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Improved Bactec MGIT 960 Pyrazinamide Test Decreases Detection of False *Mycobacterium tuberculosis* Pyrazinamide Resistance

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Pyrazinamide (PZA) is a key drug for the treatment of tuberculosis (TB). Resistance to PZA is caused mostly by mutations in the *pncA* gene, encoding pyrazinamidase, which converts the prodrug PZA to the active form, pyrazinoic acid (1, 2). For testing PZA susceptibility, the World Health Organization (WHO) recommends performance of the assay in liquid medium at pH 5.9 in the Bactec MGIT 960 (M960) system (Becton, Dickinson, Sparks, MD, USA) (3). However, false resistance to PZA was reported for this phenotypic assay (4–7) due to a high *Mycobacterium tuberculosis* inoculum, which may impair pyrazinamidase activity by increasing the pH of the medium (8). Indeed, a reduced M960 inoculum decreased detection of false resistance (9, 10) when results were compared with those of the previous reference radiometric method Bactec 460 (Becton, Dickinson) (11) and with *pncA* sequencing, a method providing from 83% to 90% sensitivity (2, 12, 13). Since 2013, the WHO has yearly offered to the global Supranational Reference Laboratory (SRL) network proficiency test panels of *M. tuberculosis* strains for PZA drug susceptibility testing (DST).

At the SRL in Rome, the PZA M960 assay is performed according to the manufacturer's instructions (14), with minor modifications. Briefly, a positive MGIT tube obtained 1 or 2 days after the positivity signal of the M960 instrument (seed tube) is vortexed for 30 s and then allowed to settle for 20 to 30 min. Thereafter, a 1-ml aliquot of the settled seed tube is taken with a 1-ml pipet from the top surface, instead of lower down. Of this aliquot, 0.5 ml is transferred to the PZA test tube containing 100 μg/ml of PZA and 0.5 ml is transferred to a tube containing 4.5 ml of sterile saline. This 1:10 dilution tube is repeatedly mixed with a new pipet, and 0.5 ml is used to inoculate the tube without PZA (growth control tube). After inversion, both the growth control and PZA test tubes are incubated in the M960 instrument. The seed tube is used first for PZA DST and then for other drugs.

In 2013 to 2016, the SRL tested the PZA susceptibility of 106 WHO *M. tuberculosis* strains (41 were PZA resistant and 65 were susceptible) with known *pncA* mutations. Using the modified M960 PZA assay (MMPA), 1 of 106 strains was falsely resistant (0.9%) and no strain was falsely susceptible. In Italy, the SRL coordinates a laboratory network (SMIRA [Italian Multicenter Study on Resistance to Antituberculosis Drugs]) periodically examined by first- and second-line drug proficiency testing exercises (15, 16). In 2016, 17 SMIRA laboratories performed in parallel the standard M960 PZA assay (14) and the MMPA on 10 *M. tuberculosis* strains from the 21st WHO round (4 were resistant and 6

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susceptible). Out of a total of 170 strains (68 were resistant and 102 susceptible) examined by each of the two methods, 8/170 showed false resistance by the standard assay (4.7%) and 2/170 showed false resistance by MMPA (1.2%); no strain was falsely susceptible. Overall, these observations suggest that the MMPA performed by withdrawing inoculum from the top surface of the settled MGIT 960 seed tubes may be useful in decreasing the finding of false phenotypic PZA resistance.

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