EVALUATION OF CHITIN-SEDIMENTATION AND SONICATION FOR CONCENTRATING *MYCOBACTERIUM TUBERCULOSIS* IN DIAGNOSTIC SPUTUM SAMPLES

R Chew, L Caviedes, P Fuentes, J Coronel, M Zimic, B Herrera, T Valencia, I Sabogal, L Huaroto, C Evans

Background. Direct sputum-smear microscopy for tuberculosis diagnosis is insensitive, although rapid and inexpensive. Chitin-sedimentation and sonication of sputum samples may concentrate mycobacteria into the volume visualised by microscopy thus improving sensitivity and slide-reading time. These relatively inexpensive interventions might therefore be of diagnostic use in high-prevalence, resource-poor settings, but their efficacies have not been fully defined.
Objective. To evaluate quantitatively the mycobacterial concentrating abilities of chitin-sedimentation and sonication, using clinical sputum samples (*n*=32) from newly-diagnosed tuberculosis patients.
Methods. For chitin-sedimentation studies (*n*=13), 1 ml of sputum was mixed with 0.25 ml of dissolved chitin, vortexed for 5 seconds and left to stand for 30 minutes before resuspension after discarding the supernatant. The remainder of the samples (*n*=19) were placed in bijou tubes and sonicated for 60 minutes in a jewellery sonicator. Triplicate slides were made from each sample pre- and post-processing using a standardised 40 µl of sputum. The slides made pre-processing were used as controls. The number of mycobacteria per 100-300 fields was counted in a blinded manner by two experienced microscopists for the total of 96 Ziehl-Neelsen stained smears produced. To ensure reliability, the microscopists cross-read approximately 20% of the slides.
Results. There was good inter-observer agreement (R=0.967, p=0.0001). Compared with controls, chitin-sedimentation and sonication caused a slight decrease in mycobacterial counts, but this was not statistically significant (Figures 1 and 2; R=-0.01, p=0.972 and R=-0.286, p=0.235 respectively). Slide-reading time was reduced by an average of 0.6 and 1.2 minutes respectively with chitin-sedimentation and sonication.
Conclusion. Using our methods, chitin-sedimentation and sonication did not concentrate mycobacteria in clinical sputum samples. The study was limited by the use of only microscopy-positive samples, as the aim of these interventions is to increase diagnostic sensitivity sufficiently to detect mycobacteria in samples currently deemed microscopy-negative but which turn out to be culture-positive. Nevertheless, these techniques have potential to be further developed for clinical use, given their positive effects on slide-reading efficiency. The next step will therefore be to identify the optimal conditions under which they will concentrate mycobacteria.

Abstract accepted for oral presentation at *the European Tropical Medicine and International Health Congress*, Verona, Italy, September 2009.

EVALUATION OF CHITIN-SEDIMENTATION AND SONICATION FOR CONCENTRATING *MYCOBACTERIUM TUBERCULOSIS* IN DIAGNOSTIC SPUTUM SAMPLES

(continued)

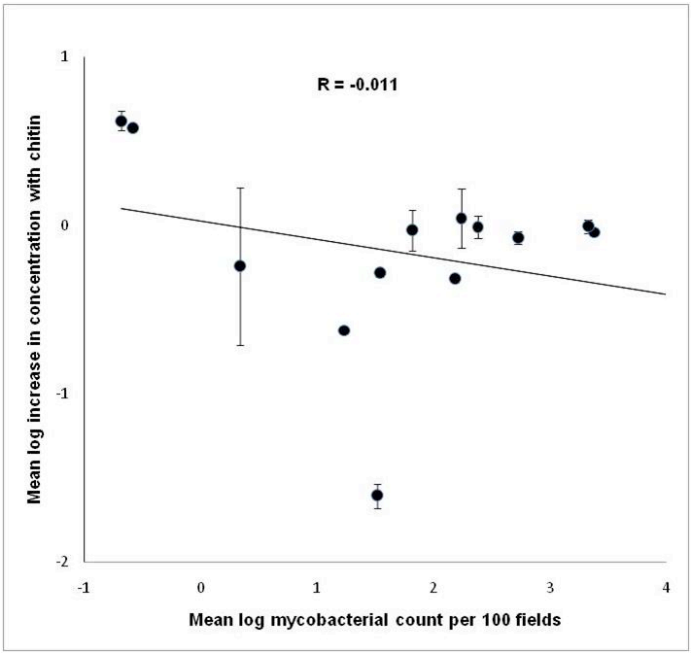

Figure 1. Correlation between mycobacterial density and concentrating efficacy of chitin-sedimentation.
Each data point represents the mean number of mycobacteria per 100 fields from one untreated sputum sample, plotted against the mean increase in concentration after chitin-sedimentation. The error bars represent the standard error of the mean with regard to the increase in mycobacterial concentration. The diagonal line is the regression line. The R value was calculated using Spearman's test.

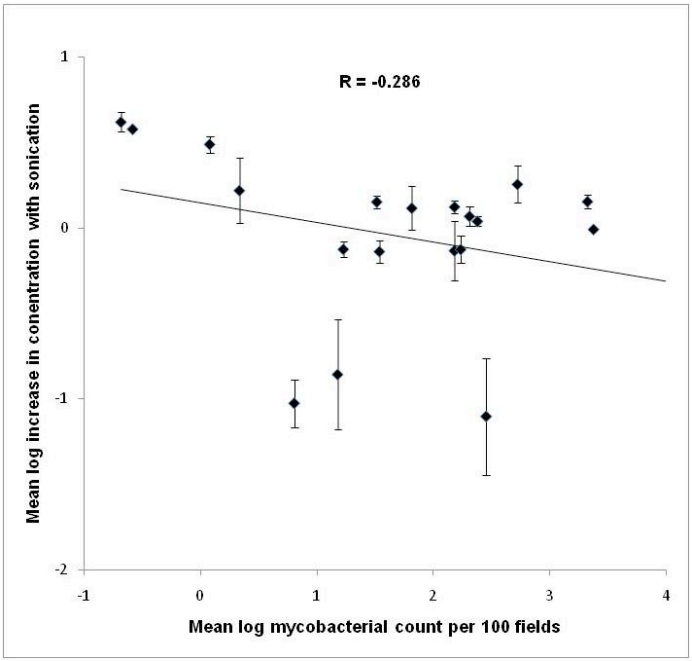

Figure 2. Correlation between mycobacterial density and concentrating efficacy of sonication.
Each data point represents the mean number of mycobacteria per 100 fields from one untreated sputum sample, plotted against the mean increase in concentration after sonication. The error bars represent the standard error of the mean with regard to the increase in mycobacterial concentration. The diagonal line is the regression line. The R value was calculated using Spearman's test.

Abstract accepted for oral presentation at *the European Tropical Medicine and International Health Congress*, Verona, Italy, September 2009.