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PII: S1201-9712(23)00011-5
DOI: https://doi.org/10.1016/j.ijid.2023.01.009
Reference: IJID 6581

To appear in: *International Journal of Infectious Diseases*

Received date: 23 May 2022
Revised date: 17 November 2022
Accepted date: 6 January 2023

Please cite this article as: Victor Santana Santos , Manuela Filter Allgayer , Konstantina Kontogianni , José Erinaldo Rocha , Beatriz Jatobá Pimentel , Maria Telma Pinheiro Amorim , Morgana Valnia Simões Cavalcanti Duarte , Polyanne de Melo Ferreira , Luciana Coelho Leite Moura , Victoria Paulino Santos de Lima , Larissa Cândido Guimarães , James Dodd , Jacob Creswell , Luis Eduardo Cuevas , Pooling of sputum samples to increase tuberculosis diagnostic capacity in Brazil during the COVID-19 pandemic, *International Journal of Infectious Diseases* (2023), doi: https://doi.org/10.1016/j.ijid.2023.01.009

Pooling of sputum samples to increase tuberculosis diagnostic capacity in Brazil during the COVID-19 pandemic

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Funding

This research was funded in part by a TB REACH grant supported by Global Affairs Canada (STBP/TBREACH/GSA/2020-04) and the UK Medical Research Council Public Health Intervention Development (PHIND) (MR/W004313/1) to LEC.

Abstract

Objectives. We assessed whether combining (pooling) four individual's samples and testing with Xpert Ultra has the same accuracy as testing samples individually, as a more efficient testing method.

Methods. We conducted a cross-sectional study of individuals with presumptive TB attending primary health care or general hospital facilities in Alagoas, Brazil. Sputum samples of four consecutive individuals were pooled and the pool and individual samples were tested with Xpert Ultra. The agreement of the tests was compared using kappa statistics. We estimated the sensitivity and specificity of pooling using the individual test as the reference standard and potential cartridge savings.

Results. Three hundred and ninety-six participants were tested. Ninety-five (24.0%) individual samples were MTB-positive, 300 (75.8%) MTB-not detected, including 20 MTB-trace, and one reported an error. Ninety-nine pools of four samples were tested, of which 62 (62.6%) had MTB-detected and 37 (37.4%) MTB-not detected, including six (6.1%) with MTB-trace. The agreement of individual and pooled testing was 96.0%. Pooling had sensitivity of 95.0% (95%CI 86.9%–99%), specificity of 97.1% (95%CI 85.1%–99.9%) and Kappa of 0.913. The method saved 12.4% of cartridge costs.

Conclusion. The pooled testing of specimens had a high level of agreement with individual testing. Pooling of samples for testing improves the efficiency of testing, potentially enabling the screening and testing of larger numbers of people more cost-effectively.

Keywords: Tuberculosis; Diagnosis; Xpert Ultra; Pooling samples; Brazil.

Introduction

Tuberculosis (TB) is second only to Coronavirus disease-19 (Covid-19) as a cause of adult death due to infection. Although TB is ubiquitous, its distribution is not even and thirty countries, including Brazil, account for 86–90% of the global TB incidence (World Health Organization (WHO) 2021a). Underreporting of TB is a major problem, as only 5.8 of the estimated 10 million people who developed TB were reported in 2020; and over 40% were missed by health services (World Health Organization (WHO) 2021a). Underreporting was exacerbated by the Covid-19 pandemic, with an 18% drop in TB notifications from 2019 to 2020 (World Health Organization (WHO) 2021a). Brazil is among countries with the largest contributions to the global shortfall in TB notifications (World Health Organization (WHO) 2021a). In 2020, the country had an estimated 96,000 people with TB, of whom 21,174 (22%) were missed by the national health services (Stop TB Partnership 2022b). Similar to other countries, notifications dropped in 2020 and were 14.3% lower than in 2019, which was accompanied by a 14% reduction in the use of rapid molecular tests (Brasil, 2021)

Access to TB treatment depends on good quality diagnosis and the World Health Organization (WHO) recommends using Nucleic Acid Amplification (NAA) assays as the initial tests for diagnosis. Although these tests are sensitive and specific, with Xpert MTB/RIF and Xpert Ultra being the most frequently used assays (Treatment Action Group 2020), only a fraction of individuals are tested with these assays, as their implementation is limited by the laboratory infrastructure required and the cost of the cartridges.

Xpert assays were approved in Brazil in 2013 and are indicated as the first tests for diagnosis of TB within the Brazilian National Health System (SUS). Cartridges are provided by the Ministry of

Health, and laboratory stocks are based on the estimated population they serve. Clinical specimens collected by the clinics are transported using sample transport networks and testing is centralized in reference laboratories. Health services however became severely strained during the pandemic and, although there have been no reports of cartridge shortages, the workload of TB and SARS-CoV-2 tests is high and often requires extended testing over the weekend.

Recent studies have reported that the sputum pooling method, in which samples from several patients are combined and tested together, could increase the efficiency of NAA TB assays (Cuevas et al. 2021; Iem et al. 2022). However, there is no data on the performance of this method from presumptive TB in primary health care in Brazil.

We therefore evaluated whether combining specimens of four individuals with presumptive TB and testing the pool with Xpert Ultra would result in the same accuracy as testing samples individually, and estimated whether pooling approach would result in cost savings.

Methods

This was a cross sectional survey of consecutive individuals attending primary health care units or general hospitals with signs and symptoms of presumptive TB in Alagoas state, Brazil from September 2021 to February 2022. Adults with presumptive TB were requested to produce two samples of expectorated sputum for examination, following the Brazilian routine procedures for TB diagnostic centres. Sputum samples were kept refrigerated and transported to the testing laboratories daily, or batched in consignments and submitted every 2-3 days depending on the local availability of transport. Samples were transported using a cold chain with cold boxes until tested. Sputum samples were routinely processed in the laboratory and tested using

Xpert Ultra, following the manufacturer's instructions with a 1:2 sputum to reagent ratio. Samples with at least 0.5 ml of left-over sputum were selected for the pooling study. We combined (or pooled) the sputum of four consecutive individuals into one pot and tested the pool with a single Xpert Ultra test. Individual samples results were used for the purpose of evaluating the performance of the approach and for modelling potential savings. Individual and pooled Xpert tests reporting invalid, error and no result were repeated, if there was enough sample left over for testing, and the repeated test result was included in the analysis. Samples with trace call results on individual tests were retested if sufficient sample was available and are described with all results to support interpretation.

Statistical analysis

We conducted the pooling assessment within the period of the COVID-19 epidemic. Sample size for the survey was not formally estimated as we were limited by the expected number of participants attending the services and the capacity of staff to conduct testing additional to their routine activities. All data was stored in anonymized databases compliant with data protection legislation. Categorical data were summarized using descriptive statistics with 95% confidence intervals (95% CI). Chi-squared tests and Chi-square for trends were used to test for statistically significant differences. Individuals unable to produce sufficient sputum were excluded from the study.

The pooled and individual tests were compared, and their agreement was tested using kappa statistics. We considered concordance if: a) the pool result was negative and all tests for the four individual samples were negative; b) the pool result was positive and at least one of the tests for the individual samples was positive. The kappa values and their interpretations were

as follows: <0, no agreement; 0–0.19, very weak agreement; 0.20–0.39, weak agreement; 0.40–0.59, moderate agreement; 0.60–0.79, substantial agreement; and 0.8–1.0, excellent agreement (Landis and Koch 1977). The MTB grades (*trace, very low, low, medium,* and *high*) of individual and pooled tests were compared to describe the effect of combining the samples. Patients with *trace* results were re-tested if there was sufficient sample left over, but were considered to be test-negative, as WHO recommends not to re-test and not to consider them as positive, unless considered with additional clinical findings and medical history (World Health Organization (WHO) 2021b). We present trace results as a separate category for clarity, and were considered negative for the purpose of test agreement. Sensitivity and specificity were estimated using the single Xpert Ultra test for a single sputum sample. The individual test was considered the reference standard. Cost differences were calculated on the bases of the number of cartridges required to test all specimens using pooled and individual testing assuming a cartridge procurement cost of USD 9.98 (Stop TB Partnership 2022a).

Ethical approval

The study was approved by the Committee on Ethics in Research with Human Beings at the Universidade Federal de Alagoas, Brazil (CAAE number 45432821.2.0000.5013) and the Liverpool School of Tropical Medicine Research Ethics Committee, UK (Ethical waiver 20-037). An informed consent waiver was obtained.

Role of the funding source

The study sponsors had no role in study design, data collection, data analysis, interpretation, writing of the report, or in the decision to submit the paper for publication.

Results

A total of 396 participants with a mean (SD) age of 49 (16.9) years were included. Of these, 252 (63.6%) were male and 144 (36.4%) female. The largest proportion of participants (152, 38.4%) were ≥ 55 years and the smallest proportion (89, 22.5%) <35 years old, as shown in table 1. The samples were considered of good quality for most participants, with only 15 (3.8%) contained saliva and 11 (2.8%) blood traces. Twenty-eight individual samples had MTB-trace results and 11 had sufficient volumes for re-testing. Three of the re-tested samples were MTB-detected (one each with very low, low and medium MTB grades), five MTB-negative and three were again reported as MTB-trace. All 20 samples reported with trace results (those not re-tested and re-tested samples reporting a repeat trace result) were considered "MTB-not detected" for analysis. Fifteen samples reported errors, with four reporting MTB-detected on re-testing, 10 MTB-not detected and one a repeated error. A further six samples with MTB-no-result were re-tested, with two having MTB-detected and four MTB-not detected.

Altogether, 95 (24.0%) samples were MTB-positive, 300 (75.8%) MTB-not detected, with the latter including 20 (5.3%) MTB-trace and one sample which reported an error. Among MTB-positive samples, 25 had very low or low and 70 medium or high MTB grade. Only two samples were RIF-positive. Males and participants <35 years old were more likely to be Xpert positive (76/252 [30.2%] males versus 19/144 [13.2%] females, $p < 0.001$; 33/89 [37.1%] <35 versus 25/152 [16.5%] ≥ 55 years old, Chi square for trend, $p = 0.0004$), as shown in Table 1.

Individual samples were tested in 99 pools of four. Sixty-two (62.6%) pools had MTB-detected and 37 (37.4%) MTB-not detected, with the latter including six (6.1%) pools with MTB-trace. One pool was RIF-positive (Table 2). Individual and pooled tests were in agreement except for one pool containing four individual MTB-negative samples, which tested MTB-positive, and

three pools containing one MTB-positive sample on individual testing which tested MTB-negative. All 26 (100%) pools containing two or more MTB-positive samples tested positive (table 4). The overall agreement was 96% with a sensitivity of 95% (95%CI 86.9% – 99%), specificity of 97.1% (95%CI 85.1% – 99.9%) and Kappa of 0.913 ("near perfect agreement"). The agreement of individual and pooled tests was associated with the MTB grade. Thirty-eight pools contained only one MTB-positive sample. The pool Xpert grade was lower than the individual grade in 13 pools, similar to the individual grade in twenty and higher in five, as shown in table 5.

Testing samples individually required 396 Xpert Ultra cartridges at a cost at source of USD 3952.08. Testing the samples using the pooling method required 99 cartridges (USD 988.02) to test the pools and 248 cartridges (USD 2475.04) to retest individual samples for the positive pools (total cost USD 3463.06), resulting in USD 489.02 (12.4%) savings in cartridge costs.

Discussion

This is the first evaluation of the pooling method for the diagnosis of TB in Brazil, a high burden country where NAAs assays are used as the first test for diagnosis. Participants in the study reflect the characteristics of people with presumptive TB in the country, with a higher proportion being male, and a higher proportion of males having positive tests than females. Our findings confirm that samples tested with Xpert Ultra using the pooling method have a high-level of agreement with individual testing, as previously reported from Cambodia (Chry et al. 2020) and Laos (Iem et al. 2022). Almost all disagreements were false negatives (3%) and occurred in samples with low MTB grades and we had only one pool containing negative samples that tested positive. Although false negatives are mostly due to low bacilli loads and

the limitations of the tests to detect paucibacillary TB, some studies have reported false positive results during pooled testing (Helb et al. 2010; Chakravorty et al. 2017). Although false positives are usually attributed to cross contamination resulting from the additional manipulation of samples, pooled testing for other pathogens (e.g. for SARS-CoV-2) has been reported to result in reduced CT values of the pools. This effect may be due to PCR efficiencies through a “carrier-RNA” effect caused by the increased total cellular RNA in the pool, or improved PCR efficiencies in samples containing PCR inhibitors, which are diluted by the pooling process (Lohse et al. 2020a, 2020b). False positive pooled results will lower potential savings, but would not affect final clinical decisions, as the samples in the pool would be tested individually.

As expected, some individual samples (n = 28, 7%) had trace results. Trace results are known to have low repeatability and WHO recommends not to re-test these samples. This recommendation is based on the difficulties in interpreting the repeat results in patients with a prior history of disease and a high false-positivity rate (World Health Organization (WHO) 2021b). A small proportion of samples (n = 11) were re-tested to try to obtain a positive or negative result and retesting resulted in only three samples being reported as MTB-positive, five as MTB-negative and three gave a second trace result. Our findings are thus in agreement with WHO guidelines and with studies in high burden countries, where it is expected that a variable but low proportion of trace results are confirmed culture-positive and patients should undertake further tests and examinations (World Health Organization (WHO) 2021c).

Our study has some limitations that need to be considered for the interpretation of results. We used the individual Xpert Ultra test as the reference standard. Although this is not a perfect

reference standard, as culture is the accepted reference standard which has a higher sensitivity, we decided to use Xpert Ultra test as the reference standard because sputum culture was not available in the study setting. In addition, most recent publications have used a similar approach. Essentially the comparison of pooling with single testing describes the sensitivity of pooling against single testing. The efficiency of the pooling method depends on the proportion of samples that are positive. If the proportion positive is low, few of the pools need to be re-tested, while if most pools are positive, re-testing a high proportion of the pools negates its potential advantages. We expected that only 10-15% of the samples would test positive, reflecting pre-pandemic testing patterns. However, samples collected during the epidemic, when access to health services was limited due to movement restrictions and laboratories were under severe strain, resulted in a high proportion of samples testing positive. This unusually high proportion limited the number of cartridges that could be saved and although savings amounted to 12%, this is lower than the 30-50% savings reported from other settings and smaller pools of 2 or 3 samples per pool could have resulted in higher savings. In addition, we only analysed the agreement of Xpert tests. This approach would miss samples with culture-positive Xpert-negative samples with low bacilli concentrations. As the agreement is dependent on the sensitivity of the test, the agreement will likely vary with the proportion of pauci-bacillary samples in the study population, which may explain why the studies from Laos (Iem et al. 2022) reported a higher level of agreement. Lastly, we tested all individual samples and compared their results to pool testing, which was needed to be able to describe the performance of the test. However, this approach precluded us to evaluate the staff

acceptability of the method and real-life time savings when applied under operational conditions.

TB continues to be a major global cause of death and long-term morbidity and a high proportion of people with TB are missed by the health services (World Health Organization (WHO) 2021a). Although it is recommended that people with presumptive TB should be tested using NAA assays, implementing this recommendation at scale has been difficult because most people attend primary health care centres with limited laboratory capacity. The most widespread used NAA platform is the 4-module GeneXpert, requires uninterrupted electricity and air conditioning, which confines it to higher level laboratories and generates the need for sputum transport. Alternative battery-operated platforms such as GeneXpert EDGE, are promoted as a point of care device. Although this one-module platform processes one test at a time and its throughput is insufficient for busy primary health care clinics, the use of pooling would allow testing a higher number of patients, while local testing would reduce sputum transportation costs. Moreover, the number of Xpert cartridges procured globally is insufficient for the number of people that should be tested. Most TB diagnostic centres require testing between 5 and 10 people with presumptive TB to confirm one person with TB and therefore this would have required testing 50 and 100 million individuals to identify the 10 million people with TB reported in 2020 (World Health Organization (WHO) 2021a). Given that only 15.4 million cartridges were procured in 2020 (Cuevas et al. 2021), the global supply of cartridges is a fraction of the number needed. Clearly, there is a need to identify methods that allow testing more patients with a limited number of cartridges and pooling could play a role by increasing the efficiency of testing.

Our study adds to the increasing body of evidence that pooling of samples for Xpert Ultra testing improves the efficiency of testing, potentially enabling the screening and testing of larger numbers of people more cost-effectively. We have shown that, as in previous studies, individual and pooled testing of samples have a high level of agreement. The pooling method has the potential to increase the number of people tested for TB at the local level by both, increasing the number of people that can be tested with a limited number of cartridges and the throughput of battery-operated platforms. Moreover, these efficiencies could be achieved while reducing the cost of testing through a reduction of sample transportation and the number of cartridges required per patient. Further implementation studies are warranted to tests these approaches on large scale.

Acknowledgements

The authors would like to thank the Tuberculosis Control Program of Maceió, Central Public Health Laboratory and the Tuberculosis Control Program of the Bureau of Health of Alagoas State for authorizing and facilizing the study. We also thank Jim Read and the Global Health Trial Unit, LSTM for generating databases and data curation.

Authors' contributions

The study was conceived and designed by VSS, JC and LEC. Xpert Ultra were conducted by MFA, JER, BJP, MTPA, PMF, LCLM, VPSL and LCG. MFA and JER performed the pooling test. Data analysis and interpretation were conducted by JD, VSS, LEC and KK. The initial manuscript was prepared by VSS, LEC and JC. All authors made substantial contributions to the writing and

editing of the manuscript. LEC is responsible for the overall content and is the guarantor of the study. The final version has been read and approved by all named authors.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Data sharing

Data will be made available upon request to Prof. Luis E. Cuevas.

References

Brasil. Ministério da Saúde. Secretaria de vigilância em saúde. Boletim Epidemiológico de Tuberculose 2021 [Internet]. Ministério da Saúde. Brasília, DF: Ministério da Saúde; 2021. Available from: http://www.aids.gov.br/pt-br/pub/2021/boletim-tuberculose-2021

Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, et al. The New Xpert MTB/RIF Ultra: Improving Detection of Mycobacterium tuberculosis and Resistance to Rifampin in an Assay Suitable for Point-of-Care Testing. Nacy CA, editor. MBio [Internet]. 2017 Sep 6;8(4). Available from: https://journals.asm.org/doi/10.1128/mBio.00812-17

Chry M, Smelyanskaya M, Ky M, Codlin AJ, Cazabon D, Eang MT, et al. Can the high sensitivity of Xpert MTB/RIF Ultra be harnessed to save cartridge costs? Results from a pooled sputum evaluation in Cambodia. Trop Med Infect Dis. 2020;5(1):27.

Cuevas LE, Santos VS, Lima SVMA, Kontogianni K, Bimba JS, Iem V, et al. Systematic review of pooling sputum as an efficient method for xpert MTB/RIF tuberculosis testing during COVID-19 pandemic. Emerg Infect Dis. 2021;27(3):719–27.

Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid Detection of Mycobacterium tuberculosis and Rifampin Resistance by Use of On-Demand, Near-Patient Technology. J Clin Microbiol [Internet]. 2010 Jan;48(1):229–37. Available from: https://journals.asm.org/doi/10.1128/JCM.01463-09

Iem V, Chittamany P, Suthepmany S, Siphanthong S, Siphanthong P, Somphavong S, et al. Pooled testing of sputum with Xpert MTB/RIF and Xpert Ultra during tuberculosis active case finding campaigns in Lao People's Democratic Republic. BMJ Glob Heal. 2022;7(2):e007592.

Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159–74.

Lohse S, Pfuhl T, Berkó-Göttel B, Gärtner B, Becker SL, Schneitler S, et al. Challenges and issues of SARS-CoV-2 pool testing – Authors' reply. Lancet Infect Dis [Internet]. 2020a Nov;20(11):1234–5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1473309920304552

Lohse S, Pfuhl T, Berkó-Göttel B, Rissland J, Geißler T, Gärtner B, et al. Pooling of samples for testing for SARS-CoV-2 in asymptomatic people. Lancet Infect Dis [Internet]. 2020b Apr 28; Available from: http://www.ncbi.nlm.nih.gov/pubmed/32530425

Stop TB Partnership. Global Drug Facility Product Catalog. Stop TB Partnership. 2022a.

Stop TB Partnership. Tuberculosis situation in 2020. Brazil TB Dashboard [Internet]. Stop TB Partnership. 2022b [cited 2022 Mar 29]. Available from: https://www.stoptb.org/static_pages/BRA_Dashboard.html

Treatment Action Group. Tuberculosis diagnostics pipeline report 2020 [Internet]. New York:

Treatment Action Group; 2020. Available from: https://www.treatmentactiongroup.org/wp-content/uploads/2020/10/pipeline_TB_Diagnostics_2020_final.pdf

World Health Organization (WHO). Global tuberculosis report 2021. Geneva: World Health Organization. 2021a.

World Health Organization (WHO). WHO operational handbook on tuberculosis. Module 3: Diagnosis - Rapid diagnostics for tuberculosis detection 2021 update [Internet]. Module 3: Diagnosis Rapid diagnotics for tuberculosis diagnosis. Geneva: World Health Organization; 2021b. Available from: https://apps.who.int/iris/bitstream/handle/10665/340256/9789240022614-eng.pdf

World Health Organization (WHO). WHO operational handbook on tuberculosis. Module 3: Diagnosis - Rapid diagnostics for tuberculosis detection 2021 update. Module 3: Diagnosis Rapid diagnotics for tuberculosis diagnosis. Geneva: World Health Organization; 2021c.

Table 1. Characteristics of study participants

Variables	**All participants N (%)**	**Xpert Ultra positive N (%)**
Sex	**396**	**95**
Male	252 (63.6)*	76 (19.1)*
Female	144 (36.4)*	19 (13.2)*
Age	**373**	**88**
Mean (SD)	49 (16.9)	42 (15.7)
<35	89 (22.5)	33 (34.7)
35-54	132 (33.3)	30 (31.6)
>=55	152 (38.4)	25 (26.3)
Sputum quality	**396**	**95**

Saliva	15 (3.8)	2 (2.1)
Mucoid	227 (57.3)	48 (50.5)
Mucopurulent	90 (22.7)	25 (26.3)
Purulent	64 (16.2)	20 (21.1)
Sputum blood	**396**	**95**
Yes	11 (2.8)	0 (0.0)
No	385 (97.2)	95 (100.0)

* Row percentage

Table 2. Laboratory results of individual and pooled Xpert Ultra tests.

	Individual	**Pooled**
Xpert MTB Result	**396**	**99**
Detected	95 (24.0)	62 (62.6)
Trace	20 (5.0)*	6 (6.1)
Not detected	280 (70.7)	31 (31.3)
Invalid	0 (0.0)	0 (0.0)
Error	1 (0.3)	0 (0.0)
No result	0 (0.0)	0 (0.0)
MTB Grade	**115**	**68**
Trace	20 (18.1%)	6 (8.8)
Very low	8 (6.8%)	4 (5.9)
Low	17 (14.7%)	13 (19.1)
Medium	11 (9.5%)	9 (13.2)
High	59 (50.9%)	36 (52.9)
Rif Resistance	**95****	**62**
Detected	2 (2.1)	1 (1.6)
Not detected	93 (97.9)	61 (98.4)
Indeterminate	0 (0.0)	0 (0.0)

*** After retesting.** Sputum samples with MTB 'trace' considered 'not detected' for the agreement analysis.

** Note none of 20 individual samples or 6 pooled samples with MTB trace results were RIF positive.

Table 3. Agreement of individual and pooled Ultra tests

	Pooled N = 99	
Individual	**Negative**	**Positive**
All four negative*	34 (34.3)	1 (1.0)
At least one positive	3 (3.0)	61 (61.6)

Agreement	95/99 (96.0%)
Kappa	0.913
Sensitivity (95% CI)	0.953 (0.869, 0.990)
Specificity (95% CI)	0.971 (0.851, 0.999)

*MTB trace classed as negative

Table 4. Number of pools with 0, 1, 2 and 3 positive individual results.

	Number of Individual Xpert Ultra-positive samples included in the pools N (%)*				
Pooled Xpert Ultra	**All negative**	**One positive**	**Two positive**	**Three positive**	**All**
	35	**38**	**21**	**5**	**99**
Detected	1 (2.9%)	35 (92.1%)	21 (100.0%)	5 (100.0%)	62 (62.6%)
Not detected	34 (97.1%)	3 (7.9%)	0 (0.0%)	0 (0.0%)	37 (37.4%)

* None of the pools contained 4-Ultra positive samples

Table 5. Individual and pooled Xpert MTB grades (includes pools with only one positive sample)

	Individual Xpert MTB grade included in pool				
	Very low n (%)	**Low n (%)**	**Medium n (%)**	**High n (%)**	**All n (%)**
Pooled Xpert Ultra	**4**	**8**	**6**	**20**	**38**
Not detected	1 (25.0%)	0 (0.0%)	2 (33.3%)	0 (0.0%)	3 (7.9%)
Very low	0 (0.0%)	3 (37.5%)	0 (0.0%)	0 (0.0%)	3 (7.9%)
Low	3 (75.0%)	3 (37.5%)	3 (50.0%)	2 (10.0%)	11 (28.9%)
Medium	0 (0.0%)	2 (25.0%)	1 (16.7%)	2 (10.0%)	5 (13.2%)
High	0 (0.0%)	0 (0.0%)	0 (0.0%)	16 (80.0%)	16 (42.1%)