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. tuber-culosis* complex, and an internal amplification and hybridization control. Similarly, the Hain LifeScience GenoType MTBDRplus (Hain MTBDRplus) (Nehren, Germany) detects the presence of mutation in rpoB, katG, and inhA.

MRIGlobal partnered with the M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry (IMBB) 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 mutations: percent agreement (95% Confi-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 98.7% (96.6-99.6); 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 resulted in one indeterminate result. Discrepant analysis between assays is ongoing with culture to adjudicate. The performance characteristics of these assays to detect specific resistance markers is further defined, while highlighting the advantages of using a practical and quick multiplexed microarray platform in a field forward setting for global surveillance.

Introduction

The MDR-TB TruArray test (Akonni Biosystems, Frederick, MD, USA) is nine primer pairs in a single multiplex amplification PCR to detect mutations known to confer resistance to rifampin, isoniazid, streptomycin, and ethambutol on a gel element microarray. The Dx2100 instrument suite, open-amplicon work flow, uses a discriminant ratio to detect the presence of a specific mutation(s); mutations detected are displayed in Table 1.

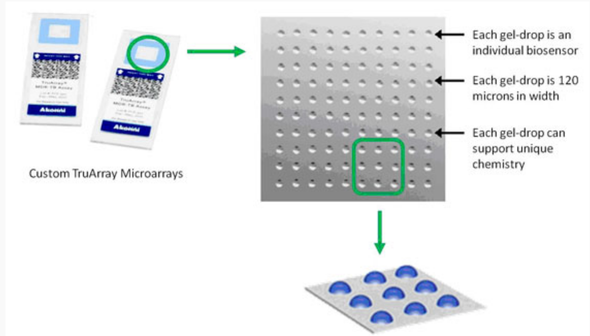

Figure 1: Design of the Akonni MDR-TB TruArray Test (Image courtesy of Akonni Biosystems)

Table 1: MDR-TB Microarray Coverage of the Akonni MDR-TB TruArray Test

Drug	Gene	Targeted Codons	Total number of mutations
RIF	*rpoB*	507, 510, 511, 512, 513, 515, 516, 522, 524, 526, 531 and 533	30
INH	*katG* *inhA*	315 8,15, and 17	2 4
EMB	*embB*	306	1
STR	*rpsL*	43 and 88	2
N/A	IS6110 IS1245 IPC	*M. tuberculosis* complex *M. avium* complex Internal amplification and hybridization control	N/A

Results

An initial comparison was performed of the MDR-TB TruArray test to the Hain 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*

Performance Characteristics	Proportion	95% Confidence Interval
RIF Percent agreement	93.6%	90.7-95.9
RIF Sensitivity	98.0%	95.4-99.3
RIF Specificity	85.3%	78.0-90.9
INH Percent agreement	97.9%	95.9-99.1
INH Sensitivity	98.7%	96.6-99.6
INH Specificity	94.7%	86.6-98.5

(RIF, Rifampin; INH, Isoniazid)

*Initial Performance of the MDR-TB TruArray Test to the Hain GenoType MTBDRplus (n = 377)

Culture results (retrospective) and the molecular tests were compared to the isolates for antimicrobial susceptibility (phenotype) to rifampin and isoniazid. An additional 33 isolates were tested and analyzed for the second analysis (n = 410). The agreement (95% Confidence Interval) between the MDR-TB TruArray test and the Hain GenoType MTBDRplus for rifampin was 94.1% (91.4-96.2) and for isoniazid 98.3% (96.5-99.3). The performance characteristics are compared in Table 3.

Table 3. Phenotypic Comparative Study

Assay	Performance Characteristics	Proportion	95% Confidence Interval
MDR-TB TruArray test	RIF Sensitivity	**92.7%**	**88.8-95.5**
	RIF Specificity	**71.1%**	**63.2-78.3**
	INH Sensitivity	**94.0%**	**90.8-96.3**
	INH Specificity	**73.4%**	**62.3-82.7**
Hain GenoType MTBDRplus	RIF Sensitivity	**90.7%**	**86.6-94.0**
	RIF Specificity	**75.8%**	**68.2-82.5**
	INH Sensitivity	**94.0%**	**90.8-96.3**
	INH Specificity	**74.7%**	**63.6-83.8**

(RIF, Rifampin; INH, Isoniazid)

Performance of the MDR-TB TruArray Test and the Hain GenoType MTBDRplus to Culture (n = 410)

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 was 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:

- 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. (who.int.) Almost a half million of the MTB infections are multidrug-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 Qiagen 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 hybridization QC mix. Microarrays were covered with a parafilm coverslip and hybridized for 3 h at 55ºC. After hybridization, microarrays were washed in post-hybridization wash buffer for 10 m 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.

Figure 2: Akonni MDR-TB TruArray Test Operational

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