Evaluation of a new reverse hybridization-based molecular method for detecting first- and second-line drug resistance in *Mycobacterium tuberculosis* in clinical samples

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Introduction / Objectives

- Drug resistant tuberculosis (TB) is a global threat.
- Conventional methods to detect drug resistance require several weeks.
- New molecular methods are needed for the rapid detection of drug resistance.
- The objetive of this study was to evaluate a rapid molecular method based on reverse hybridization with specific probes to detect *M. tuberculosis* and its resistance to first-line drugs (isoniazid [INH], rifampicin [RIF], streptomycin [STR] and ethambutol [EMB]) and second-line drugs (fluoroquinolones [FQ], kanamycin, amikacin and capreomycin [KAN, AMK, CM]) directly in clinical samples.

Material and Methods

- Sixty-five clinical samples corresponding to 32 patients were retrospectively selected.
- The corresponding isolates were characterized by BACTEC 460TB (Table 1).
- The reverse hybridization-based method AID TB Resistance (AID Diagnostika, Alemania) was performed to detect resistance to first- and second-line drugs in *M.tuberculosis.*
- This system consists on PCR amplification and reverse hybridization on probes bound to nitrocellulose strips in order to detect *M. tuberculosis* complex and mutations associated with drug resistance.
  - Resistance to **INH/RIF**: ***inhA*** -16, -15, -8; ***katG*** 315; ***rpoB*** 516, 526, 531.
  - Resistance to **FQ/EMB**: ***gyrA*** A90V, S91P, D94A, D94N, D94Y, D94G; ***embB*** M306V, M306I.
  - Resistance to **KAN/AMK/CM/STR**: ***rrs*** A1401G, C1402T, G1484C/T, ***rrs*** C513T, D514C, G515C, C517T; ***rpsL*** A43G, A88G, A88C.
- Control bands 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 (GenoType MTBDR*plus*, GenoType MTBDRs/ [Hain Lifescience, Germany] and/or pyrosequencing).

Table 1. First- and second-line drugs resistance pattern obtained by BACTEC 460TB for the clinical samples.

	Drug						
	INH	**RIF**	**FQ**	**EMB**	**KAN**	**CAP**	**STR**
Resistant	49	45	7	37	20	20	31
Sensitive	16	20	58	28	45	45	34

Results

- Valid result rate: 87.2% (170/195)
  - For smear negative samples: 22.2% (2/9)
  - For smear positive samples : 90.3% (168/186)

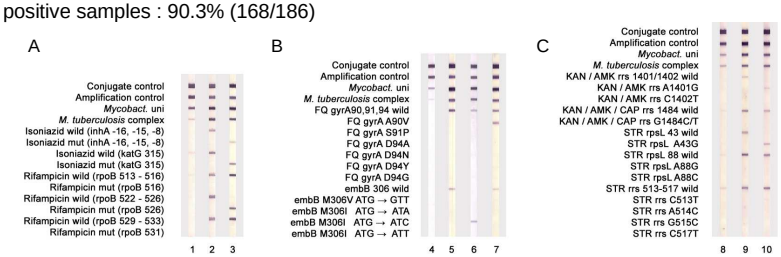

Figure 1. Examples of results obtained by AID TB Resistance assay. A) Results obtained for INH/RIF module. 1) Invalid result. 2) INH^S and RIF^S. 3) INH^R and RIF^R. B) Results obtained for FQ/EMB module. 4) Invalid result. 5) FLQ^S and EMB^S. 6) FLQ^S and EMB^R. 7) $FLQ^{R/S}$ and EMB^S. C) Results obtained for AG (aminoglycosides) module. 8) Invalid result. 9) KAN^S, AMK^S, CM^S and STR^S. 10) KAN^R, AMK^R, CM^S and STR^R.

Table 2. Sensitivity, specificity and agreement values between AID assay and BACTEC for detecting resistance to first- and second-line drugs in clinical samples[a]

	AID TB Resistance		**Agreement between AID TB Resistance and BACTEC 460TB**	
Drug	**Sensitivity (%)**	**Specificity (%)**	**Agreement (%)**	**Kappa**
INH	13/14 (92.9)	14/14 (100)	59/60 (98.3)	0.955
RIF	11/11 (100)	17/17 (100)	60/60 (100)	1.000
FQ	1/2 (50.0)	26/26 (100)	54/59 (91.5)	0.473
EMB	4/7 (57.1)	20/21 (95.2)	33/59 (72.9)	0.479
KAN/CAP	3/3 (100)	24/24 (100)	51/51 (100)	1.000
STR	6/6 (100)	20/21 (95.2)	50/51 (98.0)	0.963

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

- Regarding discordant results obtained between AID TB Resistance and BACTEC, alternative molecular methods confirmed the genotypic result in all the cases for INH and EMB and 80% for FQ. Alternative molecular methods available do not detect STR resistance.

Conclusions

The reverse hybridization-based AID TB Resistance is a rapid method that allows the detection of drug resistance in clinical samples with good sensitivity and specificity. In the clinical practice, these results may be useful to adjust the treatment until the phenotypic drug susceptibility results are available, hence potentially improving the clinical management of patients with tuberculosis.