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Presentation Abstract

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Title:	Assessment of sputum quantitative viability microscopy for predicting tuberculosis infectiousness.
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Abstract: Sputum from patients with pulmonary tuberculosis (TB) contains metabolically active and inactive *Mycobacterium tuberculosis* populations that cannot be differentiated by acid-fast microscopy and have uncertain clinical significance. These populations may be differentiated by viability microscopy with fluorescein diacetate, which fluoresces only after hydrolysis by metabolically active bacteria. We assessed the significance of *M. tuberculosis* quantitative viability microscopy (QVM) and compared it with quantitative culture and acid-fast auramine microscopy results. In a LABORATORY EXPERIMENT, unsterilized and heat-sterilized sputa were mixed in varying proportions. QVM correlated with concentrations of culturable *M. tuberculosis* (r_S=0.85, p<0.001), whereas acid-fast microscopy results were unaffected by sterilization. With each 10-times increase in the percentage of sterilized sputum, linear regression demonstrated an approximately 10-times decrease in QVM-positive bacteria (p<0.001). In 100% unsterilized samples, concentrations of acid-fast bacteria were 37-times higher than concentrations of QVM-positive bacteria. In a CLINICAL STUDY, 35 sputum-smear positive TB patients provided pre-treatment sputa. The concentration of QVM-positive bacteria was 5.1% of the concentration of acid-fast bacteria (inter-quartile range=2.4-11%). Patients' household contacts were followed-up with prevalence surveys 3 and 6 years later, that identified TB disease in 6.4% (13/209) of these contacts. Secondary TB disease was more likely for contacts of patients with lower than median QVM results (crude hazard ratio=3.8, p=0.03). This association persisted after adjusting for disease severity, drug-resistance and TB social determinants (adjusted hazard ratio=3.9, p=0.02). Thus, only a small proportion of *M. tuberculosis* in sputa was QVM positive and paradoxically, lower QVM results were associated with greater infectiousness. This may be explained by QVM-negative bacteria in untreated TB patients representing a slowly metabolising form of *M. tuberculosis* that is better adapted for disease transmission.