

# Polymerase Chain Reaction of *secA1* on Sputum or Oral Wash Samples for the Diagnosis of Pulmonary Tuberculosis

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**Background.** Nucleic acid amplification tests are sensitive and specific for identifying *Mycobacterium tuberculosis* in sputum smear–positive populations, but they are less sensitive in sputum smear–negative populations. Few studies have assessed their performance among patients infected with HIV, and no studies have assessed their performance with oral wash specimens, which may be easier to obtain than sputum samples.

**Methods.** We performed a prospective study involving 127 adults from 2 populations who were undergoing evaluation for respiratory complaints at Mulago Hospital in Kampala, Uganda. We obtained and tested sputum samples for *Mycobacterium tuberculosis*, and we simultaneously obtained oral wash specimens to test for *M. tuberculosis* DNA by polymerase chain reaction (PCR) amplification of a novel locus, the *secA1* gene. A positive mycobacterial culture of sputum was used to define cases of tuberculosis; we calculated the sensitivity and specificity of the PCR assay with sputum or oral wash specimens in reference to the standard of sputum culture results.

**Results.** Tuberculosis (75 [59%] of 127 patients) and HIV infection (58 [46%] of 126 patients) were both common in the study population. PCR of sputum samples was highly sensitive (sensitivity, 99%; 95% confidence interval, 93%–100%) and specific (specificity, 88%; 95% confidence interval, 77%–96%) for detection of pulmonary tuberculosis and performed well among HIV-infected patients and among patients with negative sputum smear results. PCR of oral wash specimens was less sensitive (sensitivity, 73%; 95% confidence interval, 62%–83%) but also detected a substantial proportion of tuberculosis cases.

**Conclusions.** PCR targeting the *secA1* gene was highly sensitive and specific for identifying *M. tuberculosis* in sputum samples, independent of smear or HIV infection status. Oral washes showed promise as an easily obtained respiratory specimen for tuberculosis diagnosis. PCR of sputum for detection of the *secA1* gene could be a rapid, effective diagnostic tool for tuberculosis referral centers.

Worldwide, 9 million new cases of active tuberculosis (TB) occur annually. In most countries with a high TB burden, national reference laboratories rely on microscopy to make a diagnosis of TB. In practice, this labor-intensive procedure is insensitive, even when specimens

are treated with mucolytic agents, concentrated by centrifugation, and examined using fluorochrome stains. Furthermore, microscopy is subjective and time consuming, and guidelines recommend that microscopists allocate at least 5 minutes to each slide and review no more than 25 slides per day [1]. Culture provides a definitive diagnosis of TB, but only after weeks of incubation, followed by identification with use of biochemical or molecular methods. Every step requires technical expertise for optimal laboratory performance [2]. Therefore, an automated molecular system for detecting *M. tuberculosis* would be useful, especially for health care facilities in countries with a high TB burden,

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because it would eliminate the subjective interpretation of microscopy and inherent delays associated with culture.

Direct amplification of *M. tuberculosis* DNA from sputum samples has been proposed as a means for improving the sensitivity of diagnostic testing for pulmonary TB [3]. Several commercial assays, including the amplified *M. tuberculosis* Direct Test (Gen-Probe), the Amplicor *M. tuberculosis* PCR assay (Roche Molecular Systems), and the BD ProbeTec ET Direct TB assay (Beckton Dickinson Diagnostics), are available for the direct detection of *M. tuberculosis* in clinical specimens. Although some studies from low-income countries have reported that sputum nucleic acid amplification is accurate and cost effective [4, 5], its performance for the diagnosis of TB has been disappointing [6–8], with a pooled sensitivity of 72% [9]. Sensitivity is especially low among patients with negative microscopy results [10]. Although smear-negative TB cases in HIV-infected patients account for ~90% of undiagnosed TB in parts of sub-Saharan Africa [11], little has been published about the performance of DNA amplification assays for TB among patients infected with HIV [12].

We previously reported that a PCR assay targeting the *secA1* gene identifies the majority of mycobacterial species [13]. In the current study, we prospectively evaluated a *M. tuberculosis*-specific *secA1* PCR assay performed on sputum samples for the diagnosis of pulmonary TB in a Ugandan population with a high prevalence of HIV infection and TB. A parallel aim was to evaluate whether PCR using a specimen more easily obtained than sputum—oral wash with sterile saline—would provide satisfactory sensitivity and specificity for detection of pulmonary TB, as demonstrated elsewhere for *Pneumocystis pneumonia* [14].

## METHODS

### Participants

We prospectively enrolled outpatients and inpatients with suspected TB at Mulago Hospital in Kampala, Uganda. From September 2006 through May 2007, a parent study of voluntary counseling and testing recruited every third outpatient who presented for initial evaluation at the Uganda National Tuberculosis and Leprosy Programme (NTLP) clinic [15]. We invited the first 2 outpatients enrolled in the parent study each day to join our study.

At a separate location on the medical wards, a research assistant recruited all inpatients aged >18 years who were admitted from March through May 2007 with chronic cough or dyspnea (experienced for >3 weeks but <6 months), excluding those receiving treatment for TB. A second, blinded research assistant randomly selected 2 inpatients from the individuals eligible for enrollment each day. Because the inpatient study

began after the outpatient study, the fixed sample size for the combined study limited the number of inpatients enrolled.

The Joint Clinical Research Centre Institutional Review Board approved the outpatient protocol. The Makerere University Research Ethics Committee and the Mulago Hospital Institutional Review Board approved the inpatient protocol. The Committee on Human Research at the University of California, San Francisco, and the Uganda National Council for Science and Technology approved both protocols.

### Patient Data and Specimen Collection

After written informed consent was obtained, participants provided demographic and clinical information in response to a standardized questionnaire and were offered HIV testing and counseling according to a standard protocol [15]. CD4<sup>+</sup> T cell counts were measured at enrollment in HIV-infected inpatients but not in outpatients.

Each participant provided expectorated sputum at enrollment. Samples obtained from outpatients were processed using 10% dithiothreitol (Prolab Diagnostics). Specimens obtained from inpatients underwent processing at the NTLP reference laboratory with use of 1% N-acetyl-L-cysteine, 2% sodium hydroxide, and 2% sodium citrate [16]. For the collection of oral wash specimens, subjects were instructed to cough vigorously 5 times, then gargle 10 mL of sterile saline for 60 s. Oral wash specimens were processed using 1% dithiothreitol. Both sputum and oral wash specimens were concentrated by centrifugation at 3000 × g, and the resulting pellet was frozen at –20°C within 8 h after collection. Specimens were stored for up to 7 months (median storage duration, 110 days; range, 7–195 days) and sent frozen to the National Institutes of Health (NIH) Clinical Center. Upon arrival, acid-fast smear and mycobacterial culture were performed on sputum samples, and PCR was performed on sputum and oral wash specimens.

### Specimen Testing

***M. tuberculosis* PCR.** All sputum and oral wash specimens were tested with a PCR assay targeting the *M. tuberculosis* gene that codes for the protein SecA1 (GenBank accession number 888860; locus tag Rv3240c), which is a component of the major pathway of protein secretion across the cytoplasmic membrane [13]. Specimens were processed by ultrasonication for 15 min in a sodium-dodecyl-sulfate-Tris-HCl buffer with zirconia/silica beads (BioSpec Products) and placed in NucliSENS lysis buffer (bioMérieux) to rupture the mycobacterial cell wall and release DNA. DNA was purified using a NucliSENS nucleic acid extraction kit. Real-time PCR was performed in a LightCycler (Roche) with use of M13-tailed primers to amplify a 490-nucleotide region of the *secA1* gene that is found in all members of the *Mycobacterium* genus [13]. Two PCRs for each sample

were performed in separate capillary tubes with use of different sets of fluorescence resonance energy transfer probes but with the same primers. One set of probes was specific for the *M. tuberculosis* complex, whereas the other set of probes was specific for the *Mycobacterium* genus. A reaction involving a third set of probes specific for a plasmid containing an internal-control sequence was performed for each sample to detect any inhibition of PCR. If a specimen demonstrated inhibition in the third, control tube without positive amplification of *M. tuberculosis* complex, all 3 capillaries were reanalyzed with use of a 1:10 dilution of the specimen DNA. For each PCR, a negative control reaction (with the same primers and probes but without template DNA) was performed to detect contamination. Additional procedures to avoid DNA carryover included unidirectional workflow that involved the use of separate rooms for extraction, master-mix preparation, PCR, and amplicon recovery; aliquoting of primers and probes in a zero-airflow cabinet; use of uracil dNTPs and uracil-N-glycosylase (Roche) in master mixes; and daily decontamination of all processing areas with 70% ethanol, Dispatch bleach detergent (Caltech Products), and ultraviolet light.

Laboratory investigators were blinded to clinical data, and those reading culture and PCR results were blinded to one another's interpretations. PCR results for each probe were independently classified as positive or negative for *M. tuberculosis* by 2 investigators (J.A.K. and C.H.), with a consensus result reached after joint review of any discrepant results.

**Smear microscopy.** NTLT staff examined sputum specimens with direct Ziehl-Neelsen microscopy on the day of enrollment, according to a standard protocol [1]. Results were collected prospectively for inpatients and by review of TB laboratory and case registries at the NTLT clinic.

**Mycobacterial culture.** Frozen sputum specimens were processed at the NIH Clinical Center Mycobacteriology Laboratory with use of a standard N-acetyl-L-cysteine-sodium hydroxide procedure [16] and concentrated by centrifugation. Mycobacterial cultures were performed using Middlebrook 7H11 agar plates (Remel) and in mycobacterial-growth-indicator tubes (Becton Dickinson). Acid-fast colonies were counted and identified using the Kinyoun stain and the Gen-Probe *M. tuberculosis* complex Accuprobe (Gen-Probe). Broth from mycobacterial growth indicator tubes with positive results was examined with acid-fast and Gram stains and subcultured to 7H11 agar for confirmation.

To assess bias in the standard-of-comparison sputum culture results that might have been caused by freezing and repeat decontamination before culture at NIH, sputum from each inpatient was plated on Lowenstein-Jensen media before freezing but after standard N-acetyl-L-cysteine-sodium hydroxide processing at the NTLT reference laboratory [16]. Cultures were

read weekly and considered to be negative if no growth was identified after 8 weeks.

### Statistical Analysis

We compared inpatient and outpatient characteristics and symptoms with use of the  $\chi^2$  test for dichotomous and categorical variables and the Mann-Whitney rank-sum test for non-normally distributed continuous variables. We used STATA, version 9.0 (Stata Corporation), for statistical analyses and defined significance to be a 2-tailed, type-I error ( $P$ ) < .05. We calculated sensitivity, specificity, and positive and negative predictive values of sputum and oral wash specimen PCR assays. We used the dichotomous results of sputum mycobacterial cultures performed at NIH as the standard of comparison for outcomes from PCR assays of both specimens. We planned in advance to compare diagnostic performance by HIV status, smear status, and inpatient-versus-outpatient status with use of McNemar's test for paired samples and a 2-sample test of proportions for unpaired samples. Assuming a TB prevalence of 60% and a sample size of 120 patients, we calculated precision for the estimated sensitivity (88%) to be  $\pm 7\%$ .

## RESULTS

**Participants.** Of 181 outpatients enrolled in the parent study, 114 were consecutively selected (figure 1). Six patients were unable to expectorate sputum, 1 was unable to perform an oral wash, 1 was missing clinical data, and 5 were missing sputum samples, which left 101 outpatients for analysis. Of 891 inpatients, 114 were eligible, and 28 were randomly selected for inclusion (figure 1). Two were unable to expectorate sputum, which left 26 inpatients for analysis. More patients were unable to expectorate sputum (8) than were unable to perform the oral wash (1). HIV status was unknown for 1 patient; 58 (46%) of the other 126 patients were infected with HIV.

Inpatients were older (median age, 33 years; interquartile range, 28–42 years) than outpatients (median age, 28 years; interquartile range, 24–35 years;  $P = .01$ ), and inpatients had a higher prevalence of HIV infection than did outpatients (81% vs. 37%;  $P < .001$ ). Most HIV-infected inpatients had advanced disease (median CD4<sup>+</sup> T cell count, 42 cells/ $\mu$ L; interquartile range, 13–296 cells/ $\mu$ L). Inpatients experienced a higher prevalence of fever and more-severe weight loss than did outpatients, but outpatients experienced a longer duration of symptoms. Frequencies of cough, sputum, dyspnea, and weight loss were similar among inpatients and outpatients (table 1; online only).

**Specimens.** Of 127 sputum samples cultured at NIH, 75 (59%) grew *M. tuberculosis* complex, including samples from 70 (69%) of 101 outpatients and from 5 (19%) of 26 inpatients.

One specimen grew *Nocardia* species. No specimens grew non-tuberculous mycobacteria.

PCR amplification of control sequences was successful for all sputum specimens and for all but 2 oral wash specimens, for which PCR was inhibited at the baseline concentration and after 1:10 dilution. Baseline inhibition of PCR was more common for oral wash specimens (39 [31%] of 127 samples) than for sputum specimens (16 [13%] of 127 samples) ( $P < .001$ ). Inhibition of PCR at baseline concentration of both sputum and oral wash specimens occurred for only 3 patients (1 inpatient and 2 outpatients).

**Sensitivity and specificity of PCR of sputum and oral wash specimens.** PCR of sputum specimens was highly sensitive (sensitivity, 99%; 95% CI, 93%–100%) and specific (specificity, 88%; 95% CI, 77%–96%) for the detection of pulmonary TB (table 2). PCR of oral wash specimens was also sensitive (sensitivity, 73%; 95% CI, 62%–83%) and specific (specificity, 88%; 95% CI, 76%–96%) for detection of pulmonary TB, although it was less sensitive than PCR of sputum samples (difference, 25%; 95% CI 13%–37%;  $P < .001$ ). Positive predictive values were high for PCR of sputum and oral wash specimens and the difference was not statistically significant (92% vs. 90%; difference, 2%; 95% CI, –7% to 12%;  $P = .64$ ). In contrast, the negative predictive value of PCR of sputum samples was significantly higher than that for PCR of oral wash samples (98% vs. 69%; difference, 29%; 95% CI, 17%–41%;  $P < .001$ ). Among oral wash specimens in which PCR inhibition occurred at baseline concentration, sensitivity was significantly lower than that among oral wash specimens in which PCR inhibition did not occur (52% vs. 83%; difference, 31%; 95% CI, 8%–53%;  $P = .006$ ). It should be noted that, because of rounding of point estimates and differences, the reported differences may appear to be inexact.

Among HIV-infected patients, the sensitivity of PCR of sputum specimens (sensitivity, 100%; 95% CI, 85%–100%) was

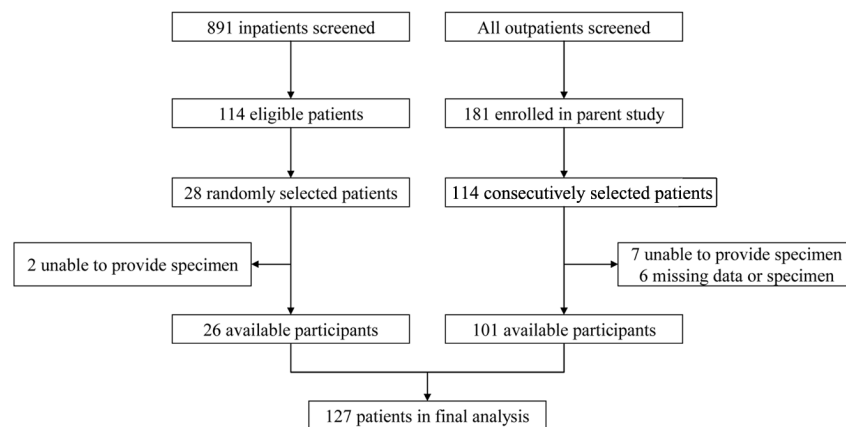
**Table 1 (online only). Clinical characteristics of 127 patients who presented to Mulago Hospital with respiratory complaints.**

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similar to that among non-HIV-infected patients (sensitivity, 98%; 95% CI, 90%–100%) (difference, 2%; 95% CI, –1% to 9%;  $P = 0.34$ ; table 2). In contrast, the sensitivity of PCR performed on oral wash specimens was lower (difference, 31%; 95% CI, 8%–53%;  $P = .006$ ) among HIV-infected patients (sensitivity, 52%; 95% CI, 31%–73%) than among non-HIV-infected patients (sensitivity, 83%; 95% CI, 70%–92%). The specificities of PCR performed on sputum and oral wash specimens were similar among HIV-infected and non-HIV-infected patients.

Among patients with negative Ziehl-Neelsen sputum smear results, the sensitivity of PCR performed on sputum specimens was 100% (95% CI, 63%–100%), and specificity was 90% (95% CI, 73%–98%) (table 2). Among patients with negative Ziehl-Neelsen sputum smear results, the sensitivity of PCR performed on oral wash specimens was 63% (95% CI, 25%–92%), and specificity was 85% (95% CI, 66%–96%). The precision of these estimates was limited by the small number of patients with smear-negative results who had positive cultures ( $n = 8$ ). Sensitivity and specificity estimates were similar among patients with unknown sputum-smear status.

Although HIV prevalence and other characteristics varied between inpatients and outpatients (table 1; online only), there were no notable differences in the sensitivity of PCR performed on either sample type, although there was a small number of inpatients, such that some differences may have been statistically undetectable. Specificity, however, was significantly lower among inpatients than among outpatients for PCR performed



**Figure 1.** Enrollment diagram

**Table 2. Diagnostic performance of PCR tests for detection of *Mycobacterium tuberculosis*, stratified by sputum acid-fast bacilli smear result and by HIV status.**

| Status and test result                         | No. of patients with culture result <sup>a</sup> |          | Comparison to reference standard, % (95% CI) |             |
|--|--|----------|--|-------------|
|  | Positive   | Negative | Sensitivity                                  | Specificity |
| Overall ( <i>n</i> = 127)                      |  |          |  |             |
| Reference standard                             | 75   | 52       | ...  | ...         |
| Sputum PCR                                     |  |          | 99 (93–100)                                  | 88 (77–96)  |
| Positive                                       | 74   | 6        | ...  | ...         |
| Negative                                       | 1  | 46       | ...  | ...         |
| Oral wash PCR <sup>b</sup>                     |  |          | 73 (62–83)                                   | 88 (76–96)  |
| Positive                                       | 55   | 6        | ...  | ...         |
| Negative                                       | 20   | 44       | ...  | ...         |
| Smear negative <sup>c</sup> ( <i>n</i> = 37)   |  |          |  |             |
| Reference standard                             | 8  | 29       | ...  | ...         |
| Sputum PCR                                     |  |          | 100 (63–100)                                 | 90 (73–98)  |
| Positive                                       | 8  | 3        | ...  | ...         |
| Negative                                       | 0  | 26       | ...  | ...         |
| Oral wash PCR <sup>b</sup>                     |  |          | 63 (25–92)                                   | 85 (66–96)  |
| Positive                                       | 5  | 4        | ...  | ...         |
| Negative                                       | 3  | 23       | ...  | ...         |
| Smear positive <sup>c</sup> ( <i>n</i> = 63)   |  |          |  |             |
| Reference standard                             | 55   | 8        | ...  | ...         |
| Sputum PCR                                     |  |          | 98 (90–100)                                  | 63 (25–92)  |
| Positive                                       | 54   | 3        | ...  | ...         |
| Negative                                       | 1  | 5        | ...  | ...         |
| Oral wash PCR                                  |  |          | 80 (67–90)                                   | 75 (35–97)  |
| Positive                                       | 44   | 2        | ...  | ...         |
| Negative                                       | 11   | 6        | ...  | ...         |
| HIV-infected <sup>d</sup> ( <i>n</i> = 58)     |  |          |  |             |
| Reference standard                             | 23   | 35       | ...  | ...         |
| Sputum PCR                                     |  |          | 100 (85–100)                                 | 86 (70–95)  |
| Positive                                       | 23   | 5        | ...  | ...         |
| Negative                                       | 0  | 30       | ...  | ...         |
| Oral wash PCR                                  |  |          | 52 (31–73)                                   | 86 (70–95)  |
| Positive                                       | 12   | 5        | ...  | ...         |
| Negative                                       | 11   | 30       | ...  | ...         |
| Non-HIV infected <sup>d</sup> ( <i>n</i> = 68) |  |          |  |             |
| Reference standard                             | 52   | 16       | ...  | ...         |
| Sputum PCR                                     |  |          | 98 (90–100)                                  | 94 (70–100) |
| Positive                                       | 51   | 1        | ...  | ...         |
| Negative                                       | 1  | 15       | ...  | ...         |
| Oral wash PCR <sup>b</sup>                     |  |          | 83 (70–92)                                   | 93 (66–100) |
| Positive                                       | 43   | 1        | ...  | ...         |
| Negative                                       | 9  | 13       | ...  | ...         |

<sup>a</sup> Reference standard mycobacterial cultures were performed at the National Institutes of Health Clinical Center Microbiology Laboratory.

<sup>b</sup> PCR was inhibited in oral wash specimens for 2 patients.

<sup>c</sup> Ziehl-Neelsen sputum smear results were not recorded in the laboratory register for 27 outpatients.

<sup>d</sup> HIV status was missing for 1 patient.

on both sputum (76% vs. 97%; difference, 21%; 95% CI, 1.3%–40%;  $P = .022$ ) and oral wash specimens (70% vs. 100%; difference, 30%; 95% CI, 10%–50%;  $P = .001$ ) (table 3). Five of 6 sputum samples with false-positive PCR results and 6 of 6 oral wash samples with false-positive PCR results were obtained from inpatients.

Of 26 sputum samples that were cultured in both laboratories, 6 samples that were positive at the NTLT reference laboratory were negative at NIH, and 1 sample that was negative at the NTLT reference laboratory was positive at NIH. To assess bias associated with the use of cultures of shipped, frozen sputum as the standard of comparison, we compared the diagnostic performance of PCR of sputum specimens with use of 2 different standards of comparison: the NTLT reference laboratory results obtained from culture of unfrozen sputum specimens and NIH results obtained from culture of previously frozen, twice-decontaminated sputum specimens (table 4; online only). Although the precision was limited by the small number of specimens, point estimates for sensitivity of PCR performed on sputum samples were similar whether they were measured in reference to sputum cultures performed at the NTLT reference laboratory (sensitivity, 90%; 95% CI, 56%–100%) or to those performed at NIH (sensitivity, 100%; 95% CI, 48%–100%). Specificity tended to be higher if culture results obtained at NTLT (specificity, 94%; 95% CI, 70%–100%) were used as the reference, compared with results obtained at NIH (specificity, 76%; 95% CI, 53%–92%). The lower bound of the 95% CI of

**Table 4 (online only). Diagnostic performance of PCR of *secA1* performed on sputum and oral wash specimens among inpatients, using cultures performed at the National Tuberculosis and Leprosy Programme Reference Laboratory in Uganda as the standard of comparison.**

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this difference suggests that NIH cultures, however imperfect, did not statistically meaningfully overestimate specificity (difference, 18%; 95% CI, –3.7% to 40%;  $P = .14$ ).

## DISCUSSION

In most TB reference laboratories, a specific diagnosis of pulmonary TB depends on sputum smear and culture results. Although microscopic examination of sputum is generally rapid and specific, microscopy misses a significant proportion of TB cases [17]. This is especially true among patients infected with HIV, for whom the sensitivity of microscopy for the detection of TB is 36%–73% [18]. Because the sputum smear, the only available rapid diagnostic method, is insensitive, many smear-negative patients with TB are not treated early enough or are not treated at all. These delays may be particularly costly in HIV-infected patients, in whom any postponement of treatment can be fatal [10].

**Table 3. Diagnostic performance of PCR tests for detection of *Mycobacterium tuberculosis*, stratified by patient population.**

| Population and test result | No. of patients with culture result <sup>a</sup> |          | Comparison to reference standard, % (95% CI) |              |
|----------------------------|--|----------|--|--------------|
|                            | Positive   | Negative | Sensitivity                                  | Specificity  |
| Outpatient ( $n = 101$ )   |  |          |  |              |
| Reference standard         | 70   | 31       | ...  | ...          |
| Sputum PCR                 |  |          | 97 (90–100)                                  | 97 (83–100)  |
| Positive                   | 68   | 1        | ...  | ...          |
| Negative                   | 2  | 30       | ...  | ...          |
| Oral wash PCR <sup>b</sup> |  |          | 76 (64–85)                                   | 100 (88–100) |
| Positive                   | 53   | 0        | ...  | ...          |
| Negative                   | 17   | 30       | ...  | ...          |
| Inpatient ( $n = 26$ )     |  |          |  |              |
| Reference standard         | 5  | 21       | ...  | ...          |
| Sputum PCR                 |  |          | 100 (48–100)                                 | 76 (53–92)   |
| Positive                   | 5  | 5        | ...  | ...          |
| Negative                   | 0  | 16       | ...  | ...          |
| Oral wash PCR <sup>b</sup> |  |          | 60 (15–95)                                   | 70 (46–88)   |
| Positive                   | 3  | 6        | ...  | ...          |
| Negative                   | 2  | 14       | ...  | ...          |

<sup>a</sup> Reference standard mycobacterial cultures were performed at the National Institutes of Health Clinical Center Microbiology Laboratory.

<sup>b</sup> PCR was inhibited in oral wash specimens for 1 outpatient and 1 inpatient.

Because sputum smears are insensitive and labor intensive, there has been considerable interest in the use of nucleic acid amplification and molecular probes for rapid diagnosis of smear-negative TB. In smear-negative specimens, commercial molecular assays are highly specific (specificity, 97%–99%) and have sensitivities ranging from 57% to 76% (table 5) [12]. Results can be obtained within 4 h, even for a large number of specimens.

In this study, we assessed 2 novel approaches for TB detection. First, we used species- and genus-specific DNA amplification assays to detect patients infected with *M. tuberculosis*. When PCR of sputum specimens was compared with culture, sensitivity and specificity were excellent among both smear-positive and smear-negative patients, with sensitivity as high as existing commercial assays (table 5). The *secA1* PCR assay also performed well among HIV-infected patients, with high positive and negative predictive values. Such robust performance data make this sequence a promising candidate for routine molecular diagnosis of TB in areas with a high prevalence of TB and HIV infection. In addition, *secA1* species- and genus-specific probes might have a higher specificity than smear microscopy; testing in populations with a higher prevalence of nontuberculous mycobacteria infection is needed to evaluate this possible advantage.

This study also assessed the use of oral wash samples for the detection of *M. tuberculosis*. In patients who cannot produce sputum, oral wash specimens may be easier and safer to obtain, compared with inducing sputum, which can be uncomfortable and dangerous for hypoxic patients and time-consuming and hazardous for staff. Because obtaining an oral wash sample involves only 5 brief coughs, it may generate fewer infectious aerosols than does sputum induction, which requires deep coughing for up to 30 min. In this study, all but 1 of the patients who could not expectorate sputum were able to provide an oral wash specimen.

PCR of *secA1* performed on oral wash specimens was not as sensitive as *secA1* PCR of sputum. This result was expected, because oral wash specimens contain fewer secretions from the lower respiratory tract and are diluted by saline. Also, baseline inhibition of PCR of oral wash specimens occurred frequently and was associated with a 31% reduction in sensitivity, compared with PCR of oral wash without inhibition. PCR performed on samples at intermediate dilutions might improve assay sensitivity in future studies.

In this study, the specificity of PCR for detection of TB may be underestimated. The standard of comparison was based on results from a single sputum specimen that was first frozen, then cultured months after collection [19]. Although freezing does not significantly damage DNA, it may reduce the viability of living organisms for culture. In addition, the decontamination of inpatient sputum twice (once before each culture)

**Table 5. Diagnostic performance of PCR of *secA1* performed on sputum specimens among patients with smear-negative tuberculosis, compared with commercially available sputum nucleic acid amplification assays.**

| Test                | Percentage (95% CI) |             |
|---------------------|---------------------|-------------|
|                     | Sensitivity         | Specificity |
| BD Probe Tec ET     | 71 (66–76)          | 97 (96–97)  |
| Cobas Amplicor MTB  | 64 (59–69)          | 99 (99–99)  |
| Gen-Probe E-MTD     | 76 (70–80)          | 97 (97–97)  |
| Roche Amplicor MTB  | 61 (57–65)          | 97 (97–97)  |
| PCR of <i>secA1</i> | 100 (63–100)        | 90 (73–98)  |

**NOTE.** BD ProbeTec ET, BD Probe Tec ET Direct Tuberculosis assay (Beckton Dickinson Diagnostics); Cobas Amplicor MTB, Amplicor *Mycobacterium tuberculosis* PCR assay (Roche Molecular Systems); Gen-Probe E-MTD, amplified *M. tuberculosis* direct test (Gen-Probe); Roche Amplicor MTB, Amplicor *M. tuberculosis* PCR assay (Roche Molecular Systems). Performance of commercial assays is based on pooled estimates from a meta-analysis published elsewhere [12].

may have further reduced mycobacterial viability; population-stratified analyses show that nearly all false-positive results were obtained from these specimens. Therefore, the specificity estimates among outpatients may best reflect the true performance of these assays. Finally, the collection of only a single sputum specimen may underestimate the prevalence of TB. Had patients provided more samples or had information about response to TB treatment been included, more patients might have been identified to be infected with *M. tuberculosis*, rather than being classified as uninfected. It is even possible that PCR of *secA1* is more sensitive than the reference standard (mycobacterial culture), such that false-positive PCR results represented actual cases of TB.

There are several strengths to this study. First, few studies have evaluated DNA amplification for TB diagnosis in sub-Saharan Africa [5, 6, 20–22], where HIV-associated smear-negative TB is a leading cause of death [10]. Our results that demonstrate that PCR of *secA1* performed well independently of smear or HIV status are promising for its use with sputum or oral wash specimens in such areas, particularly if the technique is modified into a rapid, inexpensive format.

Second, we recruited participants either randomly or consecutively from clinically relevant populations in a low-income country with high burdens of TB and HIV infection. Biased methods of recruitment and interpretation, including nonconsecutive patient sampling and nonblinded readings, are common in studies that have analyzed the usefulness of PCR for TB diagnosis and have been associated with exaggerated estimates of test performance [9, 23]. In our study, interpretations of the reference and index tests were blinded and strict quality-control measures were followed for both cultures (including identification of all mycobacteria) and PCR (including testing of positive and negative controls with each PCR performed),

in accordance with expert recommendations for studies of TB diagnostics [24, 25].

In summary, the direct PCR testing of oral wash or sputum specimens holds considerable promise to become a rapid, sensitive, specific approach to identify patients with active TB, independent of HIV or smear status. As nucleic-acid amplification technology becomes more available in low- and middle-income countries, the rapid molecular testing of sputum or oral wash specimens may reduce the transmission and morbidity associated with TB, reducing the worldwide impact of TB.

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