

# Evaluation of Molecular Tools for Detection and Drug Susceptibility Testing of *Mycobacterium tuberculosis* in Stool Specimens from Patients with Pulmonary Tuberculosis<sup>∇</sup>

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**Pulmonary tuberculosis diagnosis is difficult when patients cannot produce sputum. Most sputum is swallowed, and tuberculosis DNA can survive intestinal transit. We therefore evaluated molecular testing of stool specimens for detecting tuberculosis originating from the lungs. Paired stool and sputum samples ( $n = 159$ ) were collected from 89 patients with pulmonary tuberculosis. Control stool samples ( $n = 47$ ) were collected from patients without tuberculosis symptoms. Two techniques for DNA extraction from stool samples were compared, and the diagnostic accuracy of the PCR in stool was compared with the accuracy of sputum testing by PCR, microscopy, and culture. A heminested IS6110-PCR was used for tuberculosis detection, and IS6110-PCR-positive stool samples then underwent rifampin sensitivity testing by universal heteroduplex generator PCR (heteroduplex-PCR) assay. For newly diagnosed pulmonary tuberculosis patients, stool IS6110-PCR had 86% sensitivity and 100% specificity compared with results obtained by sputum culture, and stool PCR had similar sensitivities for HIV-positive and HIV-negative patients ( $P = 0.3$ ). DNA extraction with commercially available spin columns yielded greater stool PCR sensitivity than DNA extraction with the in-house Chelex technique ( $P = 0.007$ ). Stool heteroduplex-PCR had 98% agreement with the sputum culture determinations of rifampin resistance and multidrug resistance. Tuberculosis detection and drug susceptibility testing by stool PCR took 1 to 2 days compared with an average of 9 weeks to obtain those results by traditional culture-based testing. Stool PCR was more sensitive than sputum microscopy and remained positive for most patients for more than 1 week of treatment. In conclusion, stool PCR is a sensitive, specific, and rapid technique for the diagnosis and drug susceptibility testing of pulmonary tuberculosis and should be considered when sputum samples are unavailable.**

Tuberculosis kills approximately 2 million people per year (10), and global control is hampered by increasing HIV coinfection and multidrug-resistant tuberculosis (MDRTB) (13). Diagnosis of tuberculosis that also tests for drug resistance usually requires the isolation of mycobacteria in culture or molecular analysis. Culture and drug susceptibility testing using traditional techniques available in most developing countries take months and consequently have limited clinical relevance. In contrast, the sputum PCR method can be performed in 1 day and has a sensitivity of 60 to 100% (18, 19, 24, 28, 30). Furthermore, PCR allows direct determination of rifampin resistance (12, 22), which is particularly important because it is a marker of multidrug-resistant strains of *Mycobacterium tuberculosis* (26) and a strong predictor of treatment outcome (16). One such test, the universal heteroduplex generator PCR

(heteroduplex-PCR) assay, detects the missense mutations in the *rpoB* gene that are responsible for 96% of rifampin resistance in *M. tuberculosis* (22). This method can be completed in 6 h.

An additional consideration in the diagnosis of pulmonary tuberculosis is the inability or difficulty for patients to produce a sputum sample, a problem that is particularly common in young children and HIV-positive patients (15). In these relatively immunodeficient patient groups, a diminished inflammatory response may inhibit sputum production. Induced sputum techniques (8), nasopharyngeal aspirates (14), fiber-optic bronchoscopy (20), or the string test (25) may all be used to retrieve pulmonary secretions from patients unable to provide a sputum sample but may cause logistical, cost, or biosafety challenges. These limitations in the diagnosis of tuberculosis necessitate the development of new tests to identify *M. tuberculosis* in samples that can be obtained more easily.

Most sputum is swallowed, and the mycobacterial DNA within sputum samples may survive transit through the gastrointestinal tract, potentially allowing molecular testing of stool samples for the presence of mycobacterial DNA indicative of

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pulmonary tuberculosis (7, 9, 11, 21, 23, 32). We therefore hypothesized that stool samples may be useful for pulmonary tuberculosis molecular diagnosis and drug susceptibility testing. In order to test this hypothesis, we used two PCR assays in this study. The first was a heminested PCR of the IS6110 insert for the detection of *M. tuberculosis* (24). The second was the heteroduplex-PCR, which determines if there is a mutation or deletion in the *rpoB* gene, indicating resistance to the antibiotic rifampin (22). The results of these two molecular tests of stool samples were evaluated by comparison with sputum microscopy, culture, and PCR.

HIV infection affects the performance of diagnostic tests for tuberculosis, and we therefore wished to examine the effect of HIV coinfection on the sensitivity of stool PCR for diagnosing pulmonary tuberculosis. However, in Peru, HIV seropositivity occurs in only approximately 2% of tuberculosis patients (1). In order to recruit sufficient patients with HIV infection, we therefore recruited patient groups with and without known HIV coinfection.

In resource-poor settings, patients are often tested only for MDRTB if they have known risk factors for MDRTB, such as past tuberculosis treatment, or if their disease does not improve during the first months of therapy. The latter strategy for selective MDRTB testing of follow-up samples collected during therapy is microbiologically challenging because first-line tuberculosis therapy administered empirically for unrecognized MDRTB often causes sputum cultures to become negative despite not achieving a long-term cure (16). Stool PCR tests for the presence of *M. tuberculosis* DNA derived from living or dead mycobacteria in swallowed sputum and may be particularly well suited to MDRTB testing of follow-up samples collected during therapy. We therefore tested approximately equal numbers of "diagnostic samples" obtained from newly diagnosed patients and "follow-up samples" obtained from patients who were already established on tuberculosis treatment.

These diagnostic samples and follow-up samples obtained from patient groups with high and low rates of HIV coinfection were used to evaluate PCR tests of stool samples in comparison with sputum microscopy, culture, and PCR.

## MATERIALS AND METHODS

**Patient recruitment.** Patients were diagnosed and treated by the Peruvian national tuberculosis program that follows the World Health Organization recommendations for diagnosis and directly observed therapy short course (DOTS). Inclusion criteria for this study were that patients had to be more than 17 years old and commencing therapy for laboratory-proven pulmonary tuberculosis. All patients were either sputum microscopy and/or culture positive for tuberculosis, and the sputum sample-based diagnosis was considered to be the gold standard against which stool PCR was evaluated. The exclusion criteria were clinical symptoms or physical signs suggestive of extrapulmonary tuberculosis (including intestinal tuberculosis), as determined by questionnaire and clinical examination.

**Pulmonary tuberculosis patients.** Pairs of stool ( $n = 92$ ) and sputum ( $n = 92$ ) samples were collected from 54 pulmonary tuberculosis outpatients attending the community DOTS tuberculosis clinic at the Maria Auxiliadora Hospital. All these patients were confirmed to be HIV seronegative by enzyme-linked immunosorbent assay (ELISA) performed for the current research project. Also, pairs of stool ( $n = 67$ ) and sputum ( $n = 67$ ) samples were collected from 35 pulmonary tuberculosis patients at the Dos de Mayo Hospital HIV outpatient clinic, all of whom were HIV seropositive by ELISA and confirmatory Western blot testing done by the Peruvian Ministry of Health. This is one of the largest clinics for HIV care in Peru and serves an area of Lima that includes the Maria Auxiliadora

Hospital. Clinical characteristics of these patient populations have been reported previously (16).

**Control subjects.** Stool samples ( $n = 47$ ) were also collected from 47 control participants who were recruited at the gastroenterology department of the Peruvian-Japanese clinic that provides care for relatively wealthy individuals who have a low probability of developing tuberculosis. These patients had no cough, sputum expectoration, or other respiratory symptoms and were undergoing investigations for chronic gastroenterological (principally acid-related) symptoms that were considered inconsistent with a possible diagnosis of pulmonary, disseminated, or intestinal tuberculosis. Control subjects did not undergo HIV testing, but it was likely that they were HIV seronegative because HIV seroprevalence in Peru is less than 0.5% (1).

**Sample collection.** Paired stool and sputum samples were collected on the same day from each patient in new plastic containers prior to treatment or as soon as possible after treatment commenced. The first sample obtained from each patient was considered to be the "diagnostic sample," provided that it was collected either pretreatment or within the first 2 weeks of treatment. These diagnostic samples represented the clinical scenario when newly diagnosed patients who are considered to be at increased risk of MDRTB have samples collected around the time of treatment initiation for MDRTB testing. A similar number of "follow-up samples" were collected from patients later in their treatment to represent the clinical scenario when patients appear to be responding poorly to therapy and have samples sent later in treatment for MDRTB testing. Samples were kept at 4°C and transported to our laboratory and analyzed within 24 h of collection in the great majority of cases and within 72 h in all cases. The technicians responsible for all assays were blinded to the results obtained from the other assays.

**Sputum microscopy and culture.** Sputum samples were decontaminated with the sodium hydroxide *N*-acetyl-L-cysteine technique for 15 min (17). Staining with the auramine and Ziehl-Neelsen techniques for acid-fast bacilli was performed, and the presence of acid-fast bacilli was noted and quantified (17). Culture was performed using Lowenstein-Jensen medium (17) and MODS (microscopic observation drug susceptibility) broth culture, as described previously (2, 5, 26, 27, 29, 31), with direct drug susceptibility testing. For positive cultures, the *M. tuberculosis* isolates were then subjected to indirect drug susceptibility testing using the colorimetric tetrazolium microplate assay, as described previously (4).

**Stool sample processing.** Approximately 100 mg of feces was mixed with 6 ml of sterile distilled water and left standing for 15 min at room temperature, and 2 ml of the supernatant was decontaminated in the same way as sputum samples by using the sodium hydroxide *N*-acetyl-L-cysteine technique for 15 min (17).

**DNA extraction.** Two methods of DNA extraction were evaluated. The indirect Chelex X-100 method was used for extracting DNA from decontaminated stool and sputum samples, as described previously (6). DNA was also extracted from all stool (but not from sputum) samples using the QIAamp DNA stool minikit spin column kit (Qiagen, Valencia, CA), according to manufacturer's instructions.

**IS6110-PCR for tuberculosis detection.** To specifically detect *M. tuberculosis*, a modified heminested PCR assay, as described previously (22, 24), was used to test all DNA samples derived from sputum and stool specimens. This method is based on the amplification of the insertion segment IS6110, a 1.35-kb sequence found only in the *M. tuberculosis* complex. The heminested method has increased specificity because it involves two amplifications with two pairs of primers (22, 24). After use of the heminested PCR, amplified products were electrophoresed for 1 h and visualized as described previously (22, 24).

**PCR controls.** Every heminested PCR assay used included positive controls of genomic *M. tuberculosis* DNA and negative controls of water added to the PCR reagents in place of the sample DNA. The positive control was incorporated in order to verify the proper functioning of the PCR, while the negative control ensured that there was no contamination with amplification products from previous assays. Additionally, whenever samples were undergoing decontamination and DNA extraction, an aliquot of buffer was included as a negative-control sample to ensure that there was no contamination of samples with PCR products during processing. To reduce the risk of false-positive PCRs, separate rooms for DNA extraction, PCR mix preparation, amplification, and electrophoresis were used. Protective clothing was used to reduce the risk of PCR products contaminating diagnostic samples.

**Heteroduplex-PCR assay.** The heteroduplex-PCR assay was performed as described previously (22) using all stool samples that were positive for *M. tuberculosis* by heminested PCR. The extracted DNA from the Qiagen method was used. These DNA heteroduplex samples were separated by vertical slab gel electrophoresis using 10% polyacrylamide minigels. The susceptibility of specimens to rifampin was determined by comparing the heteroduplex patterns of the

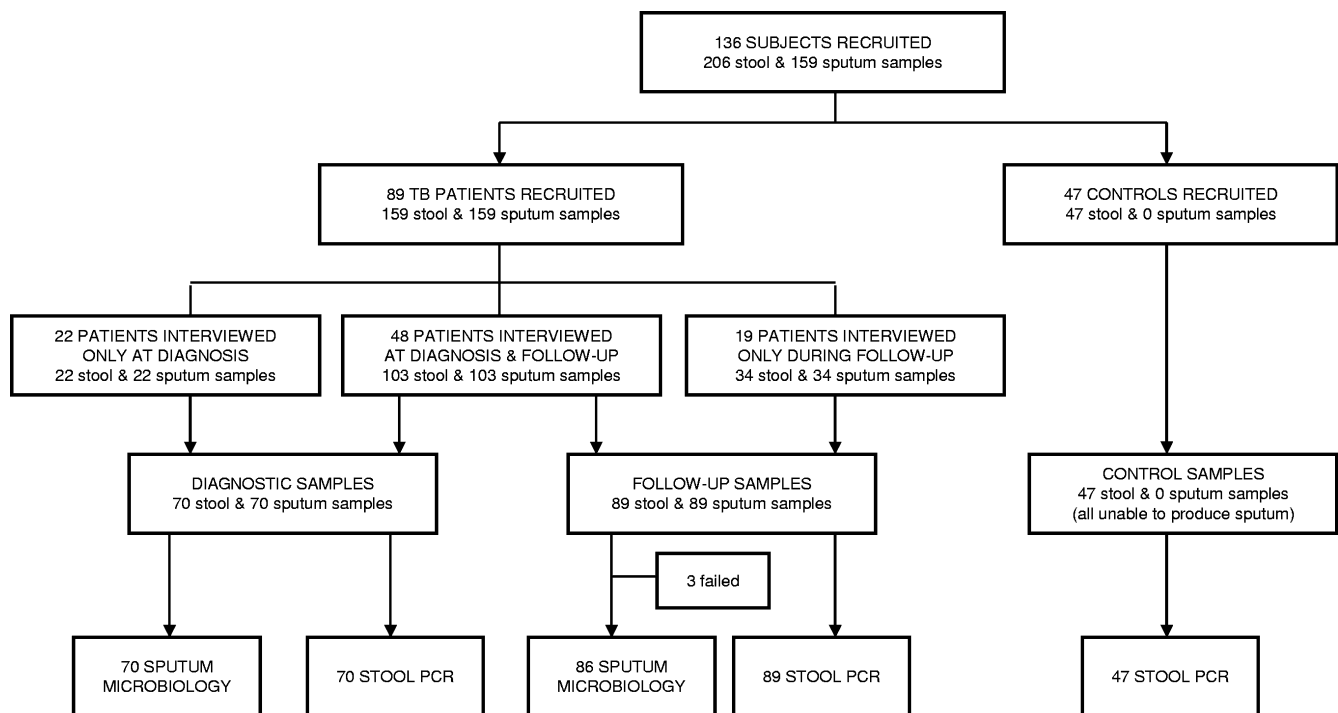


FIG. 1. The study flow sheet shows the numbers of patients in each study group.

samples to the patterns shown by the rifampin-susceptible *M. tuberculosis* H37Rv (strain ATCC 27297). The specimens with the same pattern as the rifampin-susceptible strain were scored as susceptible, and those with different patterns were considered resistant.

**Ethics.** All participants gave informed written consent, and the project was approved by the committees for human research at the Johns Hopkins Bloomberg School of Public Health and the Asociación Benéfica Prisma, Peru.

**Data analysis.** The statistical software Stata 9.0 was used for all analysis (StataCorp, College Station, TX). The proportion of positive results from each diagnostic test was determined for the overall population and subgroups to define sensitivity and specificity with their corresponding 95% confidence intervals (95% CI). Test sensitivities were compared using Z tests for proportions. Associations between stool PCR positivity and other factors were calculated as odds ratios with their 95% CI by logistic regression. The time required for each diagnostic assay was assessed approximately but was not recorded exactly for every sample. For drug susceptibility testing, sputum culture-based testing was considered to be the gold standard, and results from the stool heteroduplex-PCR assay were compared to determine sensitivity, specificity, positive and negative predictive values, the kappa statistic, and percent agreement. All tests were two tailed, and a *P* value of <0.05 was considered to be the threshold for statistical significance.

## RESULTS

A total of 206 stool samples obtained from 136 subjects were studied (Fig. 1). Forty-seven control participants each provided a single stool sample, and all of these control subjects were unable to provide sputum samples because the selection criteria required them to be free from respiratory symptoms. Eighty-nine pulmonary tuberculosis patients provided the following 159 paired stool and sputum samples: 70 were diagnostic samples and the remaining 89 paired stool and sputum samples were follow-up samples (Fig. 1).

**Sensitivity.** For the diagnostic stool samples, the sensitivity of IS6110-PCR was 86% (95% CI, 75 to 93%; 60/70 samples were PCR positive) compared with that of sputum culture, and

sensitivity levels were similar for HIV-positive and HIV-negative patients ( $P = 0.3$ ) (Table 1). Considering together all 159 diagnostic and follow-up stool samples, increasing the treatment duration decreased the likelihood of a positive stool IS6110-PCR ( $P \leq 0.02$ ) (Table 1 and Fig. 2), and when treatment duration was included as a covariate in the analysis, HIV status was not significantly associated with stool PCR sensitivity ( $P = 0.2$ ) (Table 1). Whether considering only the 70 diagnostic samples or all 159 diagnostic and follow-up samples together, the stool PCR was more likely to be positive if the paired sputum sample had a positive result for microscopy, culture, and/or PCR ( $P \leq 0.002$ ) (Table 1 and Fig. 3), and PCR sensitivity was not significantly different between paired stool and sputum samples ( $P > 0.1$ ). Stool PCR had significantly greater sensitivity than microscopy testing of the paired sputum samples, such that 19/43 patients with microscopy-negative sputum samples had positive stool PCR results (Table 1 and Fig. 3). For these 19 positive stool PCR results from sputum microscopy-negative patients, the paired sputum samples had negative culture results for 26% and negative PCR results for 36%, indicating that the sensitivity of stool PCR compared favorably with sputum culture and PCR testing.

**Specificity.** None of the 47 samples from control participants was positive, indicating a specificity of 100% (95% CI, 92 to 100%). Every PCR had appropriate positive results for the PCR-positive controls and negative results for both the PCR-negative controls and also the negative-control samples.

**DNA extraction technique.** Stool PCR sensitivity was greater when DNA was extracted directly from diagnostic stool samples using Qiagen columns than when DNA was extracted from decontaminated stool by the Chelex technique (84% versus 64% compared with those of sputum culture;  $P = 0.007$ ).

TABLE 1. Stool IS6110-PCR results and logistic regression analysis of stool IS6110-PCR result associations with clinical characteristics

Clinical characteristics for samples <sup>d</sup>	No. (%) of stool IS6110-PCR samples		Odds ratio (95% CI)	Coefficient of determination (R <sup>2</sup> ) for logistic regression	P value
	Negative <sup>e</sup>	Positive <sup>f</sup>			
<b>Diagnostic</b>					
Paired sputum sample microscopy positive (n = 56/70)	4/10 (40)	52/60 (87)	9.8 (2.2–42)	0.2	0.002
Paired sputum culture positive (n = 65/70)	6/10 (60)	59/60 (98)	39 (3.8–411)	0.2	0.002
Paired sputum PCR positive (n = 64/70)	5/10 (50)	59/60 (98)	59 (5.7–608)	0.3	0.001
HIV-seropositive patients (n = 17/70)	1/10 (10)	16/60 (27)	3.3 (0.28–28)	0.02	0.3
TB treatment duration prior to stool collection (n = 70)	3 (2–5) <sup>g</sup>	4 (1–6) <sup>g</sup>	0.0004 (–0.017–0.016)	0.001	0.9
<b>All (diagnostic and follow-up)</b>					
Paired sputum microscopy positive (n = 106/156) <sup>c</sup>	11/35 (31)	102/121 (84)	12 (4.9–28)	0.2	<0.001
Paired sputum culture positive (n = 128/155) <sup>c</sup>	13/34 (38)	115/121 (95)	32 (11–94)	0.3	<0.001
Paired sputum PCR positive (n = 129/156) <sup>c</sup>	15/35 (43)	114/121 (94)	22 (7.9–60)	0.3	<0.001
HIV-seropositive patients (n = 67/159) <sup>c</sup>	20/35 (57)	47/124 (38)	–0.10 (–0.27–0.064)	0.08	0.2
TB treatment duration prior to stool collection <sup>b</sup>	2.3 (0.71–18) <sup>h</sup>	1.0 (0.43–1.4) <sup>h</sup>	–0.010 (–0.018–0.0013)	0.08	0.02

<sup>a</sup> This logistic regression was adjusted for the tuberculosis treatment duration because HIV-positive patients had their samples collected later in treatment than HIV-negative patients (median of 1.4 and interquartile range [IQR] of 0.14 to 18 weeks versus median of 1.0 and IQR of 0.43 to 1.4 weeks, respectively; *P* = 0.058).  
<sup>b</sup> Treatment duration data were not available for 24 follow-up samples.  
<sup>c</sup> Sputum microscopy and PCR results were unavailable for 3 follow-up samples, and sputum culture was unavailable for 4 follow-up samples.  
<sup>d</sup> IQR, interquartile range; TB, tuberculosis.  
<sup>e</sup> The numbers of diagnostic samples and all (diagnostic and follow-up) samples were 10 and 35, respectively.  
<sup>f</sup> The numbers of diagnostic samples and all (diagnostic and follow-up) samples were 60 and 124, respectively.  
<sup>g</sup> These values are median numbers of days (IQR).  
<sup>h</sup> These values are median numbers of weeks (IQR).

Specifically, 44 samples were positive with both of the techniques, 10 with neither, and 15 only with the Qiagen technique compared with 1 only with Chelex extraction. Similarly, considering all 159 diagnostic and follow-up samples, sensitivity was greater using Qiagen columns (119 positive) than using the Chelex technique (87 positive; *P* < 0.0001). Specifically, 82 samples were positive by both of the techniques, 35 by neither, and 37 only with Qiagen compared with 5 only with Chelex extraction.

**Drug susceptibility testing.** The gold standard direct MODS and indirect tetrazolium microplate culture assays had 100%

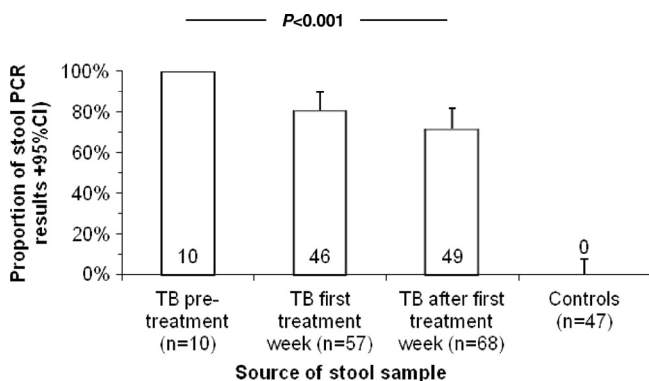


FIG. 2. Stool IS6110-PCR results for samples collected from pulmonary tuberculosis patients and from control patients that were subjected to PCR analysis and for which treatment duration was known. Treatment duration data were not available for an additional 24 samples collected during follow-up treatment. The numbers within each bar denote the numbers of samples with positive stool PCR results within that diagnostic group. The stool PCR was more likely to be positive pretreatment or early in treatment (*P* < 0.001 for trend across ordered groups).

concordant results with one another for sputum isoniazid and rifampin susceptibility. All rifampin-resistant strains were MDRTB. Considering only the 60 IS6110-PCR-positive diagnostic samples, 50 (83%) yielded detectable heteroduplex-PCR rifampin resistance results, and these had 98% agreement with the sputum gold standard (Table 2). Considering all 124 IS6110-PCR-positive diagnostic and follow-up samples, 98

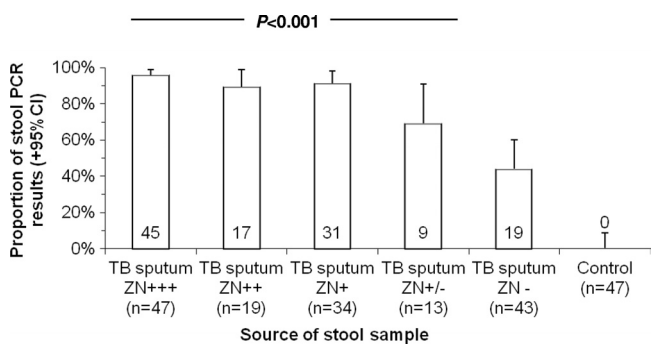


FIG. 3. Stool IS6110-PCR results for patients with pulmonary tuberculosis and for healthy controls. Data are divided by sputum auramine microscopy grade. Three plus signs denotes the highest concentration of acid-fast bacilli seen on sputum microscopy; two plus signs and one plus sign indicate samples in which fewer acid-fast bacilli were seen; a plus/minus sign indicates that the microscopy result was equivocal, with insufficient numbers of acid-fast bacilli visualized for a diagnosis of tuberculosis to be reported; and a minus sign indicates that no acid-fast bacilli were seen. The number within each bar denotes the number of stool PCR-positive samples within that group. Data are shown for 156/159 samples for which sputum microscopy data were available and for all control samples. The stool PCR was more likely to be positive when higher concentrations of acid-fast bacilli were seen using sputum microscopy (*P* < 0.001 for trend across ordered groups). TB, tuberculosis; ZN; Ziehl-Neelsen microscopy.

TABLE 2. Stool heteroduplex PCR results<sup>a</sup>

Samples	Stool heteroduplex-PCR results							
	No. of samples		Kappa statistic	P value	% sensitivity (95% CI)	% specificity (95% CI)	% positive predictive value (95% CI)	% negative predictive value (95% CI)
	Susceptible <sup>b</sup>	Resistant <sup>c</sup>						
Diagnostic			0.94	<0.0001	100 (72–100)	97 (87–100)	92 (62–100)	100 (91–100)
Culture rifampin susceptible (n = 39)	38	1						
Culture rifampin resistant (n = 11)	0	11						
All (diagnostic and follow-up)			0.90	<0.0001	96 (81–100)	96 (88–99)	90 (73–98)	99 (90–100)
Culture rifampin susceptible (n = 67)	64	3						
Culture rifampin resistant (n = 27)	1	26						

<sup>a</sup> The stool heteroduplex-PCR assay for rifampin susceptibility is compared with the gold standard culture-based rifampin susceptibility testing applied to the paired sputum sample for diagnostic samples and for all samples. Note: all sputum samples underwent two sputum culture-based tests for rifampin susceptibility that had 100% concordant results (see the text), and all rifampin-resistant strains were also MDRTB.

<sup>b</sup> The numbers of diagnostic samples and all (diagnostic and follow-up) samples were 38 and 64, respectively.

<sup>c</sup> The numbers of diagnostic samples and all (diagnostic and follow-up) samples were 12 and 29, respectively.

(79%) yielded detectable heteroduplex-PCR rifampin resistance results, and there was 96% agreement with the sputum gold standard results for the 94 samples with complete data (Table 2).

**Test speed.** Sputum and stool specimen decontamination took approximately 1 h, followed by 1 to 3 weeks for the MODS assay and a further 4 to 7 weeks for the tetrazolium microplate assay. DNA extraction required approximately 4 h, followed by a further 4 h for the IS6110-diagnostic PCR. PCR-positive samples then underwent the heteroduplex-PCR, which required 5 to 6 h.

## DISCUSSION

This study demonstrates that stool PCR is a useful method for the detection of swallowed *M. tuberculosis* for the diagnosis and drug susceptibility testing of patients with pulmonary tuberculosis. Stool PCR diagnosed tuberculosis and MDRTB in 1 to 2 days with sensitivity and specificity similar to those of the much slower sputum culture.

In our previous research, the sputum heteroduplex-PCR test had 97% agreement with culture-based testing for determination of rifampin resistance (22), although the heteroduplex-PCR was less sensitive than the IS6110-PCR for the primary diagnosis of tuberculosis (22). In the current study, the results of the stool heteroduplex-PCR method reflect a level of agreement similar of that of the sputum culture-based determination of rifampin resistance, indicating that the stool heteroduplex-PCR assay reliably identified rifampin-resistant strains of *M. tuberculosis*. It is also noteworthy that globally there is high concordance between rifampin resistance and multidrug resistance, consistent with the 100% agreement in the present study, so the heteroduplex-PCR result is also a useful marker for multidrug resistance.

The promising results from both of the PCR tests become more significant when the ease of sample collection and the rapidity of diagnosis are taken into account. Stool can be collected easily from patients whether or not they are able to

expectorate sputum. In addition, only 1 to 2 days are needed for the detection of *M. tuberculosis* and determination of drug resistance by PCR, compared to 1 to 3 weeks using rapid methods such as MODS and several months using traditional microbiological tests.

All of the patients in this study had proven pulmonary tuberculosis, and none had gastrointestinal symptoms that suggested coexistent diagnosis of intestinal tuberculosis. However, no specific gastrointestinal investigations, such as computed tomography or ultrasound, were performed, and terminal bowel tuberculosis infection may be occult, especially in people coinfecting with HIV. Stool PCR has been used to diagnose intestinal tuberculosis (3), and it is possible that in some cases in the present study, occult intestinal infection contributed to the presence of *M. tuberculosis* DNA in the stool. However, the strong association that we identified between sputum and stool findings strongly suggests that the stool PCR was detecting DNA from *M. tuberculosis* in swallowed sputum, not from occult intestinal disease.

In the present research, the cause of the false-negative PCR results was not investigated. The strong association between microscopy-based and culture-based indicators of *M. tuberculosis* concentrations in sputum and the stool PCR sensitivity indicate that inhibitors were unlikely to be the cause of most false-negative stool PCR results. We found that the stool PCR had greater sensitivity when DNA was extracted with commercially available spin columns than when DNA was extracted with the inexpensive in-house Chelex technique. This may have been because of differential DNA extraction efficiency or possibly because of differential DNA contamination with PCR inhibitors, a subject that should be investigated in future research. In some regions, strains of *M. tuberculosis* that lack the IS6110 gene have been reported, and in these locations, alternative *M. tuberculosis*-specific primers may require evaluation (3).

The stool PCR performed similarly for diagnostic samples compared with that for follow-up samples collected during treatment. This is important because in resource-poor settings, tuberculosis drug susceptibility testing is often restricted to the

minority of patients who do not respond to empirical first-line therapy, so test performance for follow-up samples has considerable clinical relevance. Furthermore, the stool IS6110-PCR had sensitivity similar to that of the same PCR technique applied to the paired sputum samples. The sensitivity of the stool PCR was unaffected by HIV coinfection, and the stool PCR was more sensitive for diagnosing TB than sputum smear microscopy.

The PCR assays that we evaluated in this study performed well for the patients who were able to produce paired stool and sputum samples for comparative testing. Because young children, some HIV-negative adults, and many HIV-positive adults with pulmonary tuberculosis are unable to expectorate sputum samples, this raises the possibility that stool PCR may be a useful tuberculosis test for patients who are unable to expectorate a sputum sample for tuberculosis testing. However, our study design compared sputum testing with stool testing and thus could not determine whether stool PCR would provide similarly sensitive tuberculosis diagnosis in patients who are unable to expectorate. Priorities for future research therefore include assessing stool PCR performance in patients who are unable to produce sputum samples, especially children and HIV-positive patients in countries with significant MDRTB prevalence, where the diagnostic speed of stool PCR may have the greatest clinical importance.

The combination of heminested PCR for the detection of *M. tuberculosis* and subsequent heteroduplex-PCR assay for determination of rifampin susceptibility makes the use of stool samples for rapid diagnosis of both drug-susceptible and MDRTB patients a promising possibility in many parts of the world. The use of stool samples for tuberculosis diagnosis may be particularly valuable in patients unable to produce sputum specimens.

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R. H. Gilman conceived the project. J. Cordova, P. Sheen, and F. Arenas led laboratory molecular work with expert assistance from D. L. Williams. L. Caviedes was responsible for the sputum sample testing. V. Kawai and G. Soto led the clinical field teams. L. Martin led data management. R. Shiloh and C. A. Evans led data analysis and article writing, with statistical consultancy from M. Zimic and editorial assistance from A. R. Escombe. We all contributed to analysis strategies and the writing of this article.

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