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**ABSTRACT BOOK**

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## **PC-95490-05 Sputum vital stain microscopy to predict sputum culture results and infectiousness**

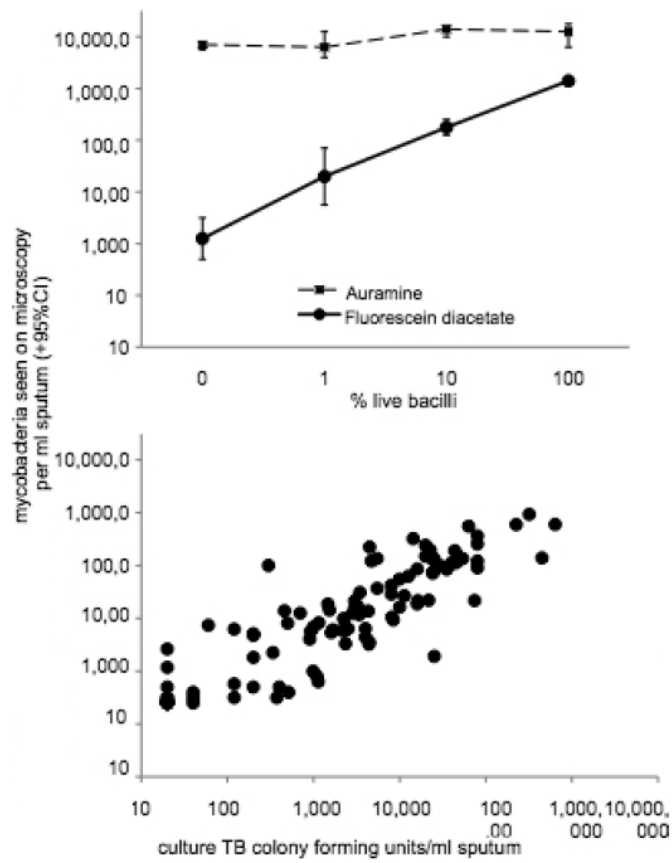
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**Background:** Assessing whether patients remain an infection risk requiring isolation after commencing tuberculosis therapy is difficult because decisions are based on culture results from sputum samples collected many weeks previously. We therefore optimized and validated fluorescein diacetate (FDA) vital staining for predicting tuberculosis culture results.

**Methods:** A protocol was optimized to stain live but not dead bacilli in decontaminated sputum samples dried onto microscopy slides using fluorescein diacetate staining and standard fluorescence microscopy. The reliability of fluorescein diacetate slide microscopy in predicting quantitative culture results was compared to that of auramine microscopy. Fluorescein diacetate was assessed in two blinded experiments: 1) validation—combination dilutions of live and sterilized, boiled sputum from untreated patients were mixed in different proportions, and 2) evaluation—sequential sputum samples from treated patients were collected before and after 3, 6, and 9 days of first-line tuberculosis treatment.

**Results:** Fluorescein diacetate slide microscopy took 40–60 minutes and required basic skills and only a microscope. Quantitative culture took 3–6 weeks and required a biosafety cabinet, centrifuge, vortex, incubator and moderate laboratory expertise. In the validation experiments, FDA-microscopy accurately reflected the proportion of live tuberculosis in each sample. In the evaluation experiments, FDA-microscopy reliably predicted quantitative culture results ( $r^2 = 0.77$ ). In contrast, auramine microscopy did not reliably predict quantitative cultures ( $r^2 = 0.33$  before treatment and  $r^2 = 0.26$  during treatment).

**Conclusions:** As compared to weeks of information lag when using quantitative cultures, fluorescein diacetate microscopy reliably predicts future cultures results in minutes, allowing for a dynamic, real-time approach to infection control.



**Figure** Validation experiment (top): FDA and auramine microscopy in live/heat killed combinations of bacilli (shown: results from decontaminated 3+ bacilli). Evaluation experiment (bottom): Correlation of FDA to CFU in sputum samples of patients before and after treatment.