Comparison of the Akonni Biosystems MDR-TB microarray test to the Hain LifeScience GenoType MTBDRplus for resistance to Rifampin and Isoniazid in Kazakh M. tuberculosis Isolates

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Abstract (Revised)

Early case detection and rapid treatment reduce Mycobacterium tuberculosis (MTB) transmission. MTB is considered multidrug-resistant TB (MDR-TB) when resistant to rifampin and isoniazid, and a test which simultaneously diagnoses MTB and multi-drug resistance (MDR) is urgently needed. The Akonni Biosystems MDR-TB microarray test (Akonni MDR-TB) (Frederick, MD, USA) specifically reports on the presence of rpoB, katG, and inhA mutations known to confer resistance to rifampin and isoniazid, respectively. The assay contains markers for the M. avium complex (MAC), M. tuberculosis complex, and an internal amplification and hybridization control. Similarly, the Hain LifeScience GenoType MTBDRplus (Hain, Germany) detects the presence of mutations in rpoB, katG, and inhA.

MRIGlobal partnered with the M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry (IMB) to initially test 411 extracted isolates from the National Center of Tuberculosis Problems in Kazakhstan. The Akonni MDR-TB was compared to the Hain LifeScience GenoType MTBDRplus (377 isolates) for rifampin and isoniazid resistance: percent agreement (95% Conf-dence Interval) rifampin 93.6% (90.7-95.9), isoniazid 97.9% (95.9-99.1); sensitivity (95% CI) rifampin 98.0% (95.4-99.3), isoniazid 97.9% (96.8-98.9); and specificity (95% CI) rifampin 85.3% (78.0-90.9), isoniazid 94.7% (86.6-98.5). Isoniazid testing on the Akonni MDR-TB was compared to the Hain GenoType MTBDRplus (377 isolates). The agreement (95% Confidence Interval) between the two assays for rifampin was 93.6% (90.7-95.9), and for isoniazid 97.9% (95.9-99.1). See Table 2.

The study confirms the ability to perform diagnostic evaluations of new technology relevant to the region where the evaluation was performed. The way forward:

• Sequence isolates for which phenotypic data and array data show disagreement, i.e., known loci (rpoB, katG)
• Whole genome sequencing to identify novel drug resistance mutations, virulence factors, and so on
• Novel assays to detect antibiotic resistance
• Epidemiology of MDR MTB strains from the FSU

Background

MTB causes 1.5 million deaths with > 95% mortality in low- and middle-income countries. Also, almost half of the MTB infections are multi-drug-resistant MTB. Global Tuberculosis Report 2015. The World Health Organization estimates that, since the year 2000, > 37 million lives have been saved by effective diagnosis followed by proper treatment. MTB incidence and prevalence in the Former Soviet Union (FSU) is high with multi-drug resistant MTB (MDR-TB) present in virtually every country surveyed. (who.int.)

Methods

MTB lysates were extracted using Qiaclean according to manufacturer’s instructions. Approximately 10 ng of extracted DNA was used in a 50 µL PCR reaction containing MDR-TB Primer Mix and control template. Post-PCR, hybridization was performed on MDR-TB v1.2 TruArray slides in a 50 µL hybridization reaction with 25 µL amplified isolate, BSA, hybridization buffer and QC mix. Microarrays were covered with a parafilm covelp and hybridized for 3 h at 55°C. After hybridization, microarrays were washed in post-hybridization wash buffer for 10 min with gentle agitation, rinsed in ultrapure water, and dried with centrifugation. Microarrays were imaged on an Akonni microarray analyzer, using Akonni Imaging Software. Scanned array images were analyzed using the Akonni Biosystems TruDx Analysis Package. See Figure 2.

Results

An initial comparison was performed of the MDR-TB TruArray test to the Hain LifeScience GenoType MTBDRplus. The agreement (95% Confidence Interval) between the two assays for rifampin was 93.6% (90.7-95.9), and for isoniazid 97.9% (95.9-99.1). See Table 2.

Table 2: Genotypic Comparative Study

<table>
<thead>
<tr>
<th>Assay</th>
<th>Performance Characteristics</th>
<th>Proportion</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR-TB</td>
<td>RIF Percent agreement</td>
<td>93.6%</td>
<td>90.7-95.9</td>
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<tr>
<td></td>
<td>RIF Specificity</td>
<td>90.8%</td>
<td>86.6-94.0</td>
</tr>
<tr>
<td></td>
<td>INH Percent agreement</td>
<td>97.9%</td>
<td>95.9-99.1</td>
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<tr>
<td></td>
<td>INH Specificity</td>
<td>97.7%</td>
<td>96.8-98.9</td>
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</tbody>
</table>

Table 3. Phenotypic Comparative Study

<table>
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<th>Performance Characteristics</th>
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<th>95% Confidence Interval</th>
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<tbody>
<tr>
<td>MDR-TB</td>
<td>RIF Sensitivity</td>
<td>93.3%</td>
<td>88.8-95.6</td>
</tr>
<tr>
<td></td>
<td>INH Sensitivity</td>
<td>97.8%</td>
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</tr>
</tbody>
</table>

Conclusions

The array assay was relatively quick and easy to use, fitting well into existing workflow with multiple steps where one could start/stop. Conventional PCR can be performed on various platforms. The hybridizations were not complex and could be accomplished using low technology solutions. The array reader was proprietary, but others could be used. Analysis was performed with an excel spreadsheet using a template within which array fluorescent data algorithms were placed. There was no statistical difference between the performance of the Akonni Assay and the Hain test.

• Simplified Microarray workflow
• Performance comparable to industry leader (Hain)
• Genotypic results not predictive of culture phenotype

The study confirms the ability to perform diagnostic evaluations of new technology relevant to the region where the evaluation was performed. The way forward:

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