**Predicting mycobacterial load from the time of positive culture using MODS (microscopic- observation drug-susceptibility assay)**

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**Background.** MODS (microscopic-observation drug-susceptibility assay) is a rapid, non-commercial method to culture *Mycobacterium tuberculosis* using 7H9 broth media, which can also identify drug sensitivity directly from sputum samples and it has been endorsed by the World Health Organization. To assess mycobacterial load in sputum with MODS, colony forming units (CFU) are counted, however this is a complex process, therefore our objective was to assess the role of time to positive culture (TTP) data to predict the number of CFU in MODS cultures.

**Methods.** Fresh sputum samples were collected from tuberculosis-affected persons in Ventanilla, Lima, Peru. Samples were processed by the standard MODS protocol with biosafety precautions. Samples were decontaminated by 4% NaOH-NALC followed by centrifugation. The supernatant was resuspended and inoculated in MODS media (Middlebrook 7H9, supplemented by OADC and PANTA). MODS cultures were read 3 times a week from 5 days until 21 days after inoculation. The TTP was calculated as the days taken for the first colony to appear in the MODS culture, and CFU was the number of colonies seen the last day of culture. Linear regression analysis was used to predict CFU from the TTP of the same sample.

**Results.** During the study period there were 1934 samples positive for *Mycobacterium tuberculosis* in MODS. The median TTP was 11 days (interquartile range, IQR 8-14), and median CFU 2.2 (IQR 1.3-3.1). There was a strong inverse correlation between TTP and CFU, that for every 4.8 (95% confidence interval 4.5 – 5.0) days needed in TTP there was a 10-fold decrease in CFU (R2=0.63, p<0.0001). Using this simple linear regression model, figure compares the actual CFU data with the predicted value from paired TTP data. 86% of actual CFU values were within 1 base-10 logarithm of the predicted CFU values.

**Conclusion.** We conclude that TTP culture data can be used as a surrogate marker for CFU. Calculating TTP is a simple and feasible technique for evaluating mycobacterial load in sputum.

