Rapid monitoring of anti-tuberculosis therapy using fluorescein diacetate microscopy: a simple method to determine infectiousness and screen for drug resistance

Jonathan M. Sherman[1,2], Rosario Montoya[2], Robert H. Gilman[1,2,3], Laura J Martin[4], Louis Grandjean[4], Teresa Valencia[1], Beatriz Herrera[1], Willi Quino[1], Eric Ramos[1], Jessica Alva[1,2], Maribel Rivero[1,2], A. Roderick Escombe[2,4] ,Carlton A. Evans[1,2,3,4]

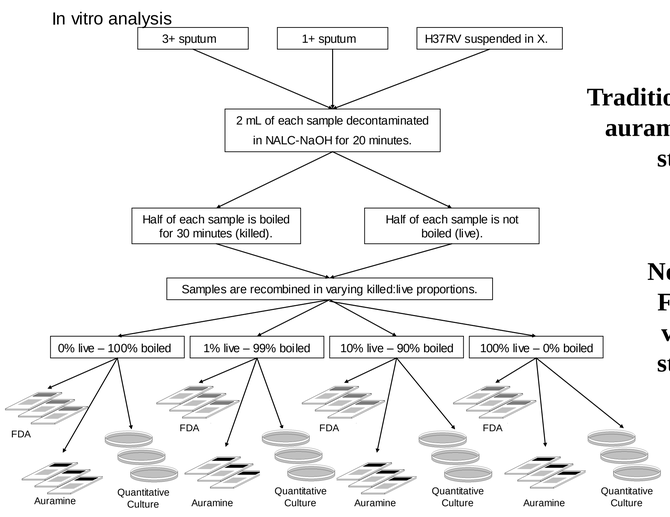
1. Laboratorio de Investigación y Desarrollo (LID), Departamento de Microbiologia, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Facultad de Ciencia, Av. Honorio Delgado #430, Urb. Ingeniería, San Martin de Porres, Lima, Peru.
2. Asociación Benéfica Prisma, Lima, Peru
3. Johns Hopkins Bloomberg School of Public Health, Baltimore, USA
4. Wellcome Centre for Clinical Tropical Medicine and Department of Infection and Immunity, Imperial College London Hammersmith Hospital Campus, 150 Du Cane Rd, London W12 0NN, UK.

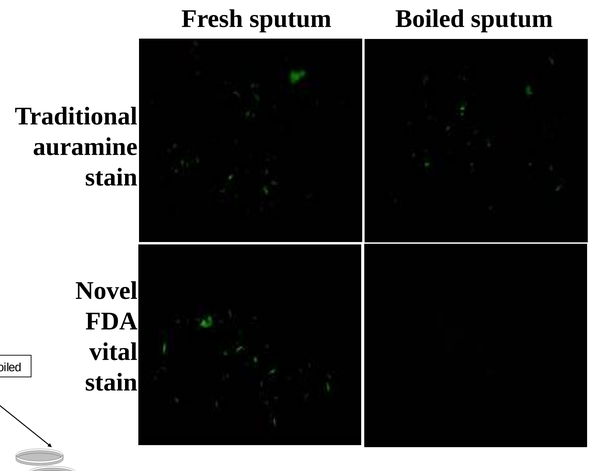
Background: Tuberculosis treatment and infection control are hampered by difficulty assessing mycobacterial viability to determine infectiousness and early treatment response. TB culture takes weeks; molecular tests are technically demanding; and acid-fast staining cannot differentiate live from dead tuberculosis.

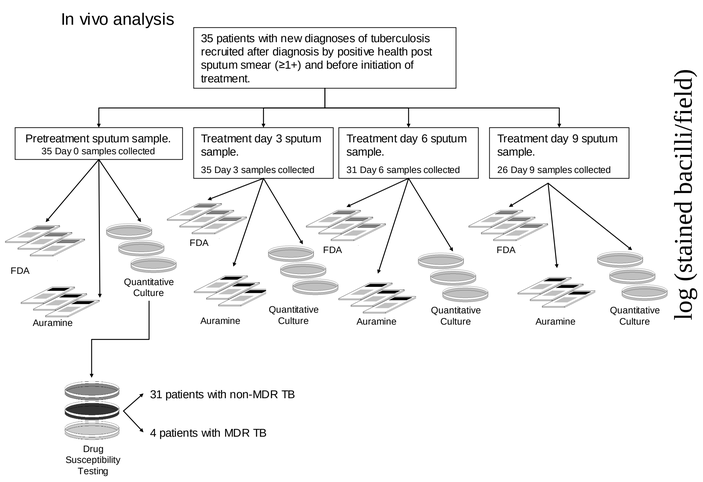
Objectives: To develop and evaluate a simple slide-microscopy test to rapidly determine tuberculosis viability.

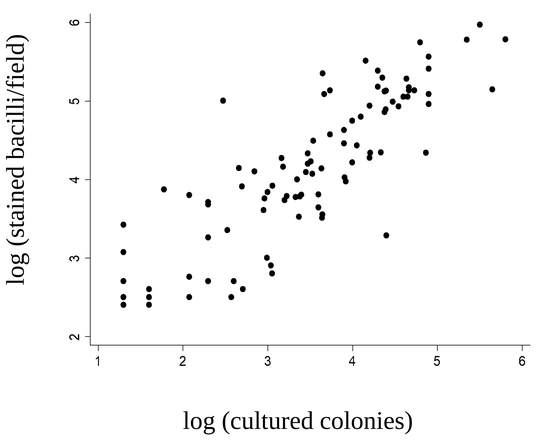
Methods: A protocol was optimized to stain viable but not dead tuberculosis in decontaminated sputum dried onto microscope slides and stained with the vital stain fluorescein diacetate (FDA). The reliability of this FDA slide microscopy for determining the concentration of viable tuberculosis in sputum was then compared with quantitative culture.

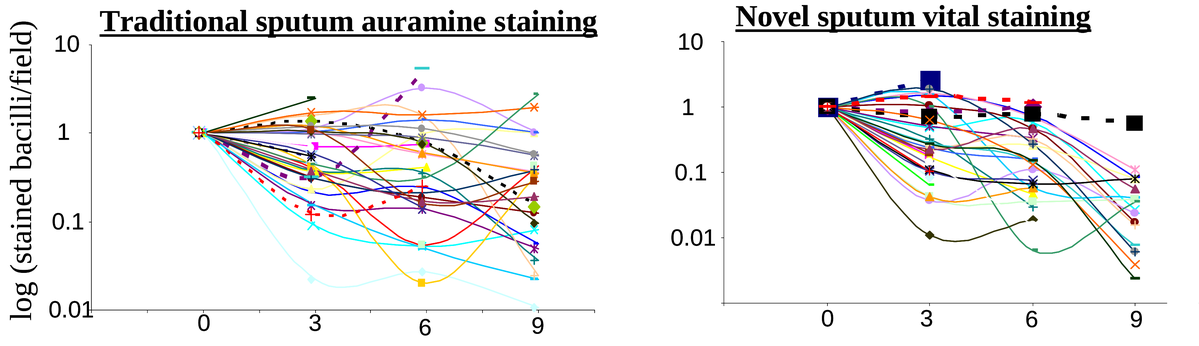
Results-laboratory evaluation: In untreated patients, tuberculosis auramine staining was unaffected whether sputum was fresh or had been sterilized by boiling, whereas FDA stained only un-boiled, viable tuberculosis. Quantification of viable tuberculosis by culture was reliably predicted by FDA, but not by auramine microscopy.

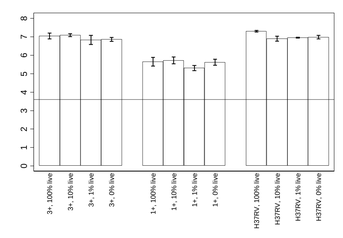


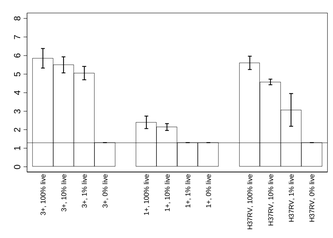


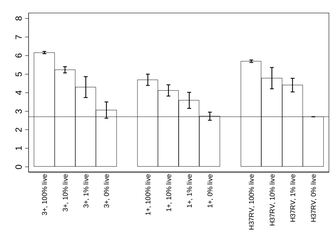












Results-clinical evaluation : Sequential sputums were collected from 35 patients before and after 3, 6 and 9 days of first-line tuberculosis treatment. Culture quantification of viable mycobacteria in sputum was predicted by slide microscopy with FDA (r^2=0.77) but not auramine (r^2=0.33). Quantification of viable tuberculosis in sputum by both quantitative culture and FDA microscopy fell 10-100 fold during the first nine days of treatment in all patients with drug-susceptible tuberculosis, whereas there was little change for patients with MDRTB. Specifically, 70% of samples from patients with drug-susceptible tuberculosis had a decline in the FDA count of viable tuberculosis of at least 0.2 logs/treatment-day, compared with none of the samples from MDRTB patients (P<0.001).

Conclusion: FDA slide microscopy determined the viability of tuberculosis in sputum in minutes, compared with >1 month required for culture. This simple and inexpensive technique rapidly assessed patient infectiousness on treatment, potentially guiding infection control measures. FDA staining also revealed differences in early treatment response between non-MDR and MDRTB and may allow early field screening for MDRTB and impending treatment failure.

Funded by the Wellcome Trust and the charity IFHAD: Innovation for Health & Development
CarltonEvans@yahoo.com