 2 and 3). TB-beads were more efficient in isolating MTB present at a low concentration with THP-1 cells.

24.7–54.4% of MTB with THP-1 cells were recovered by centrifugation at 3000*g* for 15 min. The efficiency of recovery by centrifugation was lower in the paucibacillary specimens, while the efficiency of recovery using magnetic beads was higher in the paucibacillary specimens with a maximum of 12.7%. The amount of MTB collected using TB-Beads was 51.4% of that collected using centrifugation.

Discussion

These results suggest that the efficiency of centrifugation did not depend greatly on the RCF ranging from 2000–4000*g* for 15 min with bacterial suspension in saline. Several studies indicated that a higher RCF resulted in better efficiency of recovery rate of MTB, and centrifugation at 3000*g* for 15–20 min is generally recommended [3,9,10]. However, the