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Lab-on-Chip-Based Platform for Fast Molecular Diagnosis of Multidrug-Resistant Tuberculosis

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We evaluated the performance of the molecular lab-on-chip-based VerePLEX Biosystem for detection of multidrug-resistant tuberculosis (MDR-TB), obtaining a diagnostic accuracy of more than 97.8% compared to sequencing and MTBDR*plus* assay for *Mycobacterium tuberculosis* complex and rifampin and isoniazid resistance detection on clinical isolates and smear-positive specimens. The speed, user-friendly interface, and versatility make it suitable for routine laboratory use.

Multidrug-resistant tuberculosis (MDR-TB) requires long and expensive treatment and often results in poor clinical outcome in both low- and high-income countries (1, 2). The World Health Organization (WHO) has endorsed specific molecular diagnostics to improve fast diagnosis of MDR-TB (3–5). However, the genotypic diversity and geographical distribution of *Mycobacterium tuberculosis* complex (MTBC), together with the inability to provide appropriate interpretation of silent mutations and the limited versatility are some of the restraints undermining the effectiveness of the current tools on a global scale (6–13).

In the present study, we evaluated a lab-on-chip (LoC) device, developed by STMicroelectronics (Geneva, Switzerland) and marketed by Veredus Laboratories (Republic of Singapore) as the VerePLEX Biosystem, for the diagnosis of MDR-TB and detection of common nontuberculous mycobacteria (NTM). The molecular assay was evaluated on both clinical isolates and direct specimens in low- and high-burden settings.

We tested 91 MTBC isolates (see Table S1 in the supplemental material) harboring different patterns of mutations in *rpoB*, *katG*, and *inhA* genes to evaluate the probes on the array listed in Table 1. Eighty respiratory specimens positive for acid-fast bacilli by smear microscopy and MTBC culture positive were decontaminated according to international guidelines and included in the study (Table S1) (14). An additional 116 MTBC culture-negative specimens were included in the analysis. DNA from isolates and specimens was extracted by thermal lysis and sonication as described elsewhere (15). Phenotypic drug susceptibility testing (DST) for rifampin (RIF) and isoniazid (INH) was performed according to international recommendations (16). Some of the

TABLE 1 Probes spotted onto the array and targeted mycobacterial species and MDR-TB targets included in the assay

Targeted mycobacterial species or MDR-TB target	Probe(s)
Targeted *Mycobacterium* species	
M. avium	MYC4a
M. intracellulare	MYC5a
M. simiae, *M. kansasii*, *M. scrofulaceum*	MYC6a
M. abscessus, *M. chelonae*	MYC8a
M. xenopi	MYC17a
M. haemophylum	MYC19a
M. fortuitum	MYC31a
M. tuberculosis complex	MYC15a-MYC16a
MDR-TB targets	
rpoB	
WT codons 510 to 513	L511_w3a
L511P mutant	L511P_m3
WT codons 515 to 518	D516_w5
D516V mutant	D516V_m1
WT codons 523 to 526	H526_w14
H526D mutant	H526D_m2
H526Y mutant	H526Y_m5
WT codons 530 to 533	S531L_w1
S531L mutant	S531L_m2
katG	
WT codons 313 to 317	S315_w2
S315T1 mutant	S315T1_m2
S315T2 mutant	S315T2_m1
inhA	
WT nucleotides −21 to −7	inhA_w3
T-8A mutant	InhA–8T>A_m2
T-8C mutant	InhA–8T>C_m2
C-15T mutant	InhA–15C>T_m3

Received 7 July 2015 **Accepted** 30 July 2015

Accepted manuscript posted online 5 August 2015

Citation Cabibbe AM, Miotto P, Moure R, Alcaide F, Feuerriegel S, Pozzi G, Nikolayevskyy V, Drobniewski F, Niemann S, Reither K, Cirillo DM, TM-REST Consortium, TB-CHILD Consortium. 2015. Lab-on-chip-based platform for fast molecular diagnosis of multidrug-resistant tuberculosis. J Clin Microbiol 53:3876–3880. doi:10.1128/JCM.01824-15.

Editor: K. C. Carroll

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Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.01824-15.

TABLE 2 Phenotypic DST, MTBDR*plus*, and VerePLEX Biosystem results for the 91 MTBC clinical isolates included in the study

Phenotypic DST result[a] for:		MTBDR*plus*/sequencing result[b] for the following gene:			VerePLEX Biosystem result[b,c] for the following gene:			No. of isolates[d]
RIF	INH	*rpoB*	*katG*	*inhA*	*rpoB*	*katG*	*inhA*	
R	R	S531L	S315T1	WT	S531L	S315T1	WT	15
R	R	WT	WT	WT	WT	WT	WT	1
S	R	WT	WT	WT	WT	WT	WT	5
R	R	S531L	WT	C-15T	S531L	WT	C-15T	16
R	R	S531L	WT	WT	S531L	WT	WT	7
R	S	S531L	WT	WT	S531L	WT	WT	2
R	R	H526D	S315T1	WT	H526D	WT* + S315T1	WT	1
R	R	H526D	S315T1	WT	WT + H526D	WT + S315T1	WT	1
R	R	L511P	S315N	WT	L511P	WT*	WT	1
R	R	H526D	S315R	WT	H526D	Δ 313–317 WT	WT	1
R	R	H526Y	S315N	WT	H526Y	WT*	WT	1
R	S	D516V	WT	WT	D516V	WT	WT	1
R	R	S531L	S315T1	T-8A	S531L	S315T1	T-8A	2
R	R	L530M+S531P	S315T1	T-8C	Δ 530–533 WT	S315T1	T-8C	1
R	R	S531L	S315T2	WT	S531L	S315T2	WT	2
R	R	D516V	S315T1	T-8A	D516V	S315T1	T-8A	3
R	R	D516V	S315T1	T-8C	D516V	S315T1	T-8C	1
S	R	WT	WT	C-15T	WT	WT	C-15T	11
R	R	D516V	S315T1	WT	D516V	S315T1	WT	5
S	R	WT	S315T1	WT	WT	S315T1	WT	5
R	R	H526D	S315T1	WT	H526D	S315T1	WT	1
R	R	S531L	S315T1	C-15T	S531L	S315T1	C-15T	3
R	R	Q513P	S315T1	WT	Δ 510–513 WT	S315T1	WT	1
S	R	WT	S315N	WT	WT	Δ 313–317 WT	WT	1
R	R	H526Y	S315T1	C-15T	H526Y	S315T1	C-15T	2
S	S	WT	WT	WT	WT	WT	WT	1

[a] The phenotypic drug susceptibility testing (DST) results for rifampin (RIF) and isoniazid (INH) are given as follows: R, resistant; S, sensitive.
[b] The results for the 91 MTBC isolates found by the MTBDR*plus* assay and sequencing or by the VerePLEX Biosystem are shown (wild type [WT] or mutant).
[c] Symbols: *, probe signal was on at the cutoff; Δ, no WT signal.
[d] The number of isolates apply to all the test results.

specimens were tested in a representative high-burden setting in Uganda (Nsambya Hospital, Kampala, Uganda), by trained staff.

DNA samples extracted from both isolates and specimens were tested in parallel, and results were compared with GenoType MTBDR*plus* (Hain Lifescience, Nehren, Germany) assay and Sanger sequencing performed as described elsewhere (17).

The VerePLEX Biosystem consists of a single disposable device comprising microfluidic PCR and microarray modules. The platform includes a temperature control system (TCS) and an optical reader (OR) which allows automatic analysis of the microarray, providing a user-friendly diagnostic report (see Fig. S2 in the supplemental material) (18). The protocols for MDR-TB assay are described in Text S3, and the primers are shown in Table S4. The assay allows detection of MTBC and other common NTM, together with the most frequent mutations affecting the *rpoB*, *katG*, and *inhA* genes, which are involved in phenotypic resistance to RIF and INH in MTBC.

TABLE 3 Diagnostic performance of the phenotypic DST, MTBDR*plus*, VerePLEX Biosystem, and Xpert MTB-RIF for detecting rifampin resistance (*rpoB*) in clinical isolates and specimens[a]

Parameter	Value (95% CI) for clinical isolates (n = 91)		Value (95% CI) for clinical specimens[b]		Method type and no. of indeterminate results/total (%)
	MTBDR*plus*/seq	DST	MTBDR*plus*/seq/Xpert MTB-RIF (n = 71)	DST (n = 58)	
Sensitivity (%)	100.00 (94.58, 100.00)	98.53 (92.13, 99.74)	100.00 (77.19, 100.00)	100.00 (75.75, 100.00)	Molecular 3/71 (4.23)
Specificity (%)	100.00 (86.2, 100.00)	100.00 (85.69, 100.00)	100.00 (93.47, 100.00)	100.00 (91.97, 100.00)	Phenotypic 2/58 (3.45)
PPV (%)	100.00 (94.58, 100.00)	100.00 (94.58, 100.00)	100.00 (77.19, 100.00)	100.00 (75.75, 100.00)	
NPV (%)	100.00 (86.2, 100.00)	95.83 (79.76, 99.26)	100.00 (93.47, 100.00)	100.00 (91.97, 100.00)	
Negative likelihood ratio	0.00 (0.00, ?)	0.01 (0.00, 0.10)	0.00 (0.00, ?)	0.00 (0.00, ?)	
Diagnostic accuracy (%)	100.00 (95.95, 100.00)	98.90 (94.03, 99.81)	100.00 (95.95, 100.00)	100.00 (93.58, 100.00)	

[a] The diagnostic performance of the MTBDR*plus* assay and sequencing (MTBDR*plus*/seq), phenotypic drug susceptibility testing (DST), and MTBDR*plus* assay, sequencing, and Xpert MTB-RIF assay (MTBDR*plus*/seq/Xpert MTB-RIF) for detecting rifampin resistance (*rpoB*) are shown. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and diagnostic accuracy were calculated according to the Wilson score (www.OpenEpi.com). The positive and negative likelihood ratios were also calculated. The lower and upper limits of the 95% confidence interval (95% CI) are shown in parentheses. The effective number of samples considered for the analysis is reported for each target. The positive likelihood ratio cannot be computed, since specificity is always 100%.
[b] There were a total of 80 *M. tuberculosis*-positive smear-positive clinical specimens and a total of 116 *M. tuberculosis*-negative clinical specimens.

Analysis of the diagnostic performance of the LoC assay on clinical isolates. MTBC was detected in all 91 clinical isolates (Table 2). Concerning the *rpoB* and *inhA* targets, 100% concordance was observed between the MTBDR*plus* and LoC assay results. In one case, the LoC assay revealed both wild-type (WT) and mutated signals from probes targeting positions 523 to 526 in *rpoB*, which was not confirmed by MTBDR*plus* assay. A 95.74% concordance was observed between the MTBDR*plus* and LoC assay results for the *katG* target. In two cases, probes complementary to the WT sequence of codon 315 of *katG* were detected slightly over the on/off cutoff, but the MTBDR*plus* assay showed an absence of signal from the WT probe. In another two cases, a double pattern (mutated and WT) was detected by the LoC assay, but only the mutation was identified by the MTBDR*plus* assay.

Other mutations identified by sequencing (L530M, S531P, and Q513 in *rpoB* and S315N and S315R in *katG*) were correctly detected on the chip by the absence of signal from respective WT probes.

Compared with DST, the sensitivity and specificity of the MTBDR*plus* assay for RIF were 98.53% and 100%, respectively, and the sensitivity and specificity for INH were 82.76% and 100%, respectively (Tables 3, 4, and 5).

Analysis of the diagnostic performance of the LoC assay on clinical specimens. DST results for RIF and INH were available for 58 and 57 samples, respectively. The chips presenting incomplete results were repeated once and then included in the analysis (Table 6).

Valid results were obtained in 99.00%, 95.80%, and 95.50% of the cases for MTBC, *rpoB*, *katG*, and *inhA* targets, respectively. MTBC was detected with 100% sensitivity and specificity on the LoC, as well as resistance to RIF (Tables 3, 4, and 5). One discrepant result was detected for the *katG* and *inhA* genes, leading to a sensitivity of 93.75% and 90.91%, respectively, compared to the MTBDR*plus* assay. Overall, the sensitivity and specificity of *katG* and *inhA* targets were 73.33% and 100%, respectively, compared to DST. Three specimens gave invalid values by the LoC assay. One sample gave an invalid result for PCR controls, possibly due to inhibitors affecting the reaction in the microfluidic environment. The remaining two specimens also yielded invalid results with the MTBDR*plus* assay. All 116 MTBC culture-negative specimens were classified correctly.

In the current study, we developed and evaluated a LoC-based assay for the diagnosis of MDR-TB. LoC devices represent promising tools to fill the diagnostic gap in low-income countries: they integrate many of the laboratory components on a small chip, thus reducing infrastructure and technical requirements but preserving analytical capabilities. In addition, the operating speed, ease of modification (addition/removal of probes), and ability to perform multiplex tests and to scale down costs represent other relevant features of LoCs (19, 20).

Our results showed high specificity and sensitivity of the semiautomated VerePLEX Biosystem for the MDR-TB targets, thus suggesting an usefulness of the platform for fast and simple diagnosis of MDR cases in centralized laboratories. The sensitivity and specificity of the NTM probes on the same platform were evaluated by Lazzeri et al. (21). The assay allowed us to identify correctly MTBC in 100% of the smear-positive samples tested independently of the smear microscopy score, with a small number of indeterminate results due most likely to the low quality of DNA extracted. Resistance to RIF and INH was detected by the chip with high sensitivity and specificity in agreement with the minimal requirements established by the WHO

TABLE 4 Diagnostic performance of the phenotypic DST, MTBDR*plus*, VerePLEX Biosystem, and Xpert MTB-RIF for detecting isoniazid resistance (*katG* and *inhA*) in clinical isolates and specimens[a]

Parameter	Value (95% CI) for clinical isolates ($n = 91$)			Value (95% CI) for clinical specimens[b]			Method type and no. of indeterminate results/total (%)
	MTBDR*plus*/seq			MTBDR*plus*/seq/Xpert MTB-RIF			
	katG	*inhA*	DST	*katG* ($n = 67$)	*inhA* ($n = 67$)	DST ($n = 57$)	
Sensitivity (%)	95.74 (87.75, 98.83)	100.00 (91.03, 100)	82.76 (73.48, 89.26)	93.75 (71.67, 98.89)	90.91 (62.26, 98.38)	73.33 (55.55, 85.82)	Molecular 3/67 (4.48)
Specificity (%)	100.00 (91.97, 100.00)	100.00 (93.12, 100.00)	100.00 (51.01, 100.00)	100.00 (92.59, 100.00)	100.00 (93.24, 100.00)	100.00 (86.68, 100.00)	Phenotypic 2/57 (3.5)
PPV (%)	100.00 (92.13, 100.00)	100.00 (91.03, 100.00)	100.00 (94.93, 100.00)	100.00 (79.61, 100.00)	100.00 (72.25, 100.00)	100.00 (85.13, 100.00)	
NPV (%)	95.65 (85.47, 98.90)	100.00 (93.12, 100.00)	21.05 (8.51, 43.33)	97.96 (89.31, 99.64)	100.00 (90.23, 99.67)	75.76 (58.98, 87.17)	
Negative likelihood ratio	0.04 (0.02, 0.11)	0.00 (0.00, ?)	0.17 (0.15, 0.20)	0.07 (0.009, 0.44)	0.09 (0.01, 0.65)	0.26 (0.21, 0.34)	
Diagnostic accuracy (%)	97.8 (92.34, 99.4)	100.00 (95.95, 100.00)	83.52 (74.57, 89.75)	98.44 (91.67, 99.72)	98.44 (91.67, 99.72)	85.45 (73.84, 92.44)	

[a] The diagnostic performance of the MTBDR*plus*/seq assays, phenotypic drug susceptibility testing (DST), and MTBDR*plus*/seq/Xpert MTB-RIF assays for detecting isoniazid resistance (*katG* and *inhA*) are shown. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and diagnostic accuracy were calculated according to the Wilson score (www.OpenEpi.com). The positive and negative likelihood ratios were also calculated. The lower and upper limits of the 95% confidence interval (95% CI) are shown in parentheses. The effective number of samples considered for the analysis is reported for each target. The positive likelihood ratio cannot be computed, since specificity is always 100%.

[b] There were a total of 80 *M. tuberculosis*-positive smear-positive clinical specimens and a total of 116 *M. tuberculosis*-negative clinical specimens.

TABLE 5 Diagnostic performance of the phenotypic DST, MTBDR*plus*, and VerePLEX Biosystem for detecting *M. tuberculosis* in clinical isolates and specimens[a]

Parameter	Value (95% CI) for clinical isolates ($n = 91$)		Value (95% CI) for clinical specimens ($n = 196$)[b] by MTBDR*plus*/seq/Xpert MTB-RIF	No. of indeterminate results/total (%)
	MTBDR*plus*/seq	DST		
Sensitivity (%)	100.00 (95.95, 100.00)	100.00 (95.95, 100.00)	100.00 (95.31, 100.00)	2/196 (1.02)
Specificity (%)	Undefined	Undefined	100.00 (96.79, 100.00)	
PPV (%)	100.00 (95.95, 100.00)	100.00 (95.95, 100.00)	100.00 (95.31, 100.00)	
NPV (%)	Undefined	Undefined	100.00 (96.79, 100.00)	
Negative likelihood ratio	Undefined	Undefined	0.00	
Diagnostic accuracy (%)	Undefined	Undefined	100.00 (98.06, 100.00)	

[a] The diagnostic performance of the MTBDR*plus*/seq assays, phenotypic drug susceptibility testing (DST), and MTBDR*plus*/seq/Xpert MTB-RIF assays for detecting *M. tuberculosis* are shown. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and diagnostic accuracy were calculated according to the Wilson score (www.OpenEpi.com). The positive and negative likelihood ratios were also calculated. The lower and upper limits of the 95% confidence interval (95% CI) are shown in parentheses. The effective number of samples considered for the analysis is reported for each target. The positive likelihood ratio cannot be computed, since specificity is always 100%.
[b] There were a total of 80 *M. tuberculosis*-positive smear-positive clinical specimens and a total of 116 *M. tuberculosis*-negative clinical specimens.

for molecular tools, comparable to the sensitivity and specificity of the MTBDR*plus* assay (12). The limit of detection of the assay was observed in the range of 10^1 genome copies/reaction, as reported in Table S5 in the supplemental material.

A separate array layout for spoligotyping of MTBC was also developed in the TM-REST Project (data not shown). The possibility of integrating the probes for spoligotyping, MDR- and extensively DR-TB in one medium-density microarray layout by using separate multiplex-PCR would enhance the benefits of the microarray assays and would enable the reduction of time to results compared to other available tests (22–24).

The ease of customization of the array design makes the LoC a versatile tool for easy integration of relevant targets for local genetic variants, new genes and/or mutations, and novel key drugs included in new therapeutic regimens. In addition, the LoC can be adapted for other diagnostic or research needs, thus providing a

TABLE 6 Phenotypic DST, MTBDR*plus*, Xpert MTB-RIF, and VerePLEX Biosystem *M. tuberculosis* results for the 80 smear-positive MTBC culture-positive clinical specimens included in the study

Phenotypic DST result[a] for:		MTBDR*plus*/sequencing result[b] for the following gene:			Xpert MTB-RIF result[c] for:		VerePLEX MTB result[b,d] for the following gene:			No. of clinical specimens[e]
RIF	INH	*rpoB*	*katG*	*inhA*	MTB	RIF	*rpoB*	*katG*	*inhA*	
S	R	WT	WT	C-15T			WT	WT	C-15T	9
R	R	S531L	S315T1	WT			S531L	S315T1	WT	2
R	R	S531L	WT + S315T1	WT			S531L	WT + S315T1	WT	1
S	R	WT	WT	WT			WT	WT	WT	6
R	R	D516V	S315T1	WT			D516V	S315T1	WT	2
R	R	S531L	WT	WT			S531L	WT	WT	2
S	R	WT	S315T1	WT			WT	S315T1	WT	4
R	R	S531L	S315T1/T2	WT			S531L	S315T1/T2	WT	1
R	R	Q513P	S315T1	WT			Δ 510–513 WT	S315T1	WT	1
S	R	WT	S315N	WT			WT	Δ 313–317 WT	WT	1
R	S	S531L	WT	WT			S531L	WT	WT	1
R	R	S531L	WT	C-15T			S531L	Δ 313–317 WT	WT	1
S	S	WT	WT	WT			WT	WT	WT	15
R	R	Δ 518–525 WT, Δ 530–533 WT	S315T1	WT			Δ 523–526 WT, S531L	S315T1	WT	1
		D516V	S315T1	T-8C			D516V	S315T1	T-8C	1
		WT	WT	WT			WT	WT	WT	15
		WT	S315T1	WT			WT	S315T1	WT	1
S	S						WT	WT	WT	9
					pos	WT	WT	WT	WT	4
		WT	WT	WT			PCNV	PCNV	PCNV	1
S	S	ND	S315T1	WT			MTBND	MTBND	MTBND	1
S	S	ND	WT	WT			ND	ND	ND	1

[a] The phenotypic drug susceptibility testing results for rifampin and isoniazid are given as follows: R, resistant; S, sensitive.
[b] The results for the 80 smear-positive, MTBC culture-positive isolates found by the MTBDR*plus* assay and sequencing or by the VerePLEX Biosystem are shown (wild type [WT] or mutant). Δ, no WT signal; ND, not detected.
[c] MTB, *M. tuberculosis*; pos, positive.
[d] PCNV, PCR controls not valid; MTBND, *M. tuberculosis* not detected; ND, not detected.
[e] The number of smear-positive, MTBC culture-positive clinical specimens applies to all the tests.

multipurpose platform suitable for other relevant diseases (e.g., influenza, malaria, tropical diseases) (25, 26).

ACKNOWLEDGMENTS

This study was supported by FP7 EU grant TM-REST (HEALTH-F3-2008-202145) and European and Developing Countries Clinical Trials Partnership as part of the TB CHILD project (IP.2009.32040.007).

The members of the TM-REST Consortium follow: Patrizia Di Pietro, Floriana San Biagio, Enrico Alessi, and Tony G. Barbuzzi (Analog, MEMS & Sensor Group, HealthCare Business Development Unit, STMicroelectronics, Catania, Italy); Silva Tafaj (University Hospital Shefqet Ndroqi, Tirana, Albania); Elizabetha Bachiyska (National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria); Irina Kontsevaya (Samara TB Service, Samara, Russian Federation); Yanina Balabanova (Clinical TB and HIV Group, Blizard Institute, Queen Mary University of London, United Kingdom, and Department of Infectious Diseases and Immunity, Imperial College London, United Kingdom); and Elisa Lazzeri (Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena, Siena, Italy). The members of the TB-CHILD Consortium follow: Joseph Sserunkuma, Francesco Aloi, and Martin Nsubuga (Laboratory Department, St. Raphael of St. Francis Nsambya Hospital, AISPO, Kampala, Republic of Uganda) and Mohamed Sasamalo (Ifakara Health Institute, Bagamoyo, United Republic of Tanzania).

We thank Tanja Ubben and Tanja Struwe Sonnenschein for excellent technical assistance and Enrico Tortoli for valuable support.

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