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Evaluation of GenoFlow DR-MTB Array Test for Detection of Rifampin and Isoniazid Resistance in *Mycobacterium tuberculosis*

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The aim of this study was to evaluate the GenoFlow DR-MTB array test (DiagCor Bioscience, Hong Kong) on 70 cultured isolates and 50 sputum specimens. The GenoFlow array test showed good sensitivity and specificity compared to the phenotypic Bactec 460TB. This array accurately detected mutations in *rpoB*, *katG*, and *inhA* associated with resistance to rifampin and isoniazid.

Rapid detection and drug susceptibility testing of *Mycobacterium tuberculosis* are hampered by the slow growth of mycobacteria (1). The transmission of strains resistant to both rifampin (RIF) and isoniazid (INH), i.e., multidrug-resistant (MDR) strains, remains a public health problem. These strains may harbor mutations in *rpoB* (2, 3), *katG*, and *inhA*, among other genomic regions (4, 5). The aim of this study was to evaluate the diagnostic accuracy of the GenoFlow DR-MTB array test (DiagCor Bioscience, Hong Kong) for the detection of *M. tuberculosis* molecular resistance to RIF and INH.

A total of 70 *M. tuberculosis* isolates from 70 patients and 50 sputum specimens from 25 patients (more than one specimen was obtained from nine patients) were retrospectively selected from a collection of cultured isolates and specimens recovered from the Hospital Universitari Germans Trias i Pujol (Badalona, Spain), the Instituto Aragonés de Ciencias de la Salud (Zaragoza, Spain), and Serveis Clínics (Barcelona, Spain). The isolates and specimens were selected to represent different resistance profiles. The study was approved by the institutional ethics committee at Hospital Universitari Germans Trias i Pujol.

Specimens were decontaminated using Kubica's *N*-acetyl-L-cysteine NaOH method (6, 7), stained by auramine-rhodamine, graded on a scale from 0 to 3+, and cultured on Lowenstein-Jensen and Bactec 460TB (Becton Dickinson, Sparks, MD, USA). The remaining decontaminated specimens were stored at −20°C (8). The INNO-LiPA mycobacteria version 2 assay (Innogenetics, Ghent, Belgium) was used to identify *M. tuberculosis* complex organisms for all the isolates and cultures from the specimens. Drug susceptibility testing (DST) was performed with Bactec 460TB (Bactec) using 2 μg/ml RIF and 0.1 μg/ml INH as critical concentrations (9).

For molecular drug resistance detection, DNA from isolates and specimens was extracted, as previously described (10). The GenoFlow array test consists of PCR amplification and hybridization in the FT^{PRO} flowthrough system. The mutations targeted are *rpoB* D516V, D516G, H526D, H526Y, H526L1, S531L, and S531W; *katG* S315T1 and S315T2; and *inhA* C-15T. An internal amplification control, hybridization control, and *rpoB*, *katG*, and *inhA* controls were included in each reaction. The results obtained by the array were recorded, automatically interpreted by the DiagCor software, and confirmed visually by the researcher. These

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TABLE 1 Distribution of GenoFlow DR-MTB array results according to Bactec 460TB for 70 clinical isolates and 50 sputum specimens

	Bactec 460TB result (%) for (*n*)[b]:											
	Clinical isolates (70)						Sputum specimens (50)					
	RIF		INH		MDR (23)		RIF		INH		MDR (37)	
GenoFlow result[a]	R (23)	S (47)	R (59)	S (11)	RIF	INH	R (37)	S (13)	R (40)	S (10)	RIF	INH
R	22		41		22	17	35	1	38		35	36
S	1	47	18	11	1	6	2	11	1	10	2	1
I								1[c]	1[c]			

[a] R, resistant; S, sensitive; I, invalid.
[b] RIF, rifampin; INH, isoniazid; MDR, multidrug resistant (resistant to both rifampin and isoniazid).
[c] Invalid GenoFlow results for both RIF and INH were obtained for the same specimen.

results were compared to those obtained by the Bactec. Discordant results between the array and the Bactec were compared to those obtained by alternative molecular methods. DNA sequencing targeted mutations in the *katG* gene, *oxyR-ahpC*, *mabA-inhA*, and the 81-bp core region of *rpoB* (11); the GenoType MTBDR*plus* (Hain Lifescience, Nehren, Germany) targeted mutations in *rpoB* (codons 516, 526, and 531), *katG* (codon 315), and *inhA* (positions −8, −15, and −16) (10); and pyrosequencing targeted mutations in *rpoB* (codons 516 and 526 to 531), *katG* (codon 315), and *inhA* (positions −16 to −5) (12). This diagnostic accuracy study was reported in accordance with the Standards for Reporting of Diagnostic Accuracy (STARD) statement guidelines (13).

The distribution of GenoFlow results, according to the Bactec results for clinical isolates and sputum specimens, is presented in Table 1. The sensitivity, specificity, and agreement between the GenoFlow and Bactec tests were >90% for detecting RIF resistance in cultured isolates and sputum specimens and for INH resistance in sputum specimens; however, the sensitivity of the array for INH resistance in clinical isolates was 69.5% (Table 2). A total of 23 discordant results were obtained between Bactec and GenoFlow tests for 22 isolates/specimens (for one isolate, discrepant results were obtained for both drugs) (Table 3). At least one of the results obtained by DNA sequencing, GenoType MTBDR*plus*, or pyrosequencing was in agreement with the array in 82.6% (19/23) of the cases.

Of the 50 sputum specimens selected, two were smear negative, and 48 were smear positive; eight specimens were smear 1+ (1 to 10 acid-fast bacilli [AFB] per 100 fields), nine specimens were smear 2+ (1 to 9 AFB per field), and 31 specimens were smear 3+ (>9 AFB per field). An invalid GenoFlow test result (absence of *katG* and *inhA* controls) was obtained for one specimen, which was 3+ and rifampin sensitive/isoniazid resistant. For four specimens, discordant results between the Bactec and GenoFlow tests were obtained: one specimen was smear negative, one specimen was smear 1+, and two specimens were smear 3+ (Table 3). Furthermore, for two of the specimens with a discordant result between the Bactec and GenoFlow tests, consecutive samples collected during the treatment were available, and a concordant result was obtained for those specimens. Thus, the molecular result did not appear to be affected by potential changes in the DST profile or in the different resistant/susceptible subpopulations in the sample during the treatment of the patients.

The sensitivity and specificity values of the GenoFlow test for detecting RIF resistance were comparable to those of GenoType MTBDR*plus* and INNO-LiPA Rif. TB assays (14). These high values were expected, since >95% of rifampin-resistant isolates harbor mutations in the targeted region of *rpoB* (15). Regarding INH resistance, the lower sensitivity of the GenoFlow test was partially in contrast with that of the GenoType MTBDR*plus* assay (16). The data presented here, despite the bias introduced in the selection of isolates, was more in accordance with those of another systematic review that reported a combined cumulative frequency of 79.9% for *katG* codon 315 and *inhA* position −15 mutations worldwide, which reached 83.9% when additional mutations in *inhA* and *ahpC* were included (17).

Nowadays, several molecular tests are available (18–21), but more studies are still needed to assess their clinical value. For instance, an evaluation has demonstrated the noninferiority of the GenoType MTBDR*plus* version 2.0 and Nipro line probe assays in comparison to the WHO-endorsed first version of the GenoType

TABLE 2 Sensitivity and specificity of GenoFlow DR-MTB array for detecting drug resistance, and agreement values between GenoFlow DR-MTB array and Bactec 460TB[a]

Resistance	Clinical isolates					Sputum specimens				
	Sensitivity (no. detected/total no. [%] [95% CI])	Specificity (no. detected/total no. [%] [95% CI])[b]	Agreement (no. detected/total no. [%])	Kappa[c]	SE	Sensitivity (no. detected/total no. [%] [95% CI])	Specificity (no. detected/total no. [%] [95% CI])[b]	Agreement (no. detected/total no. [%])	Kappa[c]	SE
RIF	22/23 (95.7) (76.0–99.8)	47/47 (100) (90.6–100)	69/70 (98.6)	0.967	0.032	35/37 (94.6) (80.5–99.1)	11/12 (91.7) (59.8–99.6)	46/49 (93.9)	0.839	0.090
INH	41/59 (69.5) (56.0–80.5)	11/11 (100) (67.9–100)	52/70 (74.3)	0.417	0.096	38/39 (97.4) (84.9–99.9)	10/10 (100) (65.5–100)	48/49 (98.0)	0.939	0.060
MDR	17/23 (73.9) (51.3–88.9)	47/47 (100) (90.6–100)[b]	64/70 (91.4)	0.792	0.079	34/37 (91.9) (77.0–97.9)	13/13 (100) (71.7–100)[b]	47/50 (94.0)	0.847	0.084

[a] RIF, rifampin; INH, isoniazid; MDR, multidrug resistance (resistance to both rifampin and isoniazid); CI, confidence interval; SE, standard error.
[b] For specificity calculations of MDR detection, we considered isolates/specimens sensitive to either RIF or INH or both.
[c] Kappa values of >0.6 and kappa values between 0.4 and 0.6 indicate a strong and moderate agreement, respectively.

TABLE 3 Results obtained by molecular methods for the cultured isolates and sputum specimens with a discordant result between Bactec 460TB and GenoFlow DR-MTB array[a]

Isolate or specimen	Bactec 460TB		GenoFlow DR-MTB array		DNA sequencing		GenoType MTBDR*plus*		Pyrosequencing	
	RIF	INH	RIF	INH	RIF	INH	RIF	INH	RIF	INH
Isolates	R	R	516 WTØ[b]	WT	516 TAC	WT	WT	WT	516 TAC	WT
	R	R	531 TGG	WT	531 TGG	WT	WT	WT	531 TTG	WT
	S	R	WT	WT	NP	*oxyR-aphC* G-12A	WT	WT	WT	WT
	S	R	WT	WT	NP	WT	WT	WT	WT	WT
	S	R	WT	WT	NP	*inhA* T-8C	WT	*inhA* T-8C	WT	*inhA* T-8C
	S	R	WT	WT	NP	WT (*katG* NP)	WT	WT	WT (531 NR)	WT
	R	R	WT	WT	531 TTG	WT	531 TGG	WT	WT	WT
	S	R	WT	WT	NP	WT	WT	WT	WT	WT
	S	R	WT	WT	NP	WT	WT	WT	WT	WT
	S	R	WT	WT	NP	WT	WT	WT	WT	WT
	S	R	WT	WT	NP	*inhA* C-15T	WT	WT	WT	WT
	S	R	WT	WT	NP	WT	WT	WT	WT	WT
	S	R	WT	WT	NP	WT	WT	WT	WT	WT
	S	R	WT	WT	WT	WT	WT	*katG* S315T1	WT	WT
	S	R	WT	WT	NP	*katG* S315T1	WT	WT	WT	*katG* S315T1
	R	R	531 TTG	WT	531 TTG	WT (*inhA*, *oxyR-aphC* NP)	531 TTG	WT	531 TTG	WT
	R	R	531 TTG	WT	531 TTG	WT (*inhA*, *oxyR-aphC* NP)	531 TTG	WT	531 TTG	WT
	R	R	516 GGT	WT	516 GGT	WT (*inhA*, *oxyR-aphC* NP)	516 GGT	WT	516 GGT	WT
Specimens	R[c]	R	WT	*inhA* C-15T	NP	NP	NP	NP	NR	NP
	R[d]	R	WT	*katG* S315T1	NP	NP	NP	NP	WT	NP
	S[e]	S	531 TTG	WT	NP	NP	WT	WT	WT	WT
	R[e]	R	531 TTG	WT	NP	NP	531 TTG	WT	531 TTG	WT

[a] RIF, rifampin; INH, isoniazid; WT, wild type; NP, not performed; NR, no result obtained.
[b] 516 WTØ, the GenoFlow probe targeting *rpoB* 516 wild type was absent.
[c] This specimen was smear negative.
[d] This specimen was smear 1+.
[e] This specimen was smear 3+.

MTBDR*plus* assay for the rapid detection of multidrug-resistant tuberculosis (MDR-TB) (22). Moreover, in order to improve patient management, it is important to consider not only the molecular result (presence/absence of mutation) but also the mutation detected and its correlation with the phenotypic result and clinical outcome (23).

The main advantages of the GenoFlow assay were the use of the FT^{PRO} hybridization device, which shortens the hybridization protocol to 45 min (that of the GenoType MTBDR*plus* assay is 2 h), and the specific software that facilitates the interpretation, report, and storage of the results. In addition, an automated hybridization device is under development, which may reduce the hands-on-time of the hybridization step. Another aspect that could also be improved is the low-throughput capacity.

In conclusion, the GenoFlow assay may be useful for rapid, sensitive, and specific screening of resistance to RIF and INH in isolates and specimens, and its performance is comparable to that of other molecular methods. Although molecular results should be confirmed by phenotypic testing, the identification of resistance can be helpful to rule out drugs and improve the management of tuberculosis patients.

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AUTHOR CORRECTION

Correction for Molina-Moya et al., Evaluation of GenoFlow DR-MTB Array Test for Detection of Rifampin and Isoniazid Resistance in *Mycobacterium tuberculosis*

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