Evaluation of an array-based test for the detection of isoniazid and rifampicin resistance in *Mycobacterium tuberculosis* in clinical strains and samples

Molina B (1), Kazdaglis G (2), Lacoma A (1), Prat C (1), Diaz J (1), Maldonado J (4), Samper S (5), Ruiz-Manzano J (7), Ausina V (1), Dominguez J (1).

(1) Servei de Microbiologia. Hospital Universitari Germans Trias i Pujol. Institut d'Investigació Germans Trias i Pujol, Badalona, Spain. Universitat Autònoma de Barcelona. CIBERES. (2) Department of Medical Microbiology, Aristotle University of Thessaloniki, Greece. (4) Serveis Clínics, Barcelona, Spain. (5) Instituto Aragonés de Ciencias de la Salud, Zaragoza, Spain. Hospital Universitario Miguel Servet, Zaragoza, Spain. CIBERES (7) Servei de Pneumologia. HUGTiP, Badalona, Spain.

Introduction / Objectives

- Drug resistant tuberculosis (TB) remains one of the most deadly curable infectious diseases.
- Standard phenotypic methods to detect drug resistance are slow and several weeks are required to obtain results.
- New rapid methods are needed for detection of drug resistance.
- The objetive of this study was to evaluate an array-based rapid molecular method to detect *M. tuberculosis* resistance to the first-line drugs isoniazid (INH) and rifampicin (RIF) in clinical isolates and directly in clinical samples.

Material and Methods

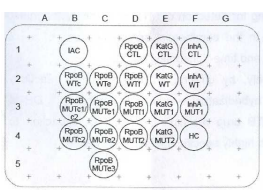
- Seventy clinical isolates from 70 patients and 50 samples corresponding to 25 patients were retrospectively selected.
- The corresponding isolates were characterized by BACTEC 460TB (Table 1)
- The array method (GenoFlow DR-MTB Array Test Kit [DiagCor Bioscience, Hong Kong]) is based on PCR and "Flow-through" hybridization technology.
- This test detects the presence of mutations associated with resistance to INH and RIF: *katG* (S315T1, S315T2), *inhA* -15C/T and *rpoB* (D516V, D516G, H526D, H526Y, H526L1, S531L and S531W).
- Hybridization control as well as *rpoB*, *katG* and *inhA* controls must be present in order to consider the result valid.
- Genotypic results obtained by this method were compared to phenotypic results obtained by BACTEC.
- Discordant results between both methods were analyzed by alternative molecular methods (DNA sequencing, GenoType MTBDR*plus* [Hain Lifescience, Germany] and/or pyrosequencing).

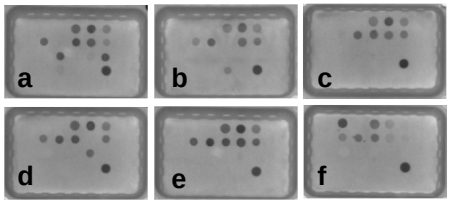
Table 1. Resistance pattern obtained by BACTEC 460TB for INH and RIF for the clinical isolates and samples.

	Clinical strains		Clinical samples	
Drug	**INH**	**RIF**	**INH**	**RIF**
Resistant	59	23	40	37
Sensitive	11	47	10	13

Results

- Valid result rate: 100% (70/70) in clinical strains and 90% (45/50) in clinical samples
  - For smear negative samples: 0% (0/2)
  - For smear positive samples : 93.8% (45/48)





a. INH^R, RIF^R
b. INH^S, RIF^R
c. INH^S, RIF^R
d. INH^R, RIF^S
e. INH^S, RIF^S
f. Invalid result

Figure 1. Examples of results obtained by GenoFlow DR-MTB Array.

Table 2. Sensitivity, specificity and agreement values between GenoFlow assay and BACTEC for detecting resistance to INH and RIF in clinical strains and samples.[a]

	Clinical strains				Clinical samples			
Drug	**GenoFlow DR-MTB Array**		**Agreement between Genoflow and BACTEC**		**GenoFlow DR-MTB Array**		**Agreement between GenoFlow and BACTEC**	
	Sensitivity (%)	**Specificity (%)**	**Agreement (%)**	**Kappa**	**Sensitivity (%)**	**Specificity (%)**	**Agreement (%)**	**Kappa**
INH	42/59 (71.2)	11/11 (100)	53/70 (75.7)	0.437	12/13 (92.3)	9/9 (100)	44/45 (97.8)	0.933
RIF	22/23 (95.7)	45/47 (95.7)	67/70 (95.7)	0.904	11/11 (100)	11/12 (91.7)	44/45 (97.8)	0.942

[a] One sample per patient was considered for sensitivity and specificity calculations.

- Regarding the discordant results obtained between GenoFlow DR-MTB Array and BACTEC, at least one of the alternative molecular methods confirmed the genotypic result in all the INH^R isolates (17/17) and samples (1/1), and in 33% (1/3) of the RIF^R isolates but not in samples (0/1).

Conclusions

The array-based GenoFlow DR-MTB allows the rapid detection of drug resistance in clinical strains and samples with good performance.
In the clinical practice, these results may be useful for an initial therapeutic approach while the phenotypic drug susceptibility results are not available, hence potentially improving the clinical management of patients with tuberculosis.