BMJ Global Health

Pooled testing of sputum with Xpert MTB/RIF and Xpert Ultra during tuberculosis active case finding campaigns in Lao People's Democratic Republic

Vibol Iem ,[1,2] Phonenaly Chittamany,[2] Sakhone Suthepmany,[2] Souvimone Siphanthong,[2] Phitsada Siphanthong,[2] Silaphet Somphavong,[3] Konstantina Kontogianni,[1] James Dodd,[1] Jahangir AM Khan,[4] Jose Dominguez,[5] Tom Wingfield,[6,7] Jacob Creswell,[8] Luis E Cuevas [9]

To cite: Iem V, Chittamany P, Suthepmany 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 Global Health* 2022;**7**:e007592. doi:10.1136/bmjgh-2021-007592

Handling editor Alberto L Garcia-Basteiro

JC and LEC are joint senior authors.

Received 2 October 2021
Accepted 1 December 2021

For numbered affiliations see end of article.

Correspondence to
Professor Luis E Cuevas; luis.cuevas@lstmed.ac.uk

ABSTRACT

Introduction Active case finding (ACF) of individuals with tuberculosis (TB) is a key intervention to find the 30% of people missed every year. However, ACF requires screening large numbers of individuals who have a low probability of positive results, typically <5%, which makes using the recommended molecular tests expensive.

Methods We conducted two ACF surveys (in 2020 and 2021) in high TB burden areas of Lao PDR. Participants were screened for TB symptoms and received a chest X-ray. Sputum samples of four consecutive individuals were pooled and tested with Xpert Mycobacterium tuberculosis (MTB)/rifampicin (RIF) (Xpert-MTB/RIF) (2020) or Xpert-Ultra (2021). The agreement of the individual and pooled samples was compared and the reasons for discrepant results and potential cartridge savings were assessed.

Results Each survey included 436 participants, which were tested in 109 pools. In the Xpert-MTB/RIF survey, 25 (sensitivity 89%, 95% CI 72.8% to 96.3%) of 28 pools containing MTB-positive samples tested positive and 81 pools containing only MTB-negative samples tested negative (specificity 100%, 95% CI 95.5% to 100%). In the Xpert-Ultra survey, all 32 (sensitivity 100%, 95% CI 89.3% to 100%) pools containing MTB-positive samples tested positive and all 77 (specificity 100%, 95% CI 95.3% to 100%) containing only MTB-negative samples tested negative. Pooling with Xpert-MTB/RIF and Xpert-Ultra saved 52% and 46% (227/436 and 199/436, respectively) of cartridge costs alone.

Conclusion Testing single and pooled specimens had a high level of agreement, with complete concordance when using Xpert-Ultra. Pooling samples could generate significant cartridge savings during ACF campaigns.

Key questions

What is already known?

- Our study adds to the emerging body of evidence that the pooling methods for testing with molecular assays can improve the efficiency of testing for tuberculosis (TB), potentially enabling the screening and testing of larger numbers of people more cost-effectively.

What are the new findings?

- These findings contribute to recognised gaps in funding sources for the procurement of sufficient cartridges for testing all individuals with presumptive TB, which jeopardises access to high sensitivity WHO-recommended rapid molecular diagnostic tests, such as the GeneXpert Xpert MTB/RIF and Xpert-Ultra.

What do the new findings imply?

- The method has not been tested during a real health crisis situation, such as the COVID-19 pandemic. The study took place at a time laboratory resources were being diverted and healthcare workers were repurposed for SARS-CoV2 testing. The higher efficiency of the pooling method can contribute to cope with these challenging times.

INTRODUCTION

Despite being treatable and curable, tuberculosis (TB) remains one of the main infectious killers in the world, as ten million people fall ill and 1.4million die from the disease each year.[1] Its diagnosis is usually reliant on passive case finding (PCF), in which health services wait for individuals with symptoms of TB to attend a health facility to initiate the diagnostic process. Although PCF identifies most people with TB in locations with adequate access to health services, it misses those unwilling or unable to attend the clinics and is a major reason only seven of the ten million people with TB are diagnosed and notified.[2] Individuals missed by passive approaches often include vulnerable populations such as internally displaced, migrant or rural populations, women, the unemployed and ethnic minorities,[3 4] who may face multiple societal and economic barriers to attend the service, including catastrophic costs.[5 6] It is,

thus, recognised that, to be inclusive and reduce the socioeconomic impact of TB,[7] health services need to include active case finding (ACF) approaches that involve proactive interventions to extend the reach of TB services for diagnosis[8] and treatment.[9] Although ACF interventions can be very effective,[10 11] they are less standardised than PCF, as they address the specific barriers of multiple target populations and are more resource and time intensive than PCF.[12]

The WHO recommends testing all individuals with presumptive TB with molecular assays, such as the Xpert Mycobacterium tuberculosis/rifampicin (Xpert MTB/RIF) and Xpert-Ultra (Cepheid Sunnyvale, California),[13] with the latter being preferred given its higher sensitivity.[14] Although the use of these assays is expanding, the assay cartridge unit costs of US$ 9.98 per test[15] remains one of the main hurdles for its wider implementation in low-income and middle-income countries. Diagnostic test costs can limit the expansion of ACF activities, as they require testing large numbers of individuals with relatively lower yields than PCF.[16]

Since 2015, the Lao National Tuberculosis Control Center (NTC) has conducted ACF by implementing intensified case finding activities to increase the detection of individuals with TB in high burden districts of the country. These activities include the sensitisation of the population, the local provision of chest X-rays for screening (independently of symptoms) and the identification of individuals with symptoms of TB who have not attended health facilities. Participants with abnormal chest X-rays or symptoms of TB are tested using Xpert MTB/RIF or Xpert-Ultra.[17] The activities have increased case detection, although the cost of the Xpert cartridges is considered high and is the main limiting factor to implement the intervention on a larger scale.

One approach that could increase the affordability of Xpert testing is to test several samples together using the pooling method.[18] This procedure combines (or pools) the sputum of several individuals into one pot and tests them together with a single test. If the test is positive, the pool's samples are retested individually to identify the positive sample(s) while if the test is negative, all samples in the pool are considered negative, resulting in 30%–40% savings in Xpert cartridge costs alone depending on the prevalence of TB in the population tested.[19] Therefore, pooling may hold great promise for ACF, but there are few reports of its performance under operational conditions.[20]

Here, we report a prospective study to assess the sensitivity and specificity of the pooling method using Xpert MTB/RIF and Xpert-Ultra during intensified case finding interventions, and its potential to increase the affordability of Xpert testing in Lao PDR.

METHODS

We conducted two independent prospective surveys embedded within the ACF activities of Lao's NTC, from March to April 2020 and from January to March 2021. Both surveys were cross-sectional and used the same recruitment and testing procedures. The 2020 survey aimed to assess the performance of the pooling method when testing samples with Xpert MTB/RIF, while the 2021 survey assessed the method when using Xpert-Ultra, after its release for routine use by Lao's NTC.

ACF was conducted in Lao's high TB burden areas, which are programmatically defined as TB incidence ≥100 cases per 100 000 population. The 2020 survey was conducted in Vientiane Capital, Luang Prabang and Savannakhet provinces with estimated populations of 890,129, 468 375 and 1 051 675 inhabitants, respectively, and TB notification rate of 134, 88 and 102 cases per 100 000 population in 2020, respectively. The 2021 survey was conducted in Saravane and Oudomxay, with 430 428 and 333 934 population and TB notification rates of 127 and 110 cases per 100 000 population in 2020, respectively.

Both surveys were conducted in the same fashion. Before an ACF activity, the NTC team met the province and district health authorities and conducted preparation visits with the provincial TB coordinator, district TB manager and village authorities, distributed health education materials, obtained the addresses of individuals with TB and line listed household contacts. At an agreed date, the NTC team set up a digital chest X-ray machine and a four-module GeneXpert platform in the village and invited all residents to complete a questionnaire on signs and symptoms, history and treatment of TB and offered chest X-rays for screening, independently of the presence of symptoms. Individuals with abnormal chest X-rays and those who indicated having cough >2 weeks duration were asked to provide sputum samples for Xpert testing and were managed according to the decision tree shown in figure 1. Sputum samples were tested with Xpert following the manufacturer's instructions.[21]

Sputum samples tested individually with Xpert MTB/RIF or Xpert-Ultra were processed in the village GeneXpert platform. Consecutive samples with remnant volumes ≥0.5 mL were included in the pooling studies and were transported to the National TB Reference Laboratory in Vientiane using a cold chain. Samples were transported after the sample reagent had been added. Turned around time to testing was <48 hours after the sample reagents had been added and samples were maintained in a cold chain at all times. Sputum samples from four participants were pooled together, with a volume of 0.5 mL of sputum each added to a pot, to obtain an aggregated volume of 2 mL.[21] Samples for a pool were selected consecutively and staff were blind to the individual Xpert test results and the pooled specimen was tested using one new Xpert cartridge.

Statistical analysis

Categorical data were summarised using descriptive statistics and χ^2 tests were used to test for statistically significant differences. Individuals unable to produce sputum were excluded from the analysis. The pooled samples were compared with the four Xpert MTB/RIF and Xpert-Ultra individual results and their agreement was tested using

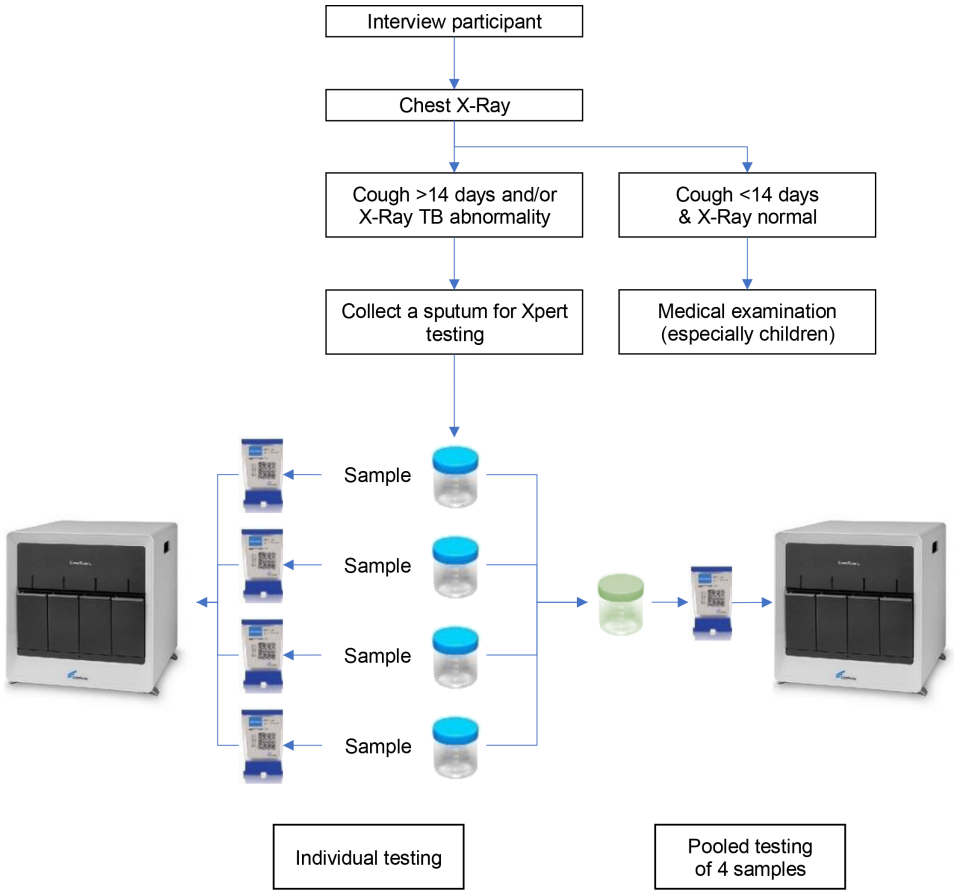

Figure 1 Flow diagram of the sputum processing.

kappa statistics. The CT values and grades (*trace, very low, low, medium* and *high*) of individual and pooled tests were compared with describe the effect of combining the samples. Cost differences were calculated on the bases of the number of cartridges required to test all specimens using pooled and individual testing.

Sample size for the surveys was not formally estimated as we were limited by the expected number of participants attending the campaigns before the COVID-19 lockdown, the capacity of staff to conduct additional testing to their routine activities and the number of spare cartridges available for research purposes.

Patient and public involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting or dissemination plans of our research.

RESULTS

The 2020 survey included 436 participants, 334 (76.6%) men and 102 (23.4%) women, and 29 (6.7%, 95% CI 4.7% to 9.4%) were Xpert MTB/RIF MTB-positive. The 2021 survey also included 436 participants, 222 (50.9%) men and 214 (49.1%) women, and 37 (8.5%, 95% CI 6.5% to 11.5%) were Xpert-Ultra MTB-positive (p value >0.1, table 1). Men were more likely to be MTB-positive than women in 2020 (26/334 (7.8%) men vs 3/102 (2.9%) women, respectively, p=0.014); but women were more likely to be MTB-positive than men in 2021 (12/222 (5.4%) vs 12/214 (11.7%), respectively, p<0.008). Each survey included 109 pools of four patients.

Xpert MTB/RIF survey

In 2020, 28 (25.7%) pools contained one or more Xpert MTB/RIF MTB-positive sample (27 pools with one and one pool with two MTB-positive samples) and 81 (74.3%) pools contained solely MTB-negative samples (table 2). The pool with two MTB-positive and 24 of 27 pools with one MTB-positive sample tested MTB-positive and three tested MTB-negative, resulting in a sensitivity of 89% (25/28, 95% CI 72.8% to 96.3%). All 81 pools containing solely MTB-negative samples tested MTB-negative in the pooled assay (specificity 100%, 95% CI 95.5% to 100%). Therefore, the accuracy performance of the 109 pools in correlation to the 436 individual results resulted in 97.3% agreement (kappa: 0.925). Among the 27 pools containing single MTB-positive samples, five contained *very low*, 15 *low*, 6 *medium* and one *high* MTB grades. The pooled MTB grade was similar to the individual test in four (14.8%), one grade lower in 21 (77.8%), two grades lower in one (3.7 %) and one grade higher in one (3.7%) of the pools. Of the five pools containing *very low* individual MTB-grades, three tested MTB-not detected and two *very low* MTB-grade in the pooled assay (table 3).

Table 1 Baseline characteristics of participants and Xpert MTB/RIF and Xpert Ultra results

	Xpert MTB/RIF		Xpert Ultra	
	Individual n (%)	Pool n (%)	Individual n (%)	Pool n (%)
Sex	**436**	–	**436**	–
Male	334 (76.6)	–	222 (50.9)	–
Female	102 (23.4)	–	214 (49.1)	–
Age	**436**	–	**436**	–
Mean (SD) (range)	45 (16.1) (12-89)	–	54 (13.7) (10-90)	–
<35	131 (30.0)	–	41 (9.4)	–
35–54	184 (42.2)	–	159 (36.5)	–
≥55	121 (27.8)	–	236 (54.1)	–
Xpert MTB result	**436**	**109**	**436**	**109**
Detected/≥1 MTB included	29 (6.7)	25/28 (22.9)	37 (8.5)	32/32 (29.4)
Not detected/≥1 MTB included	–	3 (2.7)	–	–
Not detected/only MTB-negative	407 (93.3)	81 (74.3)	399 (91.5)	77 (70.6)
Xpert MTB result by sex				
Male	26/334 (7.8)	–	12/222 (5.4)	–
Female	3/102 (2.9)	–	25/214 (11.7)	–
MTB grade	**29**	**25**	**37**	**32**
Trace	NA	NA	6 (16.2)	21 (65.6)
Very low	6 (20.7)	15 (60.0)	8 (21.6)	11 (34.4)
Low	16 (55.2)	9 (37.5)	15 (40.5)	0 (0.0)
Medium	6 (20.7)	1 (4.2)	2 (5.4)	0 (0.0)
High	1 (3.4)	0 (0.0)	6 (16.2)	0 (0.0)
RIF resistance	**29**	**25**	**37**	**32**
Detected	0	0	0	0
Not detected	27 (93.1)	24 (96.0)	30 (81.1)	10 (31.2)
Indeterminate	2 (6.9)	1 (4.0)	7 (18.9)	22 (68.8)

Bold figures are frequencies and do not indicte statistical significance.
Xpert MTB/RIF, Xpert Mycobacterium tuberculosis/rifampicin.

The CT values for the Xpert MTB/RIF probes for both individual and pooled testing are shown in table 4. The median CT values for probes A-E ranged from 23.4 to 24.8 for the individual tests and from 30.6 to 33.6 for the pooled tests, with an increase in CT values ranging from 5.4 to 7.1.

Two of the MTB-positive samples were RIF-indeterminate and 27 RIF-negative. Of the 28 pools with

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

	Individual Xpert results included in a pool					
	All negative n (%)	One positive n (%)	Two positive n (%)	Three positive n (%)	Four positive n (%)	All
Pooled Xpert MTB/RIF	**81**	**27**	**1**	**0**	**0**	**109**
Detected	0	24 (89%)	1 (100%)	0	0	25 (23%)
Not detected	81 (100%)	3 (11%)	0	0	0	84 (77%)
Pooled Xpert Ultra	**77**	**27**	**5**	**0**	**0**	**109**
Detected	0	27 (100%)	5 (100%)	0	0	32 (29%)
Not detected	77 (100%)	0	0	0	0	77 (71%)

Bold figures are frequencies and do not indicte statistical significance.
Xpert MTB/RIF, Xpert Mycobacterium tuberculosis/rifampicin.

Table 3 Correlation of Individual and pooled Xpert MTB grades (positive pools only include those with only one positive Xpert)

	Individual Xpert grade included in pool					
	Not detected n (%)	**Trace n (%)**	**Very low n (%)**	**Low n (%)**	**Medium n (%)**	**High n (%)**
Pooled Xpert MTB/RIF	**81**	**NA**	**5**	**15**	**6**	**1**
Not detected	81 (100%)	NA	**3 (60%)**	0	0	0
Very low	0	NA	2 (40%)	**12 (80%)**	0	0
Low	0	NA	0	2 (13.3%)	**6 (100%)**	**1 (100%)**
Medium	0	NA	0	**1 (6.7%)**	0	0
High	0	NA	0	0	0	0
Pooled Xpert Ultra	**77**	**2**	**5**	**13**	**1**	**6**
Not detected	77 (100 %)	0	0	0	0	0
Trace	0	2 (100%)	**4 (80%)**	**7 (54%)**	**1 (100%)**	**2 (33%)**
Very low	0	0	1 (20%)	**6 (46%)**	0	**4 (67%)**
Low	0	0	0	0	0	0
Medium	0	0	0	0	0	0
High	0	0	0	0	0	0

Bold values are frequencies and do not indicte statistical significance.
Xpert MTB/RIF, Xpert Mycobacterium tuberculosis/rifampicin.

MTB-positive samples, 25 pools contained one MTB-positive RIF-negative sample, one had two MTB-positive RIF-negative samples and two had one MTB-positive RIF-indeterminate samples. Of the 25 MTB-positive RIF-negative pools, three tested MTB-negative and did not report RIF results and 22 tested RIF-negative. The pool containing two MTB-positive RIF-negative samples tested RIF-negative and the two pools containing RIF-indeterminate samples tested RIF-negative in one and RIF-indeterminate in the other.

Xpert-Ultra survey

In 2021, 32 (29.4%) pools contained MTB-positive samples and 77 (70.6%) solely MTB-negative samples. Twenty-seven of the 32 MTB-positive pools contained one and five contained two MTB-positive samples and all tested positive in the pooled assay (sensitivity 100%, 95% CI 89.3% to 100%). All 77 pools containing only MTB-negative samples tested MTB-negative (specificity 100%, 95% CI 95.3% to 100%), resulting in 100% agreement (Kappa: 1). Among the 27

Table 4 Median CT values of individual and pooled Xpert MTB/RIF and Xpert Ultra probe results

Xpert MTB RIF					
	Individual results n=29		**Pooled results n=25**		
Probe	**CT median IQ range**	**Min–max**	**CT median IQ range**	**Min–max**	**ΔCT**
Probe D	24.6 (22.7–27.3)	19.1–35.2	32.3 (28.5–34.2)	21.0–38.2	7.1
Probe C	23.7 (22.0–26.7)	18.9–34.7	30.3 (27.4–31.8)	20.0–35.7	6.5
Probe E	24.8 (23.0–28.1)	20.5–36.2	33.9 (29.3–34.9)	21.2–39.3	7.1
Probe B	24.6 (22.9–27.3)	20.1–33.8	30.1 (27.3–32.5)	20.9–34.7	5.4
Probe A	23.3 (22.0–26.0)	20.5–34.1	31.1 (27.1–32.9)	19.7–34.6	6.6
Xpert Ultra					
	Individual results n=37		**Pooled results n=32**		
Probe	**CT median IQ range**	**Min–max**	**CT median IQ range**	**Min–max**	**ΔCT**
Probe IS1081	19.6 (17.1–22.6)	16.0–32.0	24.9 (22.2–26.5)	19.9–29.3	4.6
Probe rpoB1	21.6 (17.9–24.9)	0–32.0	0 (0–30.2)	0–34.9	NA
Probe rpoB2	21.3 (17.9–25.8)	0–32.1	0 (0–29.8)	0–35.3	NA
Probe rpoB3	23.3 (19.2–27.0)	0–33.7	0 (0–32.9)	0–39.8	NA
Probe rpoB4	25.7 (21.2–29.7)	0–35.7	0 (0–33.5b)	0–37.7	NA

Xpert MTB/RIF, Xpert Mycobacterium tuberculosis/rifampicin.

Table 5 Costs and savings to screen consecutive patients using the pooling method and number of patients that could be tested with Xpert MTB/RIF and Xpert Ultra cartridges

	Individual Xpert		Pooled Xpert	
	MTB/RIF	Ultra	MTB/RIF	Ultra
Number of individuals tested	436	436	436	436
Sensitivity	Reference	Reference	89%*	100%*
Specificity	Reference	Reference	100%*	100%*
Proportion positive	6.7%	8.5%	22.9%	29.4%
Bacteriologically confirmed	29	37	26	37
Cartridges required	436	436	209	237
Cartridge costs (USD)	4351.28	4351.28	2085.82	2365.26
Cartridge savings (USD)	NA	NA	2265.46(52%)	1986.02(46%)
Numbers tested with 436 cartridges				
Number tested	436	436	909	802
Cartridge cost per patient (USD)	9.98	9.98	4.78	5.42

*Assumes pools of 1:4; proportion positive taken from the surveys' findings.

pools with single MTB-positive samples, two contained *trace*, 5 *very low*, 13 *low*, 1 *medium* and 6 *high* MTB grades. The pooled MTB grades were the same as the individual grades in three (11%), one grade lower in 10 (42%), two grades lower in seven (29%), three grades lower in five (21%) and four grades lower in two (8%) of the pooled assays (table 3). The Xpert-Ultra probes CT values are shown in . Probe IS1081/IS6110 had median CT of 19.6 for individual and 24.9 for pooled results, with a median increase of 4.6. Probes rpoB1-B4 median CT values ranging from 19.6 to 25.7 for the individual tests, but CT values were not available for the pools. Among the 37 MTB-positive samples, 30 (81%) were RIF-negative and 7 (18.9%) were RIF-indeterminate and were distributed in 32 pools. Twenty-five of the 32 pools contained only RIF-negative and 7 contained RIF-indeterminate samples. Fifteen of the 25 pools containing only RIF-negative samples tested RIF-indeterminate and 10 RIF-negative, while all seven pools containing RIF-indeterminate samples tested pooled RIF-indeterminate.

Xpert MTB/RIF and Xpert Ultra costs

The cartridges cost for testing individually the 436 participants with Xpert at US$9.98 per test was US$4351.28 for each survey, as shown in table 5. The pooling method in 2020 required 109 Xpert MTB/RIF cartridges to test 109 pools and 100 cartridges to test individual samples of 25 MTB-positive pools. The total of 209 (109+100) cartridges for pool testing would cost US$2085.82, resulting in US$2265.46 (52%) saving in cartridge costs. Similarly, testing 109 pools with Xpert-Ultra in 2021 required 109 cartridges to test the pools and 128 cartridges to test individually the 32 positive pools. The total of 237 cartridges would cost US$2365.26, resulting in US$1986.02 (46%) savings in cartridge costs. If the number of cartridges is kept fixed, the pooling method could test more patients than testing samples individually, as 436 cartridges would allow testing 909 and 802 individuals with Xpert MTB/RIF and Xpert-Ultra, respectively—an effective test per patient cost of US$4.78.and 5.42, respectively (table 5).

DISCUSSION

Our surveys compared pooling with single testing during ACF for TB in a low-income country. Our results confirm that testing individual and pooled samples with the GeneXpert platform can achieve a high level of concordance. Concordance was higher with Xpert-Ultra than with Xpert MTB/RIF, which is in agreement to regional studies evaluating pooling with Xpert-Ultra in Cambodia[20] and Vientiane, Lao PDR (Iem *et al*, in press). Discrepancies between individual and pooled Xpert MTB/RIF tests only occurred among pauci-bacillary samples with high Xpert CT values, suggesting that some samples with low DNA concentrations fall below the assay's limit of detection and that the better agreement of Xpert-Ultra is due to its higher sensitivity. Consequently, some patients with paucibacillary disease could be missed by pooling, especially if testing is based on Xpert MTB/RIF.

The pooling strategy can lead to significant cost savings and facilitate testing of more individuals for a given number of cartridges. In our setting, pooling samples would double the number of people tested with the same number of cartridges. This is higher than in PCF studies, where pooling is reported to save up to 40% of cartridges.[19] Cartridge savings are a function of the proportion of people with MTB-positive results and their distribution within the pools. If the proportion positive is low, a low number of pools would need to be retested, resulting in higher cartridge savings. For example, in a survey in Lao's district clinics, 12% of individuals tested Xpert-positive, and pooling resulted in 38.3% and 41.7% cartridge saving costs with Xpert MTB/RIF and Xpert-Ultra, respectively (Iem *et al*, in press), while in our survey setting, the proportion of positives was 8.5%, which led

to higher savings. The proportion of participants with positive tests in ACF is often lower than reported from studies using PCF, typically below 5% depending on the target population,[16 22] and lower to 10%–20% of individuals attending TB clinics in PCF.[23 24] We have, thus, shown that pooling could be highly efficient when testing populations using ACF, and further studies among such populations are warranted. Since the pooling method is a laboratory change, it would not affect the screening algorithm and can be easily instituted without any major modifications.

Previous systematic reviews have highlighted that individual and pooled RIF results are often discordant, with pools containing RIF-negative samples often returning RIF-indeterminate pooled results,[19] and our findings are in agreement with these observations. Although samples with pooled RIF results would be routinely confirmed at the time of retesting, the samples of a positive pool to identify the individual MTB-positive samples, it is important to highlight that pooled RIF results are unreliable and should not be used for clinical management.

Further studies could explore ways to further improve the efficiency of pooling when combined with other screening tools, such as C-reactive protein (CRP)[25] and digital chest X-rays with Computer-aided diagnosis (CAD).[26 27] Both tools can identify individuals with and without the traditional symptoms of TB, although their relatively lower specificity requires confirming the diagnosis with more specific molecular assays. Although using tests combinations could increase assay costs, individuals with a positive CRP or abnormal chest X-rays CAD could be confirmed using the pooling method, and its efficiency gains could increase the affordability of tests combinations.

In conclusion, we have shown pooling samples for TB diagnoses during ACF campaigns, which can replicate testing samples with individual tests. The approach can facilitate testing higher numbers of patients with lower cartridge costs, increasing the affordability of testing with molecular assays. The high level of agreement between individual and pooled samples obtained with Xpert-Ultra demonstrates that pooling can be reliable and contribute to achieve the WHO End TB strategy targets in resource-limited settings.

Author affiliations
[1]Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK
[2]National Tuberculosis Control Center, XJ7F+P5F, Vientiane, Lao People's Democratic Republic
[3]Center of Infectology Lao Christophe Merieux, Vientiane, Lao People's Democratic Republic
[4]Health Economics and Policy Unit, School of Public Health and Community Medicine, University of Gothenburg, Gothenburg, Sweden
[5]Institut d'Investigació Germans Trias i Pujol, CIBER Enfermedades Respiratorias, and Universitat Autònoma de Barcelona, Barcelona, Spain
[6]Department of International Public Health and Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK
[7]Department of Global Public Health, WHO Collaborating Centre for Tuberculosis and Social Medicine, Karolinska Institutet, Stockholm, Sweden
[8]Stop TB Partnership, Geneva, Switzerland
[9]Clinical Sciences and Recsearch Centre for Drugs and Diagnostics, Liverpool School of Tropical Medicine, Liverpool, UK

Twitter Jacob Creswell @Jacob_Creswell

Acknowledgements The authors would like to thank the National Tuberculosis Control Center (NTC) of Lao PDR for authorising the study and facilitating samples transportation from the field until the National TB Reference Laboratory (NTRL) in Vientiane. We are also grateful to the local health authorities that allowed access to the study sites and for their coordination locally. We are thankful to the active case finding team for their engagement, screening and enrolling patients, collecting, and shipping samples from the collection sites and to laboratory technicians of the NTRL for their dedication to carefully perform these additional laboratory procedures at the time of the COVID-19 pandemic. We also thank Jim Read and the Global Health Trial Unit, LSTM for generating databases and data curation.

Contributors The study was designed by LEC, JC, VI, KK and JAMK. Patients screening and enrolling, individual sample testing on Xpert MTB/RIF and Xpert Ultra were conducted by PC, SaS, SoS, PS, and SiS in the field during the ACF campaign. VI performed the pooled testing. Data collection and analysis were conducted by VI, KK, LEC, JD and JC. VI, JD, TW, JC and LEC wrote the first draft of the manuscript. 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.

Funding This research was funded in part by a TB REACH, Stop TB Partnership grant supported by Global Affairs Canada (STBP/TBREACH/GSA/2020-04, awarded to LEC), the Global Fund to Fight AIDS, Tuberculosis and Malaria (LAO-T-GFMOH grant) and the National Tuberculosis Control Program budget from the Ministry of Health of Lao PDR. TW is supported by grants from: the Wellcome Trust, UK (209075/Z/17/Z); the Medical Research Council, Department for International Development, and Wellcome Trust, UK (Joint Global Health Trials, MR/V004832/1), the Medical Research Council, UK (MR/V028618/1); the Academy of Medical Sciences, UK; and the Swedish Health Research Council, Sweden.

Competing interests The authors have no conflicts of interest to declare.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the Lao NTC and the Liverpool School of Tropical Medicine Research Ethics Committee, UK (Ethical waiver 20–037), and informed consent waiver was obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Not applicable.

ORCID iDs
Vibol Iem http://orcid.org/0000-0001-6155-1402
Luis E Cuevas http://orcid.org/0000-0002-6581-0587

REFERENCES

1 World Health Organization. Global tuberculosis report; 2020. 2020.
2 Shewade HD, Gupta V, Satyanarayana S, *et al*. Patient characteristics, health seeking and delays among new sputum smear positive TB patients identified through active case finding when compared to passive case finding in India. *PLoS One* 2019;14:e0213345.
3 World Health Organization. Who consolidated guidelines on tuberculosis. *Module 2: screening – systematic screening for tuberculosis disease* 2021.
4 de Vries SG, Cremers AL, Heuvelings CC, *et al*. Barriers and facilitators to the uptake of tuberculosis diagnostic and treatment services by hard-to-reach populations in countries of low and medium tuberculosis incidence: a systematic review of qualitative literature. *Lancet Infect Dis* 2017;17:e128–43. Electronic).

5 Wingfield T, Boccia D, Tovar M, *et al*. Defining catastrophic costs and comparing their importance for adverse tuberculosis outcome with multi-drug resistance: a prospective cohort study, Peru. *PLoS Med* 2014;11:e1001675.
6 Chittamany P, Yamanaka T, Suthepmany S, *et al*. First national tuberculosis patient cost survey in Lao people's Democratic Republic: assessment of the financial burden faced by TB-affected households and the comparisons by drug-resistance and HIV status. *PLoS One* 2020;15:e0241862.
7 Gurung SC, Dixit K, Rai B, *et al*. The role of active case finding in reducing patient incurred catastrophic costs for tuberculosis in Nepal. *Infect Dis Poverty* 2019;8:99.
8 Ho J, Fox GJ, Marais BJ. Passive case finding for tuberculosis is not enough. *Int J Mycobacteriol* 2016;5:374–8.
9 Chan G, Triasih R, Nababan B, *et al*. Adapting active case-finding for TB during the COVID-19 pandemic in Yogyakarta, Indonesia. *Public Health Action* 2021;11:41–9.
10 Mhimbira FA, Cuevas LE, Dacombe R. Interventions to increase tuberculosis case detection at primary healthcare or community-level services. *Cochrane Database of Systematic Reviews* 2017;11.
11 Arshad A, Salam RA, Lassi ZS, *et al*. Community based interventions for the prevention and control of tuberculosis. *Infect Dis Poverty* 2014;3:27.
12 Datiko DG, Yassin MA, Theobald SJ, *et al*. Health extension workers improve tuberculosis case finding and treatment outcome in Ethiopia: a large-scale implementation study. *BMJ Glob Health* 2017;2:e000390–7908.
13 Chakravorty S, Simmons AM, Rowneki M, *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. *mBio* 2017;8. doi:10.1128/mBio.00812-17. [Epub ahead of print: 29 08 2017].
14 World Health Organization. Who meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF ultra compared to Xpert MTB/RIF 2017.
15 Foundation for Innovative New Diagnostics. Genexpert negotiated prices 2021.
16 Gurung SC, Dixit K, Rai B, *et al*. Comparative yield of tuberculosis during active case finding using GeneXpert or smear microscopy for diagnostic testing in Nepal: a cross-sectional study. *Trop Med Infect Dis* 2021;6. doi:10.3390/tropicalmed6020050. [Epub ahead of print: 14 Apr 2021].
17 Dorman SE, Schumacher SG, Alland D, *et al*. Xpert MTB/RIF ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. *Lancet Infect Dis* 2018;18:76–84.
18 Abdurrahman ST, Mbanaso O, Lawson L, *et al*. Testing pooled sputum with Xpert MTB/RIF for diagnosis of pulmonary tuberculosis to increase affordability in low-income countries. *J Clin Microbiol* 2015;53:2502–8.
19 Cuevas LE, Santos VS, Lima SVMA, *et al*. Systematic review of pooling sputum as an efficient method for Xpert MTB/RIF tuberculosis testing during the COVID-19 pandemic. *Emerg Infect Dis* 2021;27:719–27.
20 Chry M, Smelyanskaya M, Ky M, *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. doi:10.3390/tropicalmed5010027. [Epub ahead of print: 15 Feb 2020].
21 Lawn SD, Nicol MP, Xpert NMP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiol* 2011;6:1067–82.
22 Yassin MA, Datiko DG, Tulloch O, *et al*. Innovative community-based approaches doubled tuberculosis case notification and improve treatment outcome in southern Ethiopia. *PLoS One* 2013;8:e63174–6203. Electronic).
23 National Tuberculosis Control Center of Lao PDR. Lao PDR district health information software 2 (DHIS2) 2020.
24 National tuberculosis and leprosy control programme of Nigeria. *2019 Annual TB Report*;2019.
25 Yoon C, Chaisson LH, Patel SM, *et al*. Diagnostic accuracy of C-reactive protein for active pulmonary tuberculosis: a meta-analysis. *Int J Tuberc Lung Dis* 2017;21:1013–9.
26 Qin ZZ, Sander MS, Rai B, *et al*. Using artificial intelligence to read chest radiographs for tuberculosis detection: a multi-site evaluation of the diagnostic accuracy of three deep learning systems. *Sci Rep* 2019;9:15000.
27 MacPherson P, Webb EL, Kamchedzera W, *et al*. Computer-Aided X-ray screening for tuberculosis and HIV testing among adults with cough in Malawi (the prospect study): a randomised trial and cost-effectiveness analysis. *PLoS Med* 2021;18:e1003752.