Supplemental Digital Content 1. Development of an in-house urine INH metabolite test

To detect urine INH metabolites in urine, a modified Arkansas method was used to detect a color change produced when the INH metabolite isonicotinic acid reacts with cyanogen chloride and barbituric acid impregnated into the dipstick (see figure, Supplemental Digital Content 2). The INH metabolite is typically detectable in urine within 24-30 hours of ingestion.

Deposition of chemical reagents was facilitated by the dispense platform equipped with FrontLine dispensers (BioDot ZX1010). Cotton fiber membranes (Ahlstrom In Vitro Diagnostic Cotton Fiber Grade 222) were cut into 60 mm x 200 mm strips and fixed onto PVC backing cards (Diagnostic Consulting Network MIBA-020). The reagents, barbituric acid (1.5% w/v (g/mL), pH 5.2, Sigma Aldrich 185698); chloramine-T trihydrate (15% w/v, Sigma Aldrich 402869); and a solution of potassium thiocyanate (60% w/v, Sigma Aldrich P3011) with citric acid monohydrate (8% w/v, Sigma Aldrich C1909); were solubilized in filtered deionized water. Then using the striper, reagents were dispensed onto the assembled cards until each 4 mm wide section of the card contained the following amount of each reagent as shown in Supplemental Digital Content 2, Panel A (figure); barbituric acid: 1.80 mg; chloramine-T trihydrate: 2.52 mg; potassium thiocyanate: 1.08 mg. It should be noted that in order to prevent the reagents from blending together, the cards were dried for 5 minutes in an oven at 55 ˚C. Once the cards were completed and dried, they were stored in a desiccator box until they were ready to be cut into strips. The final 4 mm wide strips were cut using a guillotine strip cutter (Kinbio ZQ2002).

To verify the performance of these strips, we evaluated these strips using mock INH metabolite solutions of isonicotinic acid (Sigma Aldrich I17508) at 1, 5, 10, 25, 50, and 100 µg/mL. 0.5 mL of each sample was added to its respective culture tube (10 mm x 75 mm) before a test strip was placed in the tube. The target limit of detection was 5 µg/mL, was chosen to match the limit of detection reported by Becton Dickinson for their discontinued commercial INH detection strip BD-Taxo™ - INH Test Strips (10-231743). The strips that performed as expected were stored in sealed mylar bags with desiccant packets until used.