Investigación original / Original research

Pan American Journal of Public Health

Use and evaluation of a line probe assay in patients with tuberculosis in Peru: 2011–2013

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Suggested citation Puyén ZM, Acosta J, Obregon G, Pacheco E, Ramirez H, Mendoza A, et al. Use and evaluation of a line probe assay in patients with tuberculosis in Peru: 2011–2013. Rev Panam Salud Publica. 2016;39(1):19–25.

ABSTRACT

Objective. *To determine the use and performance of a line probe assay (LPA) compared with conventional culture and drug sensitivity testing (CDST) in patients registered with tuberculosis (TB) under routine program conditions in Peru in 2011–2013.*

Methods. *This was a descriptive, operational research, cross-sectional study of sputum specimens from patients with smear-positive pulmonary TB and mycobacterial cultures from patients with smear-negative or positive TB. Drug resistance to rifampicin and/or isoniazid detected by LPA was compared to CDST. Sensitivity, specificity, and predictive values were calculated and reliability for detecting drug resistance was assessed through kappa coefficient, with values 0.61–0.80 showing substantial correlation, and 0.81 or above showing almost-perfect correlation.*

Results. *In 2011–2013, there were 16 169 LPA tests performed, with the proportion of TB patients receiving the test increasing from 3.2% to 30.2%. In all, 2 905 LPA test results were compared to CDST. For LPA in sputum specimens, sensitivity for rifampicin was 92%; isoniazid, 94%; and MDR-TB, 88%; while specificity for rifampicin was 92%; isoniazid, 92%; and MDR-TB, 95%. For LPA in mycobacterial cultures, sensitivity for rifampicin was 95%; isoniazid, 96%; and MDR-TB, 90%; while specificity for rifampicin was 85%; isoniazid, 91%; and MDR-TB, 94%. Kappa coefficients were at 0.81 or above for all comparisons of LPA with CDST using sputum specimens and cultures, except for isoniazid in cultures, which was at 0.79.*

Conclusions. *This study suggests that LPA is a reliable and rapid screening test for drug-resistant TB and should be considered suitable for routine use and scale up in Peru.*

Keywords *Mycobacterium tuberculosis*; tuberculosis; tuberculosis, multidrug-resistant; molecular probes; molecular probe techniques; operations research; Peru.

Peru is a democratic republic divided into 25 political areas and a total population of 30 million people. Currently, the World Bank classifies Peru as an upper-middle income country with a nominal GDP per-capita of approximately US$ 7 000; however, wealth distribution is not equitable, and in 2010 about 30% the total population was classified as poor (1). Since 1990, Peru has run a national tuberculosis program following World Health Organization (WHO) guidelines (2, 3). Despite the continuous effort, tuberculosis (TB) continues to be one of the country's major infectious diseases.

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In the Region of the Americas, Peru is considered 5th in TB burden and 1st in multidrug-resistant tuberculosis (MDR-TB; resistant to both isoniazid and rifampicin) and extensively drug resistant tuberculosis (XDR-TB; MDR-TB plus resistance to a fluoroquinolone and one of the second line injectable drugs) (3). In 2013, the incidence rate was reported as 90 new cases per 100 000 population; in all, there were 31 052 patients with notified TB, of whom 1 281 had MDR-TB and 77 had XDR-TB (3, 4).

Peru's National Tuberculosis Program (NTP; 4) has a central unit in the capital city of Lima, with equivalent units in every geopolitical area of the country. The central unit determines national TB control policy for both the public and private sectors. The NTP follows WHO guidelines adapted to the country's clinical, epidemiological, and socioeconomic situation (1).

According to the most recent national drug resistance survey in Peru, 1 in every 5 TB patients diagnosed in Lima is resistant to rifampicin or isoniazid (5). TB is mainly concentrated in Lima and Callao, where 54% of national TB cases, 84% of the MDR-TB cases, and 90% of the XDR-TB cases occur (3, 4). There are another 10 geographic areas in the country that are considered to have very high or high transmission of TB. Furthermore, a high prevalence of resistance to isoniazid has been reported in the country: 11.5% in new patients and 30% in previously treated patients (5).

The time required to detect MDR-TB—in most cases, 4 months from the first positive sputum smear to results of drug sensitivity testing—has been an important obstacle to treatment because it delays initiation of second-line drugs. In order to more rapidly diagnose and treat patients with drug-resistant TB, in 2011 the Ministry of Health of Peru introduced the use of a line probe assay (LPA; 6) to detect both isoniazid and rifampicin resistance directly from sputum specimens smear-positive for acid-fast bacilli and from positive cultures of *Mycobacterium tuberculosis* (MTB). This assay is a rapid molecular test used to identify the MTB complex and to detect isoniazid and rifampicin resistance through gene mutations, allowing MDR-TB diagnosis within 3 days of sputum submission (7).

In Peru, the LPA is considered a screening test. National guidelines (6) stipulate that when a patient is found to have isoniazid or rifampicin resistance by LPA, a standardized regimen of second line drugs is initiated while another sputum specimen is obtained for culture and drug sensitivity testing (CDST) to five first-line and six second-line anti-TB drugs. CDST results are used to modify standardized MDR-TB treatment regimens to create a specific, individualized regimen for each patient.

In 2010, the LPA was validated against CDST by the *Instituto Nacional de Salud* (National Institute of Health, Lima, Peru) using 200 specimens under trial conditions (8). The LPA was found to have good sensitivity and specificity; however, it has not been compared to CDST under routine conditions in Peru—neither with sputum specimens from smear-positive pulmonary TB (PTB) patients in high drug-resistant TB burden areas nor with cultures from smear-positive PTB patients in low drug-resistant TB burden areas and smear-negative PTB/extra-pulmonary TB (EPTB) patients when rapid diagnosis of drug resistance is necessary. Furthermore, there is the possibility of MTB genetic mutations over time that could affect the comparisons obtained 4 years ago under trial conditions (8).

Given such, the present study was conducted to determine the use and performance of LPA compared to CDST in TB patients under routine program conditions, specifically by: (i) recording the number of LPA tests performed in relation to the total registered number of TB patients and the number of patients with smear-positive PTB; and (ii) evaluating the sensitivity, specificity, and predictive value of the LPA for isoniazid and/or rifampicin resistance in sputum specimens and mycobacterial cultures versus CDST.

MATERIALS AND METHODS

This was a descriptive, operational research, cross-sectional study comparing and assessing the results of LPA to CDST in Peru in 2011–2013 using data from the national TB database of the national reference laboratory of Peru.

Study setting

Laboratory procedures. The National Reference Laboratory for Mycobacteria (LRNM) belongs to the National Institute of Health and leads the TB Laboratory Network in Peru. According to national guidelines (6), all new and previously-treated patients with TB must be evaluated for resistance to isoniazid and rifampicin through rapid diagnostic tests. Following these guidelines, the LRNM implemented the LPA (Genotype® MTBDR*plus*, Hain LifeScience, Nehren, Germany) in 2011 to detect MDR-TB and MTB complex from smear positive specimens and from positive mycobacterial cultures usually from smear negative specimens or extrapulmonary TB. If the LPA shows resistance to isoniazid, rifampicin, or both, then conventional culture is carried out using Löwenstein–Jensen medium and CDST using the proportion method in Middlebrook 7H10 agar BD™ in plaque (Becton Dickinson GmbH, Heidelberg, Germany), both prepared in the reference laboratory. The immunochromatographic method, SD Bioline TB Ag MPT64 Rapid (Standard Diagnostics Incorporated, Gyeonggi-do, Republic of Korea) was used to identify MTB complex from culture samples, which would then be subjected to the national reference method, CDST (9). The immunochromatography was done from cultures that were obtained from the samples sent to the National Institute of Health.

In the first years of scaling up LPA, the decision was made to include any of its results that showed isoniazid and rifampicin sensitivity, so that correlations could be made with CDST results. The drug sensitivity profile includes: isoniazid (low and high concentration), rifampicin, ethambutol, streptomycin, ciprofloxacin, kanamicyn, capreomycin, cycloserine, and p-amino salicylic acid. Sensitivity/resistance to pyrazinamide are evaluated through the pyrazinamidase assay. The results of LPA and CDST are published in the online NETLab system, which is available to clinicians and NTP officers around the country (10). The LRNM participates in the annual Supra Laboratory Network External Quality Assurance Rounds for conventional drug sensitivity testing. LPA is performed under strict conditions following the manufacturer's instructions, as described elsewhere (11).

Linkage to treatment. Following the LPA results, the NTP guidelines (6) stipulate the use of standardized anti-tuberculosis treatment regimens according to four situations, as follows: (i) sensitive to isoniazid and rifampicin; (ii) resistant only to isoniazid; (iii) resistant only to rifampicin; and (iv) resistant to both isoniazid and rifampicin. These standardized regimens may be modified to more individualized treatment according to CDST results.

Data collection and analyses

Specimens. The study included all sputum specimens from patients with smear-positive PTB and mycobacterial cultures that had an LPA done by the LRNM with comparative CDST results available through the NETLab system in 2011–2013. More than one sample per patient could be included, but the time between samples was always 6 months or more.

Variables and data collection. Data were collected in January–June 2015 in relation to the three objectives previously mentioned, and included: (i) year, number of LPA tests, and number of patients registered with smear-positive PTB each year; and (ii) results of LPA and CDST for sputum specimens or mycobacterial cultures showing isoniazid/rifampicin sensitivity, isoniazid resistance, rifampicin resistance, and rifampicin + isoniazid resistance. The sources of data were the NETLab system. Data were compiled in a Microsoft Excel™ spreadsheet (Microsoft Corp., Redmond, Washington, United States).

Data analysis and statistics. Data from the spreadsheet were exported to Stata®/MP13 (StataCorp LP, College Station, Texas, United States). The number of LPA tests and the number of patients with TB and smear-positive PTB were presented in absolute numbers and proportions. The performance of the LPA was evaluated by calculating sensitivity, specificity, positive predictive value, and negative predictive value when compared to the CDST with 95% Confidence Intervals (95% CI) being used throughout. The kappa coefficient was used to compare the reliability of LPA against CDST in detecting drug resistance. The strength of agreement for the kappa coefficient was as follows: 0–0.20 = poor; 0.21–0.40 = fair; 0.41–0.60 = moderate; 0.61–0.80 = substantial; and 0.81 to 1.0 = almost perfect (12, 13).

Ethics

Ethics approval for this study was obtained from the International Union against Tuberculosis and Lung Disease Ethics Advisory Group (Paris, France) and from the National Institute of Health Ethics Committee (Lima, Peru).

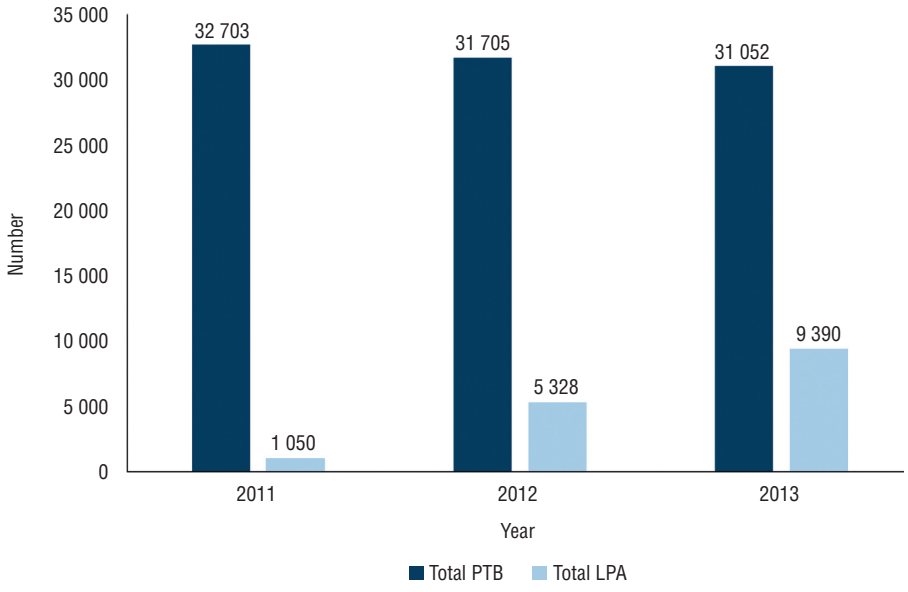
RESULTS

The numbers of LPA tests carried out in the country in relation to numbers of registered TB patients are shown in Figure 1. For all patients, the number and proportion with LPA tests done on sputum specimens and cultures increased from 3.2% in 2011 to 30.2% in 2013 (Figure 1A). For smear-positive PTB patients, the number and proportion with LPA tests done from just sputum specimens increased from 4.9% in 2011 to 34.7% in 2013 (Figure 1B).

The total number of LPA tests done in the 3-year period, along with results which were then compared with CDST, is shown in Figure 2. In total, there were 16 169 LPA tests done with more than 99% showing a definitive result of either drug sensitivity or resistance to rifampicin, isoniazid, or rifampicin+isoniazid.

FIGURE 1. Numbers of patients with line probe assay (LPA) tests performed in relation to numbers of registered tuberculosis (TB) patients, Peru, 2011–2013

1A. Total number of TB patients and total number with LPA tests performed



1B. Number of smear-positive pulmonary tuberculosis (PTB) patients and number with LPA tests performed from sputum specimens

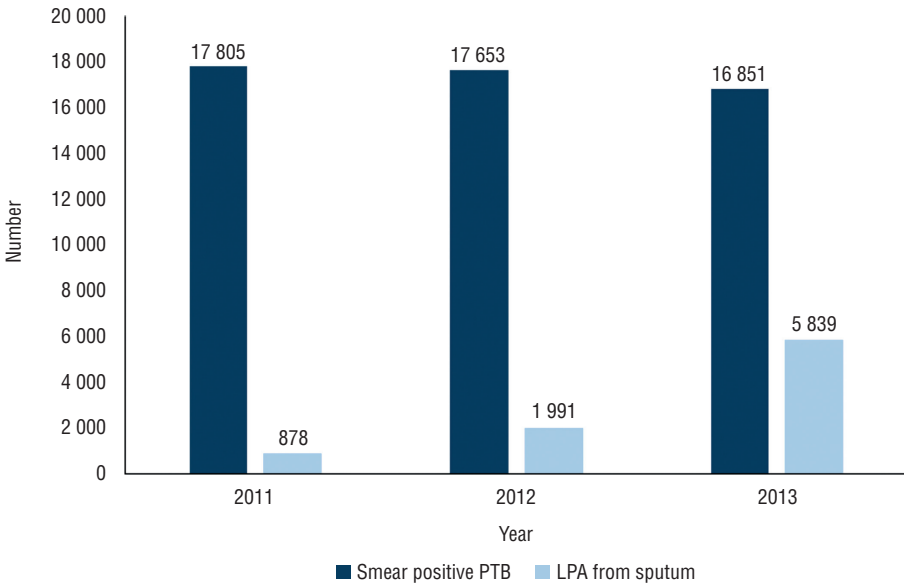
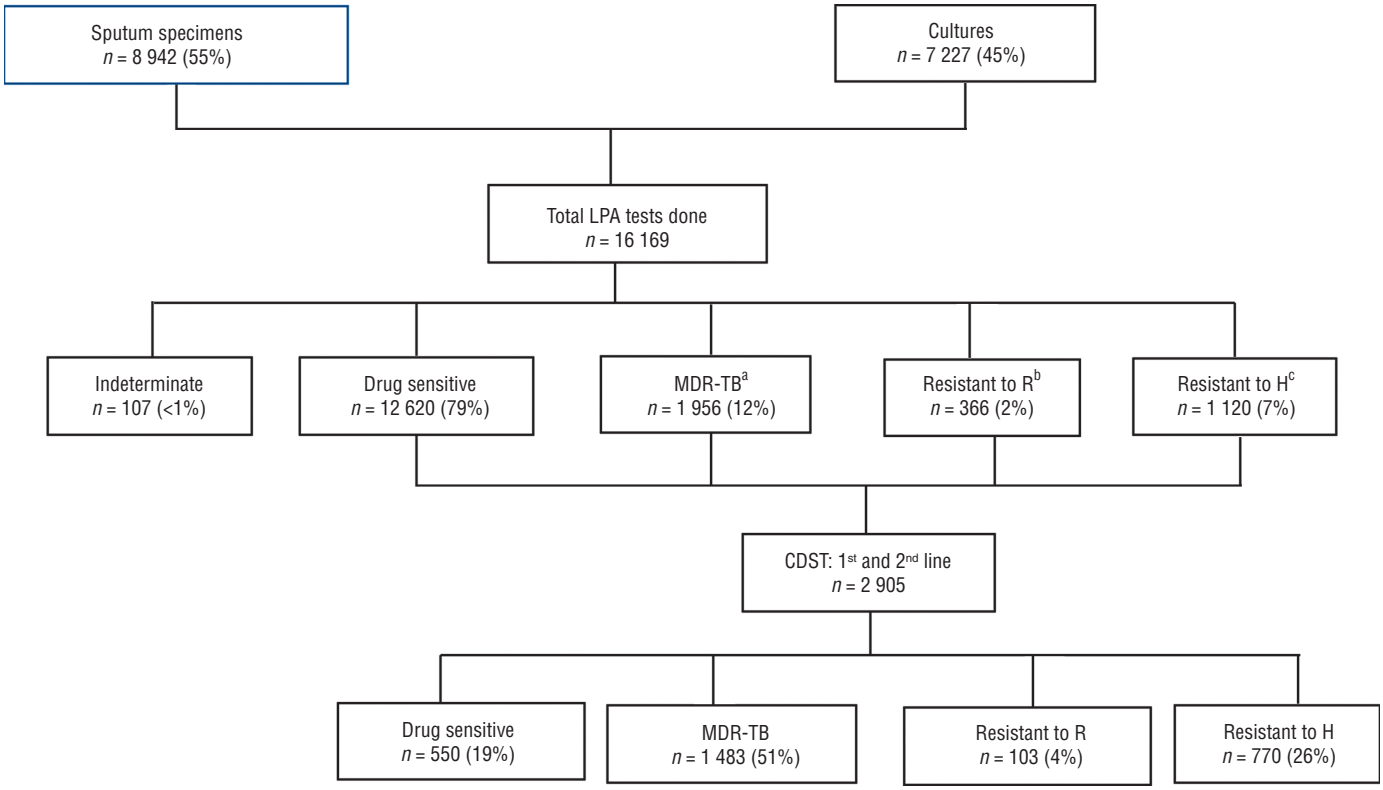


FIGURE 2. Flow diagram showing line probe assay (LPA) tests done on sputum specimens from smear-positive pulmonary tuberculosis patients and cultures from patients with smear-negative or positive tuberculosis along with the results in relation to culture and drug sensitivity testing (CDST), Peru, 2011–2013



Source: created by the authors from the study data.
[a] Multidrug resistant tuberculosis.
[b] Rifampicin.
[c] Isoniazid.

Of 12 620 LPA tests showing drug sensitivity, 493 were tested against CDST. Of 3442 LPA tests showing rifampicin, isoniazid, or rifampicin+isoniazid drug resistance, 2412 were tested against CDST, for a total of 2905 LPA tests that could be compared to CDST. There were 1 030 LPA tests with drug resistance that could not be compared due either to no mycobacterial growth or to contaminated mycobacterial cultures.

Comparisons of drug-sensitivity testing in detecting isoniazid, rifampicin, and rifampicin+isoniazid resistance using LPA and CDST for sputum specimens and cultures are shown in Table 1. The performance of the LPA in detecting rifampicin, isoniazid, and rifampicin+isoniazid resistance in sputum specimens and cultures is shown in Table 2. Sensitivity, specificity, and positive predictive value of LPA in sputum and culture for isoniazid alone, rifampicin alone, and MDR-TB were generally at 90% or above. Negative predictive values were at or above 90% for rifampicin and MDR-TB, but for isoniazid alone, they were lower at 86% for sputum and 74% for culture. Kappa coefficients were at 0.81 or above for all comparisons using sputum specimens and culture, except for isoniazid in culture which was at 0.79.

DISCUSSION

This is the first study in Peru to compare LPA to CDST under routine program conditions. Since LPA was introduced in Peru in 2011, there has been a gradual increase in the use of this rapid molecular test. By 2013, over one-third of registered TB patients received LPA as a screening test either on culture from smear-negative PTB or EPTB or on sputum specimens from smear-positive PTB. Sensitivity and specificity were generally similar and good when LPA was used with sputum or with culture, with results being slightly better for isoniazid or rifampicin alone rather than the two drugs assessed together. Kappa coefficients were all at 0.81 or above, with the exception of isoniazid in cultures, suggesting excellent overall correlation.

LPA performance in this programmatic study was inferior to that shown 4 years ago under trial conditions, where sensitivity and specificity on sputum specimens for rifampicin and isoniazid were 96%/98% and 97%/96%, respectively (8). The trial was done under ideal conditions with the type and volume of the sputum specimen and the time from submission of sputum to receipt in the laboratory being strictly controlled. Such quality control is not possible under routine program conditions.

How do these results from Peru compare with previous studies in other countries? Under trial conditions in Vietnam (14) and Thailand (15), very good sensitivity and 100% specificity were obtained when LPA was compared with CDST. Under routine program conditions in India (16), varying

TABLE 1. Comparisons of drug sensitivity testing for isoniazid and/or rifampicin using the line probe assay (LPA) and conventional culture and drug sensitivity testing (CDST) for sputum specimens from smear-positive pulmonary tuberculosis (PTB) patients and cultures from patients with smear-negative tuberculosis, Peru, 2011–2013

Drug			Total
Rifampicin (R)			
Sputum specimens:	CDST resistant to R	CDST sensitive to R	
LPA resistant to R	936	79	1 015
LPA sensitive to R	81	908	989
Total	1 017	987	2 004
Cultures:	CDST resistant to R	CDST sensitive to R	
LPA resistant to R	541	51	592
LPA sensitive to R	28	281	309
Total	569	332	901
Isoniazid (H)			
Sputum specimens:	CDST resistant to H	CDST sensitive to H	
LPA resistant to H	1 369	46	1 415
LPA sensitive to H	81	508	589
Total	1 451	554	2 004
Cultures:	CDST resistant to H	CDST sensitive to H	
LPA resistant to H	770	9	779
LPA sensitive to H	32	90	122
Total	802	99	901
Isoniazid + rifampicin (MDR-TB)[a]			
Sputum specimens:	CDST with MDR-TB	CDST with no MDR-TB	
LPA with MDR-TB	841	51	892
LPA with no MDR-TB	117	995	1 112
Total	958	1 046	2 004
Cultures:	CDST with MDR-TB	CDST with no MDR-TB	
LPA with MDR-TB	474	23	497
LPA with no MDR-TB	51	353	404
Total	525	376	901

Source: created by the authors with data exported from the NETLab database (www.netlab.ins.gob.pe/FrmNewLogin.aspx) of the National Institute of Health of Peru.

[a] Multi drug resistand tuberculosis.

TABLE 2. Performance of line probe assay (LPA) compared to conventional culture and drug sensitivity testing (CDST) for detecting isoniazid and/or rifampicin resistance in sputum specimens from smear-positive pulmonary tuberculosis patients and in cultures from patients with smear-negative or positive tuberculosis, Peru, 2011–2013

	Rifampicin (95%CI)[a]	Isoniazid (95%CI)	MDR-TB[b] (95%CI)
Sputum specimens:			
Sensitivity %	92 (90.0 – 93.6)	94 (93.1 – 95.5)	88 (85.5 – 89.8)
Specificity %	92 (90.0 – 93.6)	92 (89.1 – 93.9)	95 (93.6 – 96.3)
PPV %[c]	92 (90.4 – 93.8)	97 (95.7 – 97.6)	94 (92.6 – 95.7)
NPV %[d]	92 (89.9 – 93.4)	86 (83.2 – 88.9)	90 (87.5 – 91.2)
Kappa coefficient	0.84 (0.82 – 0.86)	0.85 (0.82 – 0.87)	0.83 (0.81 – 0.86)
Cultures:			
Sensitivity %	95 (93.0 – 96.7)	96 (94.4 – 97.3)	90 (87.4 – 92.7)
Specificity %	85 (80.0 – 88.0)	91 (83.4 – 95.8)	94 (91.0 – 96.1)
PPV %	91 (88.8 – 93.5)	99 (97.8 – 99.5)	95 (93.1 – 97.0)
NPV %	91 (87.2 – 93.9)	74 (65.0 – 81.3)	87 (83.7 – 90.5)
Kappa coefficient	0.81 (0.77 – 0.85)	0.79 (0.73 – 0.85)	0.83 (0.79 – 0.88)

Source: created by the authors from study data.

[a] 95% Confidence Interval.

[b] Multidrug resistant tuberculosis.

[c] Positive predictive value.

[d] Negative predictive value.

results were obtained, i.e., in a tertiary care center, sensitivity and specificity were excellent, while in three public-sector state laboratories results were not as good and sensitivity for isoniazid was low at 72% (17). Under routine program conditions elsewhere, results of sensitivity and specificity in Korea (18) were better than those obtained in Peru, were similar to those obtained in the Baltic states (19), and were worse in China, South Africa, and Brazil (20–22). In both China and South Africa, the kappa coefficients were less than 0.81 (20, 21).

Variations in LPA performance among countries are due to a number of factors. First, volume and quality of sputum can vary, which can be an important determinant of the result. Second, strict attention needs to be paid to following manufacturer's instructions (e.g., process, timing, sequencing, and temperature control) and laboratories that have a high throughput of tests are more likely to perform better than those with smaller workloads. Third, false-negative LPA results might be because phenotypic resistance on CDST is due to other minor resistant mutations that are not incorporated into the LPA strip or due to mutations yet to be found (23–25). There is a wide variation in circulating MTB strains across the world (26), and false negative results can also occur due to the presence of unique genetic mutations in the different settings (27). Fourth, mixed strains (sensitive and resistant) growing on culture media with growth < 1% would carry mutant genes that would be identified as drug-resistant by the LPA while conventional DST would show these strains to be drug-sensitive (i.e., false positive) (28). Drug-sensitive strains contaminated by resistant-strain DNA would also induce false-positive results. Finally, predictive values are dependent on the prevalence of drug resistance in the community being assessed. For example, a high rate of MDR-TB would increase the positive predictive value of the LPA.

Limitations

The strengths of this study were the large number of national LPA tests performed over the 3-year period, the participation of the LRNM in the annual Supra Laboratory Network external quality assurance rounds for conventional DST, and LPA being performed under strict conditions according to manufacturer's instructions. The execution and reporting of LPA performance was also in accordance with the Standards for Reporting of Diagnostic Accuracy initiative (13). Limitations related to the operational nature of the study conducted under routine program conditions were a large proportion of potential comparative samples that failed to grow mycobacteria or were contaminated. The study also suffered from the retrospective nature of the research, which led to different criteria being adopted in routine practice for selecting MTB strains or samples to be

studied by CDST; therefore, of 12 620 LPA tests showing drug sensitivity, only 493 were tested against CDST.

Conclusions

There are several programmatic implications of this study. First, it makes good sense to scale up the LPA nationally because of its rapid results. At tertiary care centers in India and in Greece, LPA results have been obtained within 48 hours of sputum receipt (13, 21). Other studies have also demonstrated a much faster time to diagnosis and to treatment of drug-resistant TB using the LPA (29, 30). Therefore, the goal in Peru should be to ensure that every patient diagnosed with TB has access to LPA technology. Second, health care workers need to be sensitized and trained in the quality of sputum collection, and laboratory staff need to be trained and supervised in its implementation. National guidelines to this effect have already been disseminated around the country (8). Third, Peru's conventional culture and DST using Löwenstein-Jensen medium and the proportion method does not always work, and this suggests the need for a different comparative gold standard approach that requires fewer steps, has less possibility of contamination, and produces quicker results. Finally, the use of LPA will detect increasing numbers of patients with MDR-TB as it allows the detection of MTB complex and its resistance to rifampicin and isoniazid directly from sputum (as long as the sputum is smear-positive) or indirectly from culture. The national TB program needs to ensure that drug supplies keep pace with increasing demand so that drug stock-outs and individual interruptions of treatment are avoided.

In conclusion, this study suggests that LPA to detect drug resistance is suitable for routine use and scale up in Peru with linkage to stipulated standardized antituberculosis treatment regimens. These standardized regimens may need to be modified to provide more individualized treatment according to CDST results.

Acknowledgments. This research was conducted through the Structured Operational Research and Training Initiative (SORT IT), a global partnership led by the Special Programme for Research and Training in Tropical Diseases at the World Health Organization (WHO/TDR). The model is based on a course developed jointly by the International Union Against Tuberculosis and Lung Disease (The Union) and *Médecins Sans Frontières*. The specific SORT IT program which resulted in this publication was jointly developed and implemented by the Communicable Diseases Research Program and the Regional Tuberculosis Control Program; Pan American Health Organization (PAHO); the Operational Research Unit (LUXOR) at *Médecins Sans Frontières*, Brussels Operational Center, Luxembourg; the Centre for Operational Research, The Union, Paris, France; the Institute of Tropical Medicine, Antwerp, Belgium and the University of Antioquia, Medellín, Colombia. The authors also thank Lely Solari, Alejandra Burela, and Carlos Castañon of the National Institute of Health (Lima, Peru); Antonieta Alarcón of Estrategia Sanitaria Nacional de Prevención y Control de la Tuberculosis-Lepra (Lima, Peru), and Rafael Van den Bergh of MSF for their invaluable help with the study.

Funding. The SORT IT programme was funded by TDR/UNICEF/UNDP/WORLD BANK/WHO, PAHO/WHO, The Union, MSF, U.S. Agency for International Development (USAID), Award No. AID-LAC-IO-11-0000,1 and the Department for International Development (DFID). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interests. None declared.

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Manuscript received on 24 July 2015. Revised version accepted for publication on 26 October 2015.

RESUMEN

Utilización y evaluación de un ensayo con sondas en línea en los pacientes con diagnóstico de tuberculosis en el Perú del 2011 al 2013

Objetivo. Definir la utilización de un ensayo con sondas en línea y evaluar su desempeño, en comparación con el método convencional de cultivo y antibiograma, en los pacientes registrados con tuberculosis en condiciones programáticas en el Perú del 2011 al 2013.

Métodos. Investigación operativa descriptiva con un estudio transversal de las muestras de esputo de los pacientes con diagnóstico de tuberculosis pulmonar y baciloscopia positiva y de los cultivos de micobacterias de los pacientes con tuberculosis y baciloscopia positiva o negativa. La farmacorresistencia a la rifampicina, la isoniacida o a ambas, detectada mediante el ensayo con sondas en línea, se comparó con los resultados obtenidos por el método de cultivo y antibiograma. Se calculó la sensibilidad, la especificidad y los valores predictivos del ensayo con sondas en línea y se evaluó su fiabilidad en la detección de la farmacorresistencia mediante el coeficiente k, cuyos valores de 0,61 a 0,80 correspondían a una fuerte correlación y los valores de 0,81 o superiores reflejaban una correlación casi perfecta.

Resultados. Del 2011 al 2013 se practicaron 16 169 ensayos con sondas en línea, y la proporción de pacientes con diagnóstico de tuberculosis en quienes se practicaba aumentó de 3,2% a 30,2%. En total, se compararon 2 905 resultados del ensayo molecular con el método convencional. En las muestras de esputo, el ensayo molecular ofreció una sensibilidad de 92% para la resistencia a la rifampicina, 94% a la isoniacida y 88% para la tuberculosis multirresistente; su especificidad fue 92% con respecto a la rifampicina, 92% a la isoniacida y 95% a la tuberculosis multirresistente. En los cultivos de micobacterias, el ensayo con sondas en línea mostró una sensibilidad de 95% para la resistencia a la rifampicina, 96% a la isoniacida y 90% para la tuberculosis multirresistente; la especificidad fue 85% para la rifampicina, 91% para la isoniacida y 94% para la tuberculosis multirresistente. El coeficiente k fue 0,81 o superior en todas las comparaciones del ensayo molecular con el método tradicional cuando se usaron muestras de esputo y cultivo, excepto con la isoniacida en cultivo, cuyo coeficiente fue 0,79.

Conclusiones. Los resultados del presente estudio indican que el ensayo con sondas en línea constituye una prueba de detección fiable y rápida para la tuberculosis multirresistente , y se debe considerar apropiada su utilización en la práctica de rutina y la ampliación de su empleo en el Perú.

Palabras clave *Mycobacterium tuberculosis*; tuberculosis; tuberculosis resistente a múltiples medicamentos; sondas moleculares; técnicas de sonda molecular; investigación operativa; Perú.