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Implementation Validation Performed in Rwanda To Determine Whether the INNO-LiPA Rif.TB Line Probe Assay Can Be Used for Detection of Multidrug-Resistant *Mycobacterium tuberculosis* in Low-Resource Countries▽

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We validated the implementation of the INNO-LiPA Rif.TB line probe assay, a diagnostic test for rapid detection of multidrug-resistant tuberculosis (MDR-TB), in Rwanda. No substantial difference was found between results obtained in Rwanda and results obtained in Belgium with the same samples. This rapid diagnostic test for MDR-TB can therefore be reliably implemented in a resource-poor setting.

Along with human immunodeficiency virus/AIDS, multidrug-resistant tuberculosis (MDR-TB) has become the most important threat to TB control (5, 13, 22). A recent survey performed in Rwanda according to WHO guidelines (27) determined the prevalence among all cases of MDR-TB to be 4.6% (25), 1.7 times higher than that reported for Africa (2.7%) (26). Left untreated or inappropriately treated, MDR-TB could become an emerging threat in Rwanda and other African countries displaying similar trends in drug resistance. Implementation of appropriate management policies is therefore crucial, especially in light of the emergence of extensively drug resistant TB (XDR-TB) (14, 21).

Late recognition of drug resistance contributes considerably to the mortality of patients and the spread of MDR-TB and XDR-TB, particularly among immunocompromised patients. The timely detection of drug-resistant TB is therefore imperative. In low-resource countries, TB is currently detected by microscopy, a tool that does not allow drug susceptibility testing (DST) (2).

Complementing microscopy with a diagnostic tool allowing DST would rapidly place patients on appropriate treatment regimens and limit the spread of MDR-TB and XDR-TB. Culture of sputum on a solid medium provides reliable DST results but is extremely lengthy and significantly delays the initiation of proper therapy. Microscopy should therefore be complemented by rapid tests to determine first-line drug resistance, providing an accurate diagnosis within a few days. One of those methods is the INNO-LiPA Rif.TB assay (Innogenetics, Zwijnaarde, Belgium). It relies on PCR amplification of mycobacterial DNA present in the sputum and subsequent hybridization of amplicons on nitrocellulose strips to detect the presence of *Mycobacterium tuberculosis* in the specimen at a sensitivity of 92%. (For detailed specifics regarding the test, see references 4 and 20.) It also has the added benefit of simultaneously detecting MDR-TB (20). Technically, the LiPA test detects rifampin (RIF) resistance only. However, in high-incidence countries, 90% of RIF-resistant isolates are also isoniazid resistant (17). RIF resistance therefore acts as a surrogate marker for the detection for MDR-TB (23). Numerous studies have previously shown that this manufactured diagnostic test is an excellent tool for the direct detection of both TB and MDR-TB from clinical samples (3, 4, 8, 11, 24), effectively bypassing the need for a culture step. These studies, however, were always carried out in molecular biology laboratories, mostly in Europe. Despite their value as rapid and accurate diagnostic tools, tests based on the LiPA technology are not yet used in developing countries because of their high cost and the assumption that such sophisticated tests cannot be implemented in low-resource settings.

In this study, we evaluate the implementation of the LiPA format in Rwanda. Given several constraints (existing facilities, local resources [LR], and personnel inexperienced in molecular techniques), we challenge the misconception that PCR technology is too sophisticated for implementation in developing countries, and we validate the INNO-LiPA assay on bacterial suspensions under low-resource conditions in two cities in Rwanda.

INNO-LiPA Rif.TB assays. Eight INNO-LiPA kits purchased from Innogenetics (Zwijnaarde, Belgium) were used in accordance with the manufacturer's instructions (4). Each run contained a negative control using water instead of DNA.

Samples. Eighteen *M. tuberculosis* strains (Table 1) were obtained from the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. Samples were chosen to include both RIF-sensitive and -resistant strains and to encompass different mutations causing RIF resistance, as determined by sequencing of the *rpoB* gene (19). Samples were prepared as follows. A loopful of bacilli grown on Löwenstein-Jensen

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TABLE 1. Samples analyzed for the validation of the INNO-LiPA Rif.TB line probe assay in Rwanda

Sample ID[a]	Sample origin	S/R[b]	Mutation
M04-1150	Rwanda	R	Leu531 (TTG)
M04-1144	Rwanda	R	Leu531 (TTG)
M04-1130	Rwanda	R	Leu531 (TTG)
M04-1105	Rwanda	R	Leu531 (TTG)
M04-1086	Rwanda	R	Leu531 (TTG)
M04-1226	South Korea	R	Pro511 (CCG)
M04-1224	South Korea	S	Wild type
M04-1221	South Korea	R	Tyr526 (TAC)
M04-1679	Nepal	R	Gly512 (GGC)
M04-1668	Germany	S	Wild type
M04-1663	Germany	R	Asp526 (GAC)
M04-1251	South Korea	S	Wild type
M04-1244	South Korea	R	Tyr526 (TAC)
M04-1243	South Korea	R	Arg526 (CGC)
M04-1674	Nepal	S	Wild type
M04-1232	South Korea	S	Wild type
M04-1458	Rwanda	R	Val516 (GTC)
M04-1681	Nepal	R	Leu531 (TTG)

[a] ID, identification.
[b] S/R, susceptibility or resistance, respectively, to RIF.

slants was added to 400 μl of 1× TE buffer (10 mM Tris, 1 mM EDTA [pH 8]), mixed for about 1 min, and boiled (100°C) for 5 min. The heat-inactivated bacterial suspensions (approximately 1 mg/ml) were blinded by members of the ITM, transported to Rwanda at room temperature, and subsequently stored at +4°C.

Location of the study. The study was performed at two locations in Rwanda: the National Reference Laboratory (NRL) in Kigali and the University Hospital in Butare (UHB). The NRL is furnished with modern equipment and routinely performs PCR for infectious diseases other than TB. The UHB, a facility that analyzes hospital specimens using traditional methodologies, does not currently perform PCR diagnosis. All required equipment was transported from Kigali. A universal power supply connected to the thermocycler prevented disruption of the cycles and loss of samples and reagents in case of power outages during PCR.

Training of Rwandan technicians. Two Rwandan technicians, one working at the NRL and one at the UHB, were first trained for 2 days at the NRL to perform PCR and use the INNO-LiPA kits. Neither Rwandan technician had prior experience using these techniques or any other training in molecular biology. Under supervision, each technician successfully amplified a DNA amplicon of the appropriate size starting from *M. tuberculosis* DNA and using primers and reagents from the LiPA kit. Both trainees correctly distinguished a TB sample from a negative control after hybridization and visualization on the LiPA strip. Training was therefore deemed successful, and the technicians were subsequently required to independently analyze all 18 samples by using INNO-LiPA kits under different working conditions.

Analysis of samples. Blinded samples were analyzed by testing three working conditions: the facility, the resources used, and the skills of the operator. In each setting, the samples were always analyzed in parallel by a Rwandan technician and a trained molecular biologist, whose results served as a control. To determine whether the INNO-LiPA assay could be carried out in Rwanda using LR, we tested two key materials required for the PCR step but not provided in the kit: *Taq* polymerase and water. Thus, we analyzed each sample, in each setting, using either PCR-grade distilled water and *Taq* polymerase manufactured by Roche Diagnostics, both items purchased from the United States (referred to as imported materials [IM]), or locally distilled water and a cheaper *Taq* polymerase manufactured by Bioline (Luckenwalde, Germany) and purchased from Hass Scientific in Kenya, the geographically closest distributor of the enzyme (referred to as LR). All remaining consumable materials were purchased from Fisher Scientific (St. Louis, MO). The thermocycler (T3 thermocycler; Biometra, Goettingen, Germany) and universal power supply (1000VA; Picace, Weiswampach, Luxembourg) were provided by the NRL. At the end of the study, the results from all the different working conditions were sent to the ITM, where codes were lifted. The performance rate was calculated as a percentage of the agreement between the results obtained in Rwanda and those obtained in Belgium.

Impact of the facility. Despite differences in working conditions, 100% correlation was obtained both by the molecular biologist at the NRL (Table 2) and by the molecular biologist in Butare (Table 3).

Impact of skills. After a 2-day training, the Rwandan technicians were able to reach performance rates as high as 94.4%, and performances never fell below 88%. Decreased performance was systematically caused by inconclusive LiPA strips with unreadable patterns due to low-yield amplicons following a suboptimal PCR amplification, as demonstrated by very weak bands on the agarose gel.

Impact of materials. Complete agreement was found between the results obtained by the molecular biologists with IM and those obtained with LR in both Kigali (Table 2) and Butare (Table 3): However, the performance of the technician in Kigali dropped when LR were used instead of IM. For logistical reasons, all samples were tested in Butare first and later in Kigali. We suspect that the *Taq* polymerase (Bioline) purchased from Kenya had been transported at a suboptimal temperature, possibly leading to a loss in activity. Quality control of new batches of reagents should be systematically made part of general laboratory quality management. Nevertheless, the results obtained by both the molecular biologists and the Rwandan technicians in both settings, using either IM or LR, were similar enough to suggest that current LR could be sufficient to implement this diagnostic test in Rwanda.

Statistical analysis. Based on McNemar's test for matched pairs, there does not appear to be an association between test accuracy and working conditions (i.e., facility, resources used, and skills of operator). Additionally, given the 95% confidence intervals (95% CI) (Tables 2 and 3), the accuracy of testing under each working condition is not significantly different from the 97% pooled sensitivity of the INNO-LiPA test (15).

In this study, we have shown that PCR-based assays such as the INNO-LiPA Rif.TB assay can be implemented in a low-income setting and require limited equipment and skills. Although the number of samples analyzed in the study was small, the results demonstrated that local personnel can be rapidly trained to successfully use the LiPA platform. We used heat-inactivated bacterial suspensions for this initial validation. Therefore, one step in the diagnostic procedure for rapid de-

TABLE 2. Results obtained in Kigali by the molecular biologist and the Rwandan technician at the NRL using IM or LR

Sample ID[a]	Expected LiPA pattern[b]	Result[c] obtained by:			
		M-IM	R1-IM	M-LR	R1-LR
M04-1150	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5
M04-1144	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5
M04-1130	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5
M04-1105	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5
M04-1086	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	Indeterminate
M04-1226	ΔS1	ΔS1	ΔS1	ΔS1	ΔS1
M04-1224	wt	wt	wt	wt	wt
M04-1221	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a	Indeterminate
M04-1679	ΔS1 and ΔS5	ΔS1 and ΔS5	ΔS1 and ΔS5	ΔS1 and ΔS5	ΔS1 and ΔS5
M04-1668	wt	wt	wt	wt	wt
M04-1663	ΔS4/R4b	ΔS4/R4b	ΔS4/R4b	ΔS4/R4b	ΔS4/R4b
M04-1251	wt	wt	wt	wt	wt
M04-1244	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a
M04-1243	ΔS4	ΔS4	Indeterminate	ΔS4	ΔS4
M04-1674	wt	wt	wt	wt	wt
M04-1232	wt	wt	wt	wt	wt
M04-1458	ΔS2/R2	ΔS2/R2	ΔS2/R2	ΔS2/R2	ΔS2/R2
M04-1681	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5

[a] ID, identification.
[b] wt, wild type.
[c] M, molecular biologist; R1, Rwandan technician employed at the NRL. Performances (95% CI) were 100% (0.845, 1.0) for M-IM, 94.4% (0.723, 1.0) for R1-IM, 100% (0.845, 1.0) for M-LR, and 88.8% (0.659, 0.981) for R1-LR. Intervals were calculated using the adjusted Wald technique presented by Agresti and Coull (1).

tection of MDR-TB in TB patients was not validated: the direct extraction of DNA from clinical samples. However, we do not foresee this as a limitation to successful implementation of the tool, because DNA extractions from clinical specimens are currently performed at the NRL. Nevertheless, further validation on spiked sputum specimens is recommended as a final step prior to implementation.

Although the INNO-LiPA test displays higher sensitivity and specificity than microscopy (22), the current unit price of the test remains too high for widespread rollout of the technology in low-resource settings. However, prices of line probe assays are expected to decrease significantly in the coming years, since the Foundation for Innovative New Diagnostics (FIND) recently signed an agreement with one of the manufacturers of line probe assays in Europe to provide the diagnostic tools at a preferential rate for developing countries (7).

It should therefore be possible in the coming years to integrate this platform into a centralized strategy in the context of a reference laboratory as a complement to microscopy. The INNO-LiPA Rif.TB assay could then be used to screen treatment failures and single out cases of MDR-TB and possibly XDR-TB. Those patients can subsequently undergo the regu-

TABLE 3. Results obtained in Butare by the molecular biologist and the Rwandan technician at the UHB using IM or LR

Sample ID[a]	Expected LiPA pattern[b]	Result[c] obtained by:			
		M-IM	R2-IM	M-LR	R2-LR
M04-1150	ΔS5/R5	ΔS5/R5	Indeterminate	ΔS5/R5	ΔS5/R5
M04-1144	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5
M04-1130	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5
M04-1105	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5
M04-1086	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5
M04-1226	ΔS1	ΔS1	ΔS1	ΔS1	ΔS1
M04-1224	wt	wt	wt	wt	wt
M04-1221	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a
M04-1679	ΔS1 and ΔS5	ΔS1 and ΔS5	ΔS1 and ΔS5	ΔS1 and ΔS5	ΔS1 and Δ S5
M04-1668	wt	wt	wt	wt	wt
M04-1663	ΔS4/R4b	ΔS4/R4b	ΔS4/R4b	ΔS4/R4b	ΔS4/R4b
M04-1251	wt	wt	wt	wt	wt
M04-1244	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a	ΔS4/R4a
M04-1243	ΔS4	ΔS4	ΔS4	ΔS4	ΔS4
M04-1674	wt	wt	wt	wt	wt
M04-1232	wt	wt	wt	wt	wt
M04-1458	ΔS2/R2	ΔS2/R2	ΔS2/R2	ΔS2/R2	Indeterminate
M04-1681	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5	ΔS5/R5

[a] ID, identification.
[b] wt, wild type.
[c] M, molecular biologist; R2, Rwandan technician employed at the UHB. Performances (95% CI) were 100% (0.845, 1.0) for M-IM, 94.4% (0.723, 1.0) for R2-IM, 100% (0.845, 1.0) for M-LR, and 94.4% (0.723, 1.0) for R2-LR. Intervals were calculated using the adjusted Wald technique presented by Agresti and Coull (1).

lar DST and benefit from second-line-drug regimens tailored to their infections. This critical need for laboratory tests for detection of drug resistance highlights the importance of strengthening laboratory capacities in some high-burden countries. High-quality laboratory activities complement other strategies and contribute to better control of TB worldwide (18). Only after implementation, with subsequent quality controls and continuous monitoring of contamination and performance, can a final evaluation of the use of this assay in resource-poor settings be made.

In countries such as Rwanda, the major barrier to addressing the MDR-TB threat, besides selection of the appropriate DST tool, is the high cost of second-line drugs, which are 300 times more expensive than first-line drugs (9). Despite this significant hindrance, several authors have argued that deliberately overlooking the treatment of MDR-TB cases based on the cost of drugs is both unethical and dangerous (6, 12, 16). Others, however, claim that treatment of MDR-TB cases should not be considered in Africa, because it could potentially reallocate funds destined for treatment of susceptible TB and therefore divert money from the directly observed therapy, short course (DOTS) programs in place (10). Nevertheless, with the creation of funding bodies such as the Global Fund, countries can currently apply for grants that would allocate special resources specifically for the diagnosis and treatment of MDR-TB, provided the treatment success rates are high enough to ensure no further spread of resistance. In Rwanda, funds for second-line drugs are already available from the Belgian Damien Foundation. This further confirms the need for quick implementation of rapid diagnosis of MDR-TB in a country that has both the need for the technology and the means to implement it.

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