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ABSTRACT BOOK

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FA-95601-07 Rapid pyrazinamide susceptibility testing with the MODS technique directly from sputum

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Background: Pyrazinamide susceptibility testing is usually too slow to guide initial therapy. Consequently,

many patients with pyrazinamide-resistant TB receive inappropriate pyrazinamide therapy. We therefore optimized the microscopic-observation drug-susceptibility (MODS) assay as a test for TB drug susceptibility to pyrazinamide.

Methods and results: Pyrazinamide susceptibility testing is done in acid media (pH = 6.0) because pyrazinamide usually has no anti-tubercular activity at neutral pH and requires acid pH to prevent TB growth. All TB strains could be sub-cultured in this acid culture media, but when this acid media was used with decontaminated sputa in the MODS assay, 77% (158/204) of positive sputa failed to grow. However, pyrazinamide was found to be active against TB at neutral pH when testing freshly decontaminated sputa. For an OPTIMIZATION experiment, patients with suspected MDR-TB gave 204 sputa that underwent conventional NaOH decontamination and were cultured in MODS at neutral pH in 0, 50, 100, 400 and 800 ug/ml pyrazinamide. 100 and 400 ug/ml pyrazinamide prevented the growth of 85% and 91% of pyrazinamide-susceptible TB, respectively. A subsequent evaluation experiment involved 102 patients with suspected MDR-TB who had sputum tested with the MODS assay using pyrazinamide 300 ug/ml that was compared with subsequent testing of their TB isolates with Bactec-460, Wayne, sequencing and TEMA culture. MODS agreed with other tests as often as they agreed with each other (graph). The MODS assay took 1–3 weeks and cost \$2US vs. 4–13 weeks for other assays that cost \$5–50US.

Conclusions: The alkaline stress of conventional NaOH decontamination caused *M. tuberculosis* to become susceptible to pyrazinamide in subsequent broth culture at neutral pH. This discovery provides new insight into the mechanism of action of pyrazinamide and allows the MODS assay to be used for inexpensive pyrazinamide susceptibility testing sufficiently rapidly to guide initial therapy.

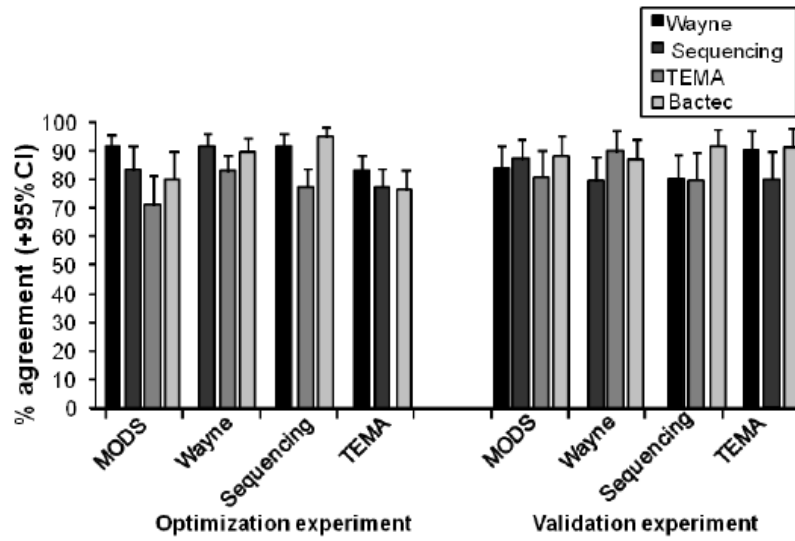


Figure Accuracy of rapid, direct pyrazinamide susceptibility testing with MODS: agreement between tests.