

Supporting Information

A bench-top automated sputum-to-genotype system using a Lab-on-a-Film assembly for detection of multidrug-resistant *Mycobacterium tuberculosis*

Alexander V. Kukhtin*, Ryan Norville*, Ariel Bueno*, Peter Qu*, Nicole Parrish†, Megan Murray‡, Darrell P. Chandler*, Rebecca C. Holmberg*, and Christopher G. Cooney**

* Akonni Biosystems, Inc., 400 Sagner Avenue, Suite 300, Frederick, Maryland, 21701, USA

† Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, 21287, USA

‡ Harvard Medical School, Department of Global Health and Social Medicine, Boston, Massachusetts, 02115, USA

** Corresponding author.

S1. Hybridization ratios for all markers for DNA-to-Genotyping tests.

Supporting Information S1

| Sample | | H37Rv | H37Rv | TDR-0015 | TDR-0015 | TDR-0018 | TDR-0018 | | | |
|--|-------------------|---------------|--------------|---------------|---------------|---------------|---------------|-------|-------|-------|
| Resistance Profile | | Wild Type | Wild Type | INH Resistant | INH Resistant | RIF Resistant | RIF Resistant | | | |
| Amount | | 1 pg | 1 pg | 1 pg | 1 pg | 1 pg | 1 pg | | | |
| RESULTS | | | | | | | | | | |
| Final Results | | Test Validity | VALID | VALID | VALID | VALID | VALID | | | |
| M. tuberculosis Complex | | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | | | |
| Rifampin Resistance | | Not Detected | Not Detected | Not Detected | Not Detected | DETECTED | DETECTED | | | |
| Isoniazid Resistance | | Not Detected | Not Detected | DETECTED | DETECTED | Not Detected | Not Detected | | | |
| Suspected Mutation | | rpoB | N/A | N/A | N/A | N/A | S531W | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | katG | N/A | N/A | S315T (ACC) | S315T (ACC) | N/A | | | |
| | | inhA | N/A | N/A | -15T | -15T | N/A | | | |
| M13 (90, 103, 106) | | Absent | Absent | Absent | Absent | Absent | Absent | | | |
| IS6110 (71) | | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | | | |
| rpoB UN (137, 176) | | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | | | |
| katG UN (178) | | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | | | |
| inhA UN (139) | | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | PRESENT | | | |
| rpoB | | Not Detected | Not Detected | Not Detected | Not Detected | DETECTED | DETECTED | | | |
| katG | | Not Detected | Not Detected | DETECTED | DETECTED | Not Detected | Not Detected | | | |
| inhA | | Not Detected | Not Detected | DETECTED | DETECTED | Not Detected | Not Detected | | | |
| Discrimination Ratios (WT - MU) / (WT + MU) | | | | | | | | | | |
| Target | Probe Description | | WT | MU | | | | | | |
| rpoB | 507-wt | 507-DEL | 1 | 2 | 0.94 | 0.88 | 0.97 | 0.90 | 0.89 | 0.83 |
| rpoB | 510-wt | Q510H | 3 | 4 | 0.73 | 0.69 | 0.78 | 0.75 | 0.76 | 0.70 |
| rpoB | 511-wt | L511P | 5 | 6 | 0.64 | 0.58 | 0.66 | 0.49 | 0.56 | 0.62 |
| rpoB | 511-wt | L511R | 5 | 7 | 0.76 | 0.65 | 0.65 | 0.57 | 0.61 | 0.69 |
| rpoB | 512-wt | S512T | 8 | 9 | 0.67 | 0.62 | 0.59 | 0.58 | 0.57 | 0.49 |
| rpoB | 512-wt | S512R | 8 | 10 | 0.85 | 0.72 | 0.78 | 0.74 | 0.78 | 0.71 |
| rpoB | 513-wt | Q513L | 11 | 12 | 0.90 | 0.95 | 0.96 | 0.88 | 0.96 | 0.99 |
| rpoB | 513-wt | Q513K | 11 | 13 | 0.91 | 0.94 | 0.86 | 0.86 | 0.87 | 0.88 |
| rpoB | 513-wt | Q513P | 11 | 14 | 0.83 | 0.75 | 0.79 | 0.83 | 0.80 | 0.80 |
| rpoB | 515-wt | M515I | 15 | 16 | 0.78 | 0.57 | 0.65 | 0.61 | 0.68 | 0.41 |
| rpoB | 516-wt | D516E | 17 | 20 | 0.69 | 0.80 | 0.54 | 0.76 | 1.05 | 0.62 |
| rpoB | 516-wt | D516Y | 17 | 23 | 0.50 | 0.24 | 0.65 | 0.41 | 0.53 | 0.36 |
| rpoB | 516-wt | D516G | 17 | 24 | 0.69 | 0.54 | 0.25 | 0.81 | 0.83 | 0.50 |
| rpoB | 516-wt | D516V | 17 | 25 | 0.56 | 0.53 | 0.65 | 0.60 | 0.14 | 0.54 |
| rpoB | 522-wt | S522L | 26 | 28 | 0.79 | 0.86 | 0.91 | 0.83 | 0.84 | 0.77 |
| rpoB | 524-wt | L524S | 29 | 30 | 0.87 | 1.05 | 0.92 | 0.92 | 0.84 | 0.83 |
| rpoB | 526-wt | H526D | 31 | 32 | 0.87 | 0.83 | 0.83 | 0.85 | 0.87 | 0.90 |
| rpoB | 526-wt | H526R | 31 | 34 | 0.92 | 0.93 | 0.87 | 0.84 | 0.97 | 0.93 |
| rpoB | 526-wt | H526L | 31 | 35 | 0.90 | 0.92 | 0.85 | 0.86 | 0.91 | 0.89 |
| rpoB | 526-wt | H526Q (CAA) | 31 | 36 | 0.89 | 1.00 | 0.99 | 0.86 | 0.97 | 0.98 |
| rpoB | 526-wt | H526Q (CAG) | 31 | 37 | 0.95 | 0.98 | 0.90 | 0.94 | 0.96 | 0.98 |
| rpoB | 526-wt | H526C | 31 | 38 | 0.97 | 0.98 | 0.89 | 0.93 | 0.96 | 0.98 |
| rpoB | 526-wt | H526N | 31 | 39 | 0.91 | 0.92 | 0.92 | 0.94 | 0.97 | 0.95 |
| rpoB | 526-wt | H526P | 31 | 41 | 0.75 | 0.82 | 0.83 | 0.87 | 0.90 | 0.84 |
| rpoB | 526-wt | H526Y | 31 | 43 | 0.57 | 0.58 | 0.60 | 0.62 | 0.74 | 0.71 |
| rpoB | 531-wt | S531W | 44 | 46 | 0.74 | 0.85 | 0.65 | 0.77 | -0.96 | -0.92 |
| rpoB | 531-wt | S531L | 44 | 47 | 0.74 | 0.56 | 0.89 | 0.72 | -0.42 | -0.20 |
| rpoB | 531-wt | S531Q | 44 | 48 | 0.86 | 1.08 | 0.93 | 0.99 | 0.76 | 0.47 |
| rpoB | 531-wt | S531C | 44 | 50 | 0.61 | 0.75 | 0.56 | 0.56 | -0.89 | -0.79 |
| rpoB | 533-wt | L533P | 51 | 52 | 0.83 | 0.79 | 0.75 | 0.76 | 1.18 | 0.78 |
| katG | 315-wt | S315T (ACC) | 59 | 63 | 0.94 | 0.91 | -0.79 | -0.48 | 0.93 | 0.90 |
| katG | 315-wt | S315T (ACA) | 59 | 78 | 0.93 | 0.92 | -0.15 | 0.30 | 0.93 | 0.91 |
| katG | 315-wt | S315N | 59 | 79 | 0.95 | 0.90 | 0.14 | 0.57 | 0.92 | 0.89 |
| inhA | -8T-wt | -8A | 82 | 83 | 0.82 | 0.82 | 0.50 | 0.63 | 0.90 | 0.85 |
| inhA | -8T-wt | -8C | 82 | 84 | 0.74 | 0.71 | 0.59 | 0.54 | 0.77 | 0.75 |
| inhA | -15C-wt | -15T | 85 | 86 | 0.94 | 0.92 | -0.58 | -0.49 | 0.97 | 0.95 |
| inhA | -17G-wt | -17T | 87 | 88 | 0.79 | 0.79 | 0.79 | 0.84 | 0.87 | 0.82 |

Ratios in black are called wild-type.

Ratios in red are called mutants.

Ratios in gray are not called in deference to more negative ratio in the same grouping.