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Improving the Sensitivity of the Xpert MTB/RIF Assay on Sputum Pellets by Decreasing the Amount of Added Sample Reagent: a Laboratory and Clinical Evaluation

Nila J. Dharan,[a] Danielle Amisano,[a*] Gerald Mboowa,[b] Willy Ssengooba,[b] Robert Blakemore,[a*] Rachel W. Kubiak,[c] Derek T. Armstrong,[d] Martin Jones,[e] Yukari C. Manabe,[d,f] Moses L. Joloba,[b] Jerrold J. Ellner,[c,g] Susan E. Dorman,[d,f] David Alland[a]

New Jersey Medical School—Rutgers, the State University of New Jersey, Newark, New Jersey, USA[a]; Makerere University College of Health Sciences Mycobacteriology Laboratory, Kampala, Uganda[b]; Boston Medical Center, Boston, Massachusetts, USA[c]; Johns Hopkins University School of Medicine, Baltimore, Maryland, USA[d]; Cepheid, Sunnyvale, California, USA[e]; Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA[f]; Boston University School of Medicine, Boston, Massachusetts, USA[g]

The Xpert MTB/RIF (Xpert) assay permits rapid near-patient detection of *Mycobacterium tuberculosis* in sputum; however, the test sensitivity remains suboptimal in paucibacillary specimens that are negative for acid-fast bacilli using smear microscopy. Xpert testing includes dilution with sample reagent, and when processed sputum pellets are tested, the recommended sample reagent/pellet ratio is 3:1. We evaluated whether a decreased sample reagent/pellet ratio of 2:1 increased Xpert sensitivity compared to the recommended 3:1. The limit of detection was determined by inoculating serial dilutions of *M. tuberculosis* into sputum samples, preparing sputum pellets, and testing each pellet by Xpert at both sample reagent ratios. Processed sputum pellets obtained from *M. tuberculosis* culture-positive clinical specimens were also tested by Xpert at both ratios. Among spiked sputum pellets, the limit of detection was 1,478 CFU/ml (95% confidence interval [CI], 1,211 to 1,943) at a 3:1 ratio and decreased to 832 CFU/ml (95% CI, 671 to 1,134) at 2:1. The proportion of specimens in which *M. tuberculosis* was detected was greater at 2:1 than at 3:1 for almost all numbers of CFU/ml; this difference was most prominent at lower numbers of CFU/ml. Among 134 concentrated sputum pellets from the clinical study, the sensitivity of Xpert at 2:1 was greater than at 3:1 overall (80% versus 72%; $P = 0.03$) and for smear-negative specimens (67% versus 58%; $P = 0.12$). For Xpert testing of sputum pellets, using a lower sample reagent/pellet ratio increased *M. tuberculosis* detection, especially for paucibacillary specimens. Our study supports use of a 2:1 sample reagent/pellet dilution for Xpert testing of sputum pellets.

A major challenge to the reduction of the global burden of tuberculosis (TB) (1) has been the poor sensitivity and timeliness of conventional diagnostic tests, such as smear microscopy and mycobacterial culture. The Xpert MTB/RIF (Xpert) assay is a PCR-based diagnostic that detects *Mycobacterium tuberculosis* and rifampin (RIF) resistance within 2 h (2). Xpert was endorsed by the WHO in December 2010 for use in high-burden countries and is the first rapid near-point-of-care diagnostic to be widely implemented in TB-endemic settings (3). In clinical studies among adults, the sensitivity of the Xpert assay was 95 to 100% in smear-positive, culture-positive TB cases and 43 to 100% in smear-negative, culture-positive TB cases; specificity was 99 to 100% (2, 4–6).

The Xpert assay procedure for the detection of *M. tuberculosis* from sputum has been well described (2, 5). In brief, the sputum sample is mixed with a "sample reagent" (SR) that is used to liquefy the sample, reduce biohazard, and inactivate PCR inhibitors. Two milliliters of the mixture is placed into the Xpert cartridge, and the cartridge is inserted into the GeneXpert instrument, where fully automated PCR is completed to detect both *M. tuberculosis* and RIF resistance (2, 5). An estimate of the *M. tuberculosis* concentration in the sample is provided by the threshold cycle (C_t), which is the number of PCR cycles required to obtain a positive result (a lower C_t means a higher burden of *M. tuberculosis*). The Xpert can be performed on either raw, unprocessed sputa or concentrated sputum pellets obtained after the sputum is liquefied and decontaminated with *N*-acetyl cysteine-NaOH, followed by centrifugation. The sensitivities of the Xpert assay performed on raw sputum samples and sputum pellet samples have been reported to be similar (5, 7). The Xpert assay package insert recommends that raw sputum should be diluted with SR at a ratio of 2:1 (SR/sample) and that concentrated sputum pellets should be diluted with SR at a ratio of 3:1 (8).

To our knowledge, no studies have examined the effect of the SR/sputum ratio on Xpert sensitivity. It is therefore unknown whether the increased (i.e., 3:1) SR/sputum ratio recommended when pellets are tested reduces assay sensitivity by overdilution of

Received 22 December 2014 **Returned for modification** 23 January 2015
Accepted 27 January 2015

Accepted manuscript posted online 4 February 2015

Citation Dharan NJ, Amisano D, Mboowa G, Ssengooba W, Blakemore R, Kubiak RW, Armstrong DT, Jones M, Manabe YC, Joloba ML, Ellner JJ, Dorman SE, Alland D. 2015. Improving the sensitivity of the Xpert MTB/RIF assay on sputum pellets by decreasing the amount of added sample reagent: a laboratory and clinical evaluation. J Clin Microbiol 53:1258–1263. doi:10.1128/JCM.03619-14.

Editor: B. A. Forbes

Address correspondence to Nila J. Dharan, dharannj@njms.rutgers.edu.

* Present address: Danielle Amisano, New York Institute of Technology College of Osteopathic Medicine, Old Westbury, New York, USA; Robert Blakemore, Sackler School of Graduate Biomedical Science-Tufts University, Boston, Massachusetts, USA.

N. J. Dharan and D. Amisano contributed equally to this work.

doi:10.1128/JCM.03619-14

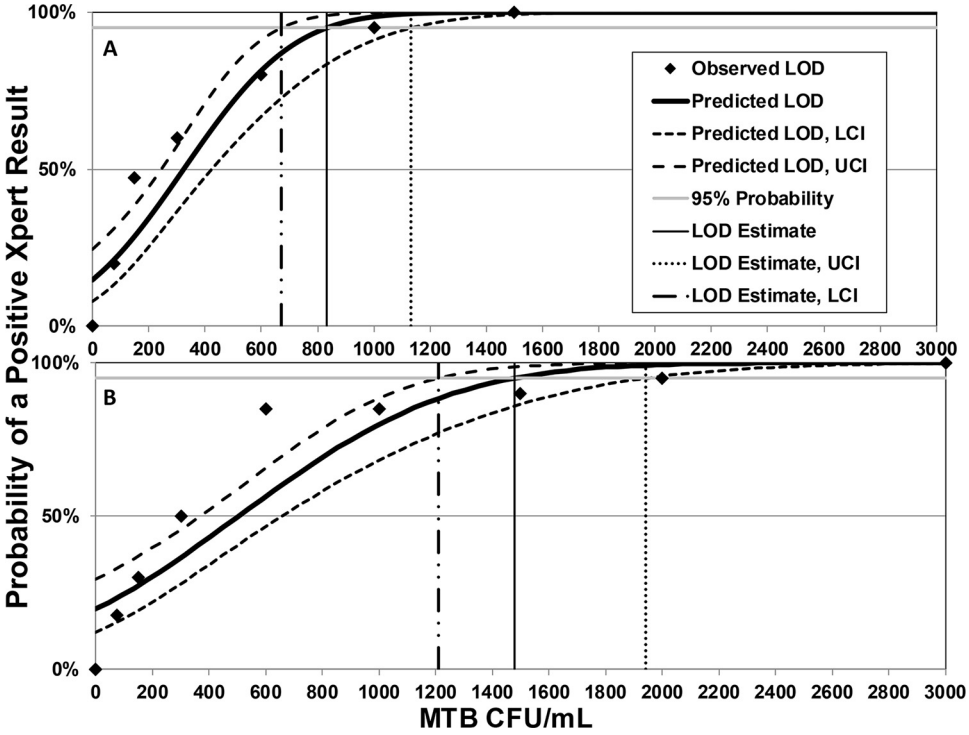

FIG 1 Analytic study. Shown are the probabilities of a positive Xpert result at SR/pellet dilutions of 2:1 (A) and 3:1 (B) by increasing concentrations. LCI, lower 95% confidence interval; UCI, upper 95% confidence interval; MTB, *M. tuberculosis*; LOD, limit of detection.

the sample or direct inhibition by the higher SR concentration in the tested specimen. Here, we present analytical and clinical data comparing the Xpert assay yield for sputum pellets diluted 3:1 versus 2:1 with SR. The overall aim of this analysis was to determine whether using a lower ratio of SR to sputum pellets (2:1 compared with the currently recommended 3:1 ratio) would improve Xpert detection of *M. tuberculosis* in sputum pellets.

MATERIALS AND METHODS

Analytic study. (i) *M. tuberculosis* strains and culture methods. The *M. tuberculosis* strain H37Rv used in all the experiments was obtained from the American Tissue Culture Collection (ATCC), Manassas, VA. The cells were grown in Middlebrook 7H9 (Difco, USA) liquid medium supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC), 0.05% Tween 80, and 0.5% glycerol and incubated at 37°C until they reached an optical density at 550 nm (OD_{550}) of 0.6 to 0.8. The cultures were aliquoted and stored at −80°C. The number of CFU per milliliter of stock solution was estimated by plating serial dilutions on Middlebrook 7H10 agar and incubating them for at least 21 days at 37°C. For all experiments, frozen stocks were either sonicated or vortexed for 1 to 2 min before making serial dilutions in 7H9 medium. Analytical studies that required the addition of known numbers of *M. tuberculosis* CFU into tuberculosis-negative sputum were performed with sputa obtained from SeraCare Life Sciences, Milford, MA.

(ii) LOD. The limit of detection (LOD) for the assay at both dilutions was defined as the minimum concentration (CFU/ml) at which 95% of the samples tested positive. To determine the LOD, known concentrations of *M. tuberculosis* were prepared from previously quantified frozen stocks of *M. tuberculosis* H37Rv and were added to 3-ml aliquots of SeraCare sputum samples. The samples were tested with 20 replicates at the following concentrations: 0, 75, 150, 300, 600, 1,000, 1,500, 2,000, and 3,000 CFU/ml. Two milliliters of concentrated sputum pellet was made from each 3-ml sample following the BBL MycoPrep Specimen Digestion/Decontamination kit protocol (9). Each sputum pellet was split and tested at both 2:1 and 3:1 SR/sample dilutions. To prepare the 3:1 dilutions, 1.5 ml SR was mixed with 500 μl sample. To prepare the 2:1 dilutions, 1.3 ml SR was mixed with 650 μl sample. Both were incubated for 15 min at room temperature, and the entire final volume of sample (2 ml) was loaded into the Xpert cartridge and placed into the Xpert machine for automated PCR detection of *M. tuberculosis* and RIF resistance. To determine the LOD for each SR dilution, we repeated this procedure 20 times at each CFU concentration and recorded the number of times that the Xpert assay was positive for *M. tuberculosis* at each CFU concentration.

Clinical study. (i) Design and specimens. The clinical study described here used samples collected as part of a previously described multicenter prospective diagnostic-accuracy study of a urine lipoarabinomannan (LAM) test for tuberculosis (referred to here as the parent study) (10). Subjects were recruited into the parent study from South Africa and Uganda from January to November 2011. Symptomatic outpatient and inpatient HIV-positive adults (≥18 years old) suspected to have TB were eligible for participation in the study. Subjects were excluded if they had received more than 2 days of antituberculosis treatment within 60 days prior to enrollment. After written informed consent, medical and demographic information was obtained and the subjects provided clinical specimens for laboratory testing, including two expectorated or induced sputum samples.

(ii) Substudy laboratory testing methods. All laboratory assessments completed as part of the parent study were described previously (10). In this substudy, only samples tested at the Uganda laboratory site were included. Briefly, raw sputum specimens were smeared, stained, and graded for the presence of acid-fast bacilli (AFB) under microscopy using the Ziehl-Neelsen (ZN) staining method (11). Smear examination followed the WHO scale for AFB smear grading. Raw samples were decontaminated using *N*-acetyl-L-cysteine-NaOH; 0.5 ml of the pellet was cultured with the Bactec MGIT (mycobacterial growth indicator tube) 960 system (Becton Dickinson, Franklin Lakes, NJ), and 0.2 ml was inoculated

TABLE 1 Comparison of subject characteristics between subjects who had at least one specimen included versus none included[a]

Characteristic	Value[b] for participants for whom:		
	Sputum tested at both dilutions (included in analysis) ($n = 134$)	Sputum not tested at both dilutions (excluded from analysis) ($n = 36$)	*P* value[c]
Demographics			
Male	60 (44.8)	11 (30.6)	0.12
Black race	134 (100)	36 (100)	NA
Clinical parameters			
Wt (kg) [median (IQR)]	50.0 (45.0–54.0)	48.0 (43.0–52.5)	0.64
Karnofsky score [median (IQR)]	60.0 (60.0–70.0)	60.0 (60.0–70.0)	0.75
Cough within prior 4 weeks	132 (98.5)	35 (97.2)	0.60
Cough duration (days) [median (IQR)]	30.0 (21.0–60.0)	30.0 (14.0–60.0)	0.57
Fever within prior 4 weeks	121 (90.3)	34 (94.4)	0.44
Wt loss	129 (96.3)	35 (97.2)	0.78
Currently taking antiretrovirals	49 (36.6)	8 (22.2)	0.11
Currently taking cotrimoxazole	124 (92.5)	34 (94.4)	0.69
CD4 [median (IQR)]	69.0 (18.0–171.0)	59.0 (15.0–195.0)	0.91
TB treatment history			
Ever prescribed anti-TB treatment	9 (6.7)	4 (11.1)	0.38
Currently taking anti-TB treatment	0 (0.0)	0 (0.0)	NA
Microbiologic studies			
Blood culture positive for MTB	38 (28.4)	9 (25.0)	0.69
Time to growth on MGIT (days) [median (IQR)]	9.0 (6.0–13.0)	11.8 (10.0–14.0)	0.02
Urine LAM positive	82 (61.2)	16 (44.4)	0.07
Chest radiograph findings			
Bilateral infiltrates	76 (56.7)	12 (33.3)	0.01
Miliary infiltrates	29 (21.6)	3 (8.3)	0.07
Cavitary disease[d]	16 (11.9)	3 (8.3)	0.70

[a] Every subject was counted only once. Subjects for whom both specimens were tested at both dilutions are included only one time in the table; IQR, interquartile range; TB, tuberculosis; MTB, *Mycobacterium tuberculosis*; MGIT, mycobacterial growth indicator tube; LAM, lipoarabinomannan; NA, not applicable.
[b] Values are number (percent) unless otherwise indicated.
[c] The chi-square test was used where *n* is given; the Wilcoxon-Mann-Whitney test was used where a median is given.
[d] One or more cavities were present on chest radiograph.

onto Lowenstein Jensen (LJ) medium. A concentrated fluorescent (CF) smear was made from the pellet, stained, and examined. Cultures were incubated at 37°C for up to 8 weeks (9). A concentrated sputum sample with a total remaining volume of 1.8 ml was stored at −80°C for future use. The time to detection of culture growth on MGIT (time to a positive culture result [TTP]) was defined as the time elapsed from the date of culture inoculation to the date of detection of mycobacterial growth.

To compare the sensitivity of Xpert for the detection of *M. tuberculosis* from sputum pellets using the manufacturer's recommended (8) 3:1 SR/pellet dilution versus a reduced dilution of 2:1, frozen samples were thawed to room temperature and vortexed for 15 s. Standard dilution volumes were used as recommended in the package insert (8). For the standard 3:1 dilution, at least 0.5 ml of the total resuspended pellet and 1.5 ml of SR were added to a tube. For the experimental 2:1 dilution, 0.7 ml of resuspended sputum pellet and 1.4 ml of SR were added to a tube. The tubes were then shaken vigorously 10 to 20 times and incubated for 15 min at room temperature. After 5 to 10 min, the sample was shaken again 10 to 20 times until the samples were liquefied with no visible clumps of material. The remaining time of incubation was completed, and 2 ml of sample was then transferred into the Xpert cartridge and loaded into the automated Xpert instrument.

(iii) Statistical methods. Statistical comparisons of categorical and continuous variables were made using Pearson's chi-square test and the Wilcoxon-Mann-Whitney test, respectively. Smears were considered positive if graded 1+, 2+, or 3+, as defined by the World Health Organization (11). The first available specimen was defined as the first specimen collected from a subject that was LJ and/or MGIT culture positive for *M. tuberculosis* and tested by Xpert at both dilutions. Culture-positive specimens were excluded from analyses if not tested at both 3:1 and 2:1 dilutions. Sensitivity was calculated using MGIT and LJ culture as a composite reference standard wherein a specimen was considered *M. tuberculosis* positive if either culture was *M. tuberculosis* positive. Comparisons between sputum dilution ratios from paired samples were made using McNemar's test. Analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA). All *P* values reported are two sided, with statistical significance defined as a *P* value of <0.05.

Ethics statement. The parent clinical study was approved by the appropriate regulatory bodies as previously described (10). Ethical approval for this substudy was obtained from the Uganda National Council for Science and Technology and the Institutional Review Boards at Johns Hopkins University, the Joint Clinical Research Centre (Kampala, Uganda), and New Jersey Medical School (NJMS).

RESULTS

Analytic study: effect of SR treatment ratios on LOD. We performed an analytic study to test the effects of the two SR ratios on the sensitivity of the Xpert assay (Fig. 1). With the standard 3:1 SR-to-sputum pellet treatment ratio, the LOD was 1,478 CFU/ml (95% confidence interval [CI], 1,211 to 1,943). The LOD decreased to 832 CFU/ml (95% CI, 671 to 1,134) when the ratio was decreased to 2:1. The proportion of specimens in which *M. tuber-*

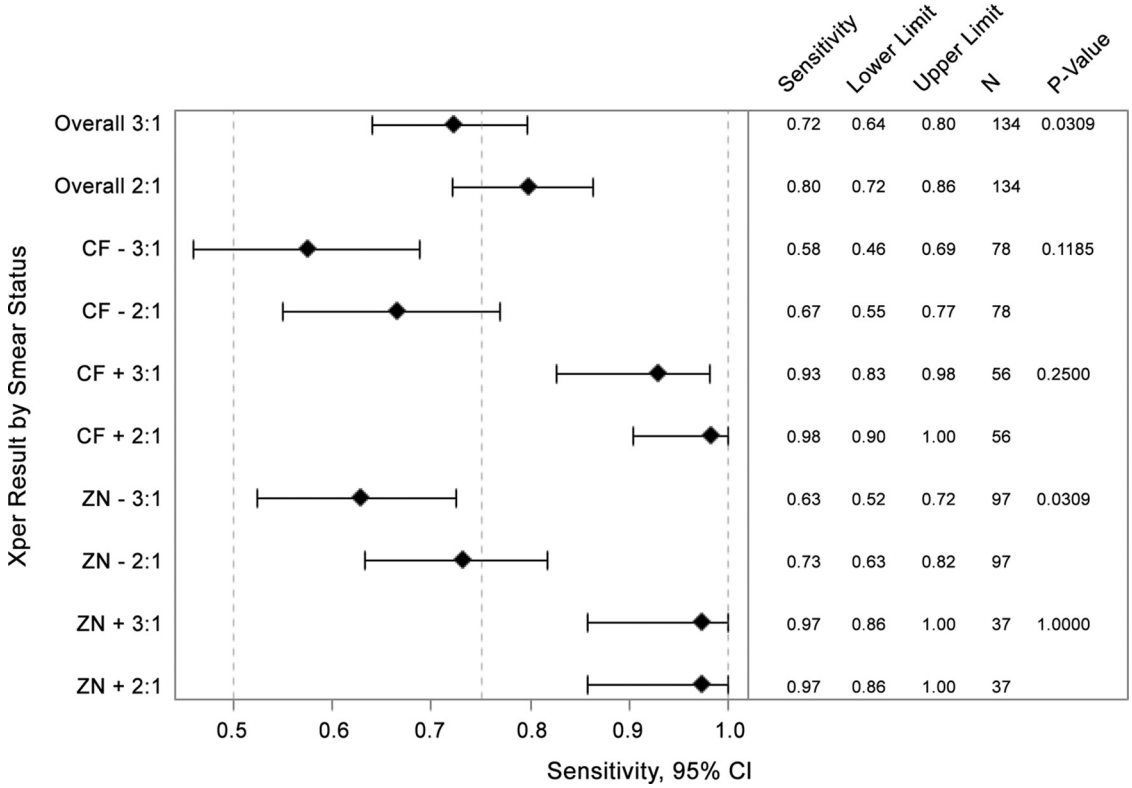

FIG 2 Sensitivity of Xpert for the detection of *M. tuberculosis* in sputum pellet specimens at each dilution, overall and stratified by smear microscopy status. ZN, direct Ziehl-Neelsen smear; CF, concentrated fluorescent acid-fast smear from pellet.

culosis was detected was greater at 2:1 dilution than at 3:1 dilution at almost all CFU dilutions; this difference was most prominent at lower concentrations. None of the negative-control samples was positive for *M. tuberculosis*. There were no errors at 2:1 and 3 errors at 3:1 ($P = 0.25$). There was one *M. tuberculosis* detected/RIF resistance indeterminate result at 2:1 dilution and 9 *M. tuberculosis* detected/RIF resistance indeterminate results at 3:1 dilution ($P = 0.02$).

Clinical substudy. (i) Clinical study participants. Having identified a possible advantage of 2:1 over 3:1 SR treatment in the analytic study, we then performed a similar study using clinical sputum samples from known TB patients. Of the 506 subjects enrolled in the parent study, 5 were excluded because they did not produce any sputum samples. The remaining 501 subjects produced 957 sputum samples, of which 690 were culture negative for *M. tuberculosis* and therefore excluded from the analysis. Of the 267 sputum samples that were culture positive for *M. tuberculosis*, 101 were not tested at the two dilutions because of insufficient sample volume. Of the 166 remaining sputum samples that were tested at both dilutions, 32 subjects had two samples tested at each dilution. For this analysis we considered only the first specimen, resulting in a total of 134 subjects who were included in the comparison of Xpert *M. tuberculosis* detection in sputum pellets at a 2:1 versus a 3:1 SR/sputum pellet dilution.

A comparison of baseline study characteristics between subjects included in this substudy analysis and subjects enrolled in the parent study but excluded from this analysis (due to insufficient pellet volume) is provided in Table 1. Compared with the excluded subjects, subjects included in this analysis had a lower median number of days to growth on culture by MGIT ($P = 0.02$), and a larger proportion of included subjects had bilateral infiltrates on chest radiograph ($P = 0.01$). A larger proportion of substudy subjects were positive by urine TB LAM test and had miliary infiltrates on chest radiograph; however, these differences did not reach statistical significance ($P = 0.07$ for both).

(ii) Results of Xpert testing at 2:1 and 3:1 dilutions with sample reagent on clinical samples. There were no "invalid" or "error" Xpert results. The overall sensitivity of Xpert using 2:1 dilu-

TABLE 2 Detection of *M. tuberculosis* in clinical samples at both dilutions stratified by smear status

Smear status	Detection at 2:1 concn	No. of samples positive for *M. tuberculosis*			
		ZN smear (3:1 concn) ($n = 134$)		CF acid-fast smear (3:1 concn) ($n = 134$)	
		Not detected	Detected	Not detected	Detected
Negative[a]	Not detected	22	4[c]	22	4[d]
	Detected	14[c]	57	11[d]	41
Positive[b]	Not detected	1	0	1	0
	Detected	0	36	3	52

[a] ZN (Ziehl-Neelsen) smear, $n = 97$; CF (concentrated fluorescent) acid-fast smear, $n = 78$.
[b] ZN smear, $n = 37$; CF acid-fast smear, $n = 56$.
[c] P value for difference (McNemar's test), 0.03.
[d] P value for difference (McNemar's test), 0.12.

TABLE 3 Time to positive growth on MGIT among clinical samples

Sample	*n*	Median TTP (days)	IQR	Sensitivity
All	127[a]	9	6–13	
Ziehl-Neelsen smear[b]				
Negative	92	11	9–14	
Positive	35	5	4–7	
Concentrated acid-fast smear[b]				
Negative	73	12	9–15	
Positive	54	6	4–8	
3:1				
Negative	34	14	11–17	
Positive	93	8	5–11	0.73
2:1				
Negative	25	17	11–17	
Positive	102	8	5–11	0.80
Discordant Xpert results				
3:1 positive, 2:1 negative	4	14	12–16.5	
3:1 negative, 2:1 positive	13	12	9–14	

[a] Three specimens were contaminated on MGIT; four were nontuberculous mycobacteria on MGIT (but positive for *M. tuberculosis* on Lowenstein-Jensen medium).
[b] Scanty was considered negative.

tion was 80% (107/134; 95% CI, 72% to 86%) versus 72% (97/134; 95% CI, 64% to 80%) using 3:1 dilution ($P = 0.03$). Among 78 concentrated smear-negative specimens, sensitivity using 2:1 dilution was 67% (52/78; 95% CI, 55% to 77%) versus 58% (45/78; 95% CI, 46% to 69%) using 3:1 dilution ($P = 0.12$) (Fig. 2). Among all specimens, 14 were positive for *M. tuberculosis* at 2:1 dilution but negative at 3:1 dilution. Conversely, four specimens were positive for *M. tuberculosis* at 3:1 dilution but negative at 2:1 dilution ($P = 0.03$). By each smear methodology, there were significantly more specimens with *M. tuberculosis* detected by Xpert at 2:1 dilution than at 3:1 among smear-negative specimens ($P = 0.049$) and almost no difference in detection of *M. tuberculosis* by Xpert for smear-positive specimens (Table 2 and Fig. 2).

(iii) Relationship between Xpert results and time to positivity in liquid culture. The above-mentioned results suggest that the 2:1 SR treatment ratio would be most beneficial in sputum samples with low *M. tuberculosis* bacterial loads. Although we did not perform quantitative CFU plating of the clinical samples, we did record the TTP for each sputum sample, which has been used as an indication of the bacillary burden in clinical samples (12–14). Of 134 samples tested in our study, three MGIT liquid cultures were contaminated with nonmycobacterial organisms and four were positive only for nontuberculous mycobacteria (these samples were confirmed to have *M. tuberculosis* by solid culture). For this analysis, they were excluded, leaving a total of 127 specimens with TTP results. As expected, specimens that were smear positive by either ZN and/or CF and/or positive by Xpert at either dilution were correlated with shorter TTP (i.e., higher burdens of mycobacteria; $P < 0.0001$ for each) (Table 3). As with smear status, the higher Xpert sensitivity associated with a lower dilution with SR (2:1 versus 3:1) was most apparent on samples with the longest TTP, indicating that the increased yield at lower SR dilution is greatest among paucibacillary samples (Fig. 3).

DISCUSSION

We found that the sensitivity of Xpert tests on concentrated sputum pellets can be increased by decreasing the ratio of SR that is added to the sputum pellets from the recommended 3:1 to 2:1. The improvement in detection was greatest among specimens that had lower burdens of *M. tuberculosis*; this finding was consistent for nonclinical samples spiked with lower numbers of CFU/ml, as well as for clinical samples that were AFB smear negative and had greater TTP on MGIT culture. These findings support further

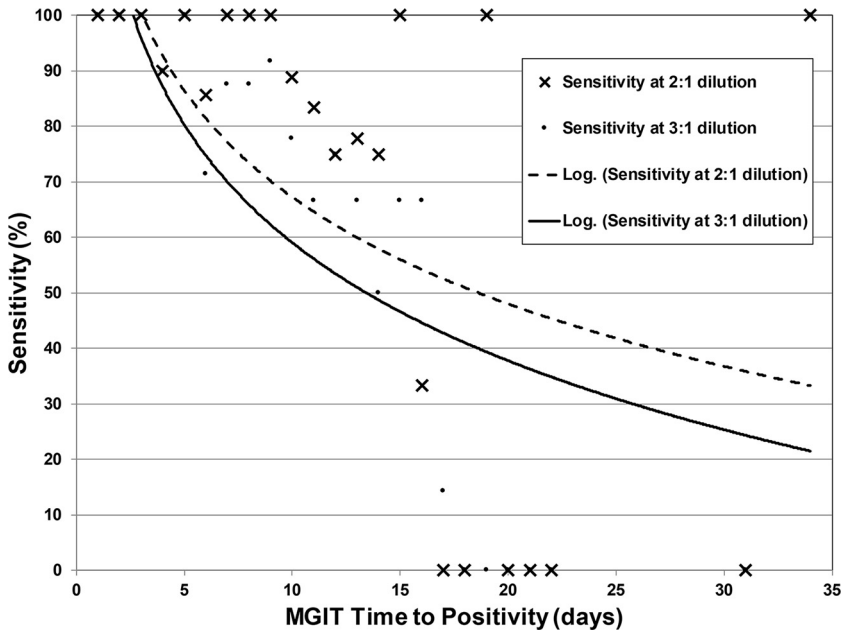

FIG 3 Time to growth on MGIT and detection of *M. tuberculosis* by Xpert. MGIT, mycobacterial growth indicator tube.

studies to evaluate recommended procedures for sample pretreatment with SR to optimize the yield of Xpert, particularly among samples with low *M. tuberculosis* bacillary burdens.

Overdilution of clinical samples prior to Xpert testing could lead to a decreased concentration of *M. tuberculosis* bacilli in the Xpert cartridge and therefore lead to lower detection. In fact, lower sensitivity of Xpert for detection of *M. tuberculosis* has been reported for sputum samples that have been induced with saline than for those that were expectorated (13), possibly because of sample dilution. In the Cepheid package insert (8), clinical data are reported comparing Xpert sensitivity between 535 expectorated specimens and 234 induced specimens. There was no difference in sensitivity between AFB smear-positive induced versus expectorated specimens (sensitivity, 100% versus 99.6%, respectively). However, among AFB smear-negative subjects, Xpert had lower sensitivity among induced specimens than expectorated specimens (sensitivity, 40% versus 79%), though the numbers of induced samples tested were low. Similar to our findings, this suggests that the risk of saline induction causing a dilution of clinical specimens that affects detection of *M. tuberculosis* by Xpert may be greatest among paucibacillary specimens.

Another possible explanation for decreased yield in samples with higher SR/pellet ratios is that the SR material itself causes direct inhibition in the samples being tested. In general, SR is thought to inactivate PCR inhibitors (2, 8) and is not thought to inhibit the Xpert test at the recommended SR concentrations, but this possibility should be investigated in further studies.

Our findings are subject to several limitations. First, all clinical samples were obtained from HIV-positive patients who, especially those with advanced immunosuppression (15), are more likely to be sputum AFB smear negative due to a higher frequency of noncavitary pulmonary disease (13) and lower bacterial burden (16). Therefore, our results may not be relevant to HIV-negative patients, who have a higher burden of *M. tuberculosis* and whose sputum samples are more likely to be smear positive and to have a shorter time to growth on MGIT. Our analyses were performed only on culture-positive specimens, and our findings should be validated in subjects who are culture negative but have positive Xpert results and a clinical diagnosis of TB (defined by clinical response to anti-TB therapy).

In conclusion, we found an increase in Xpert detection of *M. tuberculosis* using a lower dilution of analytical samples and concentrated clinical sputum pellets with SR, especially among specimens with lower *M. tuberculosis* burdens. Although these findings should be investigated in larger studies, strong consideration should be given to revision of the manufacturer's suggested procedures for sample pretreatment. Increasing the detection of *M. tuberculosis* by Xpert is especially important in paucibacillary disease, where diagnosing TB is the most challenging.

ACKNOWLEDGMENTS

David Alland reports receiving grants from Cepheid during the conduct of the study and has a patent related to molecular beacons with royalties paid. Martin Jones reports that he is employed by Cepheid and is a shareholder in Cepheid. We have no other conflicts of interest to disclose.

REFERENCES

1. **World Health Organization.** 2014. Global tuberculosis report. World Health Organization, Geneva, Switzerland. http://www.who.int/tb/publications/global_report/en/index.html. Accessed 11 November 2014.
2. **Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens MR, Rodgers R, Banada P, Safi H, Blakemore R, Lan NT, Jones-Lopez EC, Levi M, Burday M, Ayakaka I, Mugerwa RD, McMillan B, Winn-Deen E, Christel L, Dailey P, Perkins MD, Persing DH, Alland D.** 2010. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol **48:**229–237. http://dx.doi.org/10.1128/JCM.01463-09.
3. **World Health Organization.** 2014. WHO monitoring of Xpert MTB/RIF roll-out. World Health Organization, Geneva, Switzerland. http://who.int/tb/laboratory/mtbrifrollout/en/. Accessed 9 November 2014.
4. **Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, Gler MT, Blakemore R, Worodria W, Gray C, Huang L, Caceres T, Mehdiyev R, Raymond L, Whitelaw A, Sagadevan K, Alexander H, Albert H, Cobelens F, Cox H, Alland D, Perkins MD.** 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet **377:**1495–1505. http://dx.doi.org/10.1016/S0140-6736(11)60438-8.
5. **Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD.** 2010. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med **363:**1005–1015. http://dx.doi.org/10.1056/NEJMoa0907847.
6. **Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N.** 2014. Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev **1:**CD009593. http://dx.doi.org/10.1002/14651858.CD009593.pub3.
7. **Shenai S, Amisano D, Ronacher K, Kriel M, Banada PP, Song T, Lee M, Joh JS, Winter J, Thayer R, Via LE, Kim S, Barry CE, III, Walzl G, Alland D.** 2013. Exploring alternative biomaterials for diagnosis of pulmonary tuberculosis in HIV-negative patients by use of the GeneXpert MTB/RIF assay. J Clin Microbiol **51:**4161–4166. http://dx.doi.org/10.1128/JCM.01743-13.
8. **Cepheid.** 2013. Xpert MTB/RIF assay package insert. Cepheid, Sunnyvale, CA. http://www.ipaqt.org/wp-content/uploads/2013/02/Xpert_mtb_rif_product_insert.pdf. Accessed 9 November 2014.
9. **Kent PT, Kubica GP.** 1985. Public health mycobacteriology: a guide for the level III laboratory. Centers for Disease Control, Atlanta, GA.
10. **Nakiyingi L, Moodley VM, Manabe YC, Nicol MP, Holshouser M, Armstrong DT, Zemanay W, Sikhondze W, Mbabazi O, Nonyane BA, Shah M, Joloba ML, Alland D, Ellner JJ, Dorman SE.** 2014. Diagnostic accuracy of a rapid urine lipoarabinomannan test for tuberculosis in HIV-infected adults. J Acquir Immune Defic Syndr. http://dx.doi.org/10.1097/QAI.0000000000000151.
11. **WHO.** 2014. Global TB programme, World Health Organization. Laboratory services in TB control. Part II. Microscopy. WHO/TB/98.258. http://whqlibdoc.who.int/hq/1998/WHO_TB_98.258_(part2).pdf. Accessed 9 November 2014.
12. **Jindani A, Dore CJ, Mitchison DA.** 2003. Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. Am J Respir Crit Care Med **167:**1348–1354. http://dx.doi.org/10.1164/rccm.200210-1125OC.
13. **Theron G, Peter J, Calligaro G, Meldau R, Hanrahan C, Khalfey H, Matinyenya B, Muchinga T, Smith L, Pandie S, Lenders L, Patel V, Mayosi BM, Dheda K.** 2014. Determinants of PCR performance (Xpert MTB/RIF), including bacterial load and inhibition, for TB diagnosis using specimens from different body compartments. Sci Rep **4:**5658. http://dx.doi.org/10.1038/srep05658.
14. **Palaci M, Dietze R, Hadad DJ, Ribeiro FK, Peres RL, Vinhas SA, Maciel EL, do Valle Dettoni V, Horter L, Boom WH, Johnson JL, Eisenach KD.** 2007. Cavitary disease and quantitative sputum bacillary load in cases of pulmonary tuberculosis. J Clin Microbiol **45:**4064–4066. http://dx.doi.org/10.1128/JCM.01780-07.
15. **Chamie G, Luetkemeyer A, Walusimbi-Nanteza M, Okwera A, Whalen CC, Mugerwa RD, Havlir DV, Charlebois ED.** 2010. Significant variation in presentation of pulmonary tuberculosis across a high resolution of CD4 strata. Int J Tuberc Lung Dis **14:**1295–1302.
16. **Hanrahan CF, Theron G, Bassett J, Dheda K, Scott L, Stevens W, Sanne I, Van Rie A.** 2014. Xpert MTB/RIF as a measure of sputum bacillary burden. Variation by HIV status and immunosuppression. Am J Respir Crit Care Med **189:**1426–1434. http://dx.doi.org/10.1164/rccm.201312-2140OC.