Scientific Research Publishing

Advances in Infectious Diseases, 2023, 13, 183-192
https://www.scirp.org/journal/aid
ISSN Online: 2164-2656
ISSN Print: 2164-2648

Pooled Sputum for Xpert MTB/RIF Testing: A Cost-Saving Strategy in Mwanza, Tanzania

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How to cite this paper: Henerico, S., Makapa, R.V., Okamo, B.C., Japhet, G., Gerwing-Adima, L., Kidenya, B.R. and Kasang, C. (2023) Pooled Sputum for Xpert MTB/RIF Testing: A Cost-Saving Strategy in Mwanza, Tanzania. *Advances in Infectious Diseases*, **13**, 183-192.
https://doi.org/10.4236/aid.2023.132017

Received: February 11, 2023
Accepted: May 16, 2023
Published: May 19, 2023

Abstract

Background: Tuberculosis (TB) diagnostics, such as Xpert MTB/RIF, is still limited by cost. Testing of pooled samples from presumptive TB patients has been thought as a cost-saving strategy to diagnose TB. We assessed the utility and cost-saving of pooled Xpert MTB/RIF testing strategy for the diagnosis of TB in Mwanza, Tanzania. **Methods:** Sputum samples from Presumptive TB patients were submitted to TB laboratory for routine diagnosis of TB using Xpert MTB/RIF. The TB results from the individual sputum samples were used as the reference standard and were concealed to the investigating laboratory technicians. The remainder of samples were collected serially and were pooled (5 samples per pool) for testing. The agreement of the results between individual sample testing against pooled sample testing and cost-savings was assessed. **Results:** A total of 250 sputum samples from presumptive TB patients were analyzed and 50 pools were made with each pool containing 5 samples. Of the 50 sputum pools made, *Mycobacterium tuberculosis* (MTB) was detected in 17 (34.0%) pools. Results from the individual sputum samples MTB/RIF testing were retrieved for all 250 samples and there were 28 (11.2%) samples in which MTB was detected whereas 222 (88.8%) samples had no MTB detected. Following re-analysis of positive pools, all 28 (100%) individual positive MTB samples were detected within the 17 positive pools, with 1 to 3 individual MTB positive samples per pool. The individual sputum samples were correctly identified by pooled sputum on Xpert MTB/RIF testing, with the sensitivity of 100% and specificity of 100%. Using pooling of sputum samples strategy, we saved 46.0% (115/250) of the cartridges. **Conclusion:** The pooled sputum testing strategy reduced cartridge costs by 46.0% and has

the potential to increase the affordability of Xpert MTB/RIF testing in countries with limited resources, such as Tanzania.

Keywords

Tuberculosis, Pooled Sputum, Xpert MTB/RIF, Cost-Saving, Diagnostic Strategy, Mwanza, Tanzania

1. Introduction

Despite the reduction in Tuberculosis (TB) associated deaths of up to 47.0% since 1990, TB remains one of the deadliest infectious diseases in the world. Ranking above HIV as the world's greatest health threat, TB is on top of the list of causes of death from a single infectious agent accounting for around 1.4 million deaths in 2019 [1]. This death toll is unacceptably high and early detection and treatment is of paramount importance. Despite the decline of TB incidence from 306 per 100,000 populations in 2015 to 208 per 100,000 in 2021, Tanzania remains one of the high TB burden countries [2]. The introduction of the Xpert/MTB RIF in the diagnosis of TB and detection of the bacterial resistance to rifampicin in 2010, by the WHO, has been seen as a major diagnostic revolution as far as TB is concerned [3]. This is a fully automated real-time PCR assay that simultaneously detects *Mycobacterium tuberculosis* (MTB) and resistance to rifampicin in less than 2 hours [4].

However, despite the achievements brought about by the introduction of the Xpert MTB/RIF, the running costs and difficulties in supply chains are still a major challenge in low- and middle-income countries like Tanzania. Furthermore, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) disease pandemic has aggressively destabilized the health systems, abrogated supply chains and threatens the efforts made previously by the National TB control programs in many Countries [5]. Therefore, a very sensitive and affordable diagnostic strategy must be in place to achieve the goal of TB by 2035 [5] [6]. The Xpert MTB/RIF is very sensitive but fails to address the problem of affordability in the context of low-income countries. Pooling of sputum samples to test for Xpert MTB/RIF has been investigated as a cost-saving strategy to be able to serve as many patients as possible with the available limited resources. This strategy has been assessed for its effectiveness in South Africa, Nigeria, Vietnam, Cambodia and Brazil and there has been a high agreement between the pooled and individual samples [5] [7] [8]-[12]. Moreover, this strategy demonstrated the salvage of cartridges and time required to process samples [5]-[12]. The purpose of this study was to determine the effectiveness of this strategy to be able to employ it in the screening and/or diagnosis of TB in our setting where the prevalence of pulmonary tuberculosis among people with productive cough is 6.4% [13]. This was done by assessing the diagnostic utility as well as the cost-savings of this strategy.

2. Materials and Methods

2.1. Study Design and Setting

This was a cross sectional study performed between late May to early July 2018, which utilized the remaining sputum samples submitted and tested routinely for TB diagnosis at Bugando Medical Centre TB laboratory. Bugando Medical Centre is a 1000-bed tertiary hospital located in Mwanza, Tanzania serving about 15.0% of the Country's population of about 60 million.

2.2. Study Participants, Sample Size and Sampling Technique

Sputum samples from Presumptive TB patients attending TB clinic at Bugando Medical Centre were submitted to TB laboratory for routine diagnosis of TB using Xpert MTB/RIF (Cepheid Inc., Sunnyvale, CA, US). The TB results from the individual sputum sample were used as a reference standard to examine the pools results and were concealed from the investigating laboratory technicians. A total of 250 samples from the remainder of samples from presumptive TB patients submitted for routine Xpert MTB/RIF testing at Bugando Medical Centre Laboratory were used serially for pooling analysis. All methods were carried out in accordance with standard operating procedures adopted from manufacturer's instructions [14].

2.3. Inclusion Criteria

Residualsputum samples with sufficient volume to make up a pool were serially collected until the sample size was reached.

2.4. Laboratory Analyses

The residual sputum samples were de-identified and used to make pools of five samples per pool with each sample contributing 0.5 mL. Buffer solution was added to each sputum sample before splitting. These pooled samples were transferred into 5 mL falcon tubes, each tube making one pool. The laboratory registration (identification) number for each sample in a pool was noted in linkage with the pool number to ensure no mix up of samples and pools during analysis. The laboratory personnel working with the MTB/RIF testing for pooled samples was blinded for the results from the individual MTB/RIF sputum testing. The MTB/RIF testing for both individual and pooled samples was carried out following the tertiary hospital laboratory standard operating procedures adopted from the manufacturer's instructions (Cepheid Inc., Sunnyvale, CA, US, 2010) [14].

2.5. Data Analysis

Data from questionnaires and laboratory forms were entered into the computer using Microsoft Excel 2016 (Microsoft Corporation, WA, US) and then imported to STATA version 13 (StataCorp, College Station, TX, US) for analysis. Continuous data were summarized using median with interquartile range [IQR]

whereas categorical data were summarized using frequency and proportions (percent). To determine the sensitivity and specificity of pooled 5 samples testing strategy we employed 2 × 2 contingency using individual sputum samples Xpert MTB/RIF results as the reference standard.

2.6. Ethics Approval and Consent to Participate

The ethical approval to conduct this study was obtained from the joint Bugando Medical Centre/Catholic University of Health and Allied Sciences (BMC/CUHAS) ethics review board (CREC 672/2018). A waiver of informed consent was obtained from the ethics committee/institutional review board of Central Pathology Laboratory to use the samples for this study.

3. Results

3.1. Patient Demographics

Of the 250 samples, 143 (57.2%) were from male patients. The median age for all patients was 35 (IQR 27 - 40) years. Table 1 shows the demographic information.

3.2. Pooled Sputum Samples MTB/RIF Results

Of the 50 pooled sputum samples, MTB was detected in 17 (34.0%) pools whereas 33 pooled sputum samples (66.0%) had no MTB detected. Of the 17 MTB positive pools, 10 (58.8%) were detected as medium while the remaining 7 (41.2%) pools were detected as low MTB. Of note, there was no pool that had a high MTB detected. Furthermore, none of the pools was detected to have Rifampicin resistance.

3.3. Analysis of Pooled Samples Using Individual Sputum Samples MTB/RIF Results as a Reference Standard

Finally results from the individual sputum samples MTB/RIF testing were retrieved for all 250 samples and there were 28 (11.2%) samples in which MTB was detected whereas 222 (88.8%) samples had no MTB detected. Of the 28 samples in which MTB was detected, 2 (7.1%) had high MTB detected results, 13 (46.4%) medium MTB detected and the rest 13 (46.4%) had low MTB detected. None of the sputum samples was Rifampicin resistant. Following re-analysis of 17 positive pools for MTB using individual sputum samples MTB/RIF results as a reference standard, all 28 (100%) individual sputum samples tested positive for

Table 1. Demographic information for patients whose samples were used in the study.

Patient characteristic	Median	IQR
Median age	35	27 - 40
Sex	**Number (n)**	**Percent (%)**
Male	143	57.2
Female	107	42.8

Xpert MTB/RIF was detected by pooling. The number of individual MTB positive samples ranged from 1 to 3 per pool, with 3 (17.6%) pools contained two positive individual samples whereas 4 (23.5%) pools contained three positive individual samples. Furthermore, of the 33 negative pools for MTB, and none of the negative pools contained the positive individual sample for MTB. These results showed that all the positive and negative individual sputum samples were correctly identified, with an agreement of 100% between individual and pooled sputum on Xpert MTB/RIF testing. Table 2 summarizes the comparison of Cycle Thresholds Results of Pooled versus Individual sputum samples.

3.4. The Dilution Effect after Pooling Samples

The pooling of samples has the effect of increasing the dilution (decreasing MTB concentration) as compared to the individual sample analysis. This is shown by the increase in cycle thresholds whereby pooled samples had slightly higher cycle thresholds than the original individual sample cycle threshold. Table 3 summarizes the comparison of cycle thresholds and dilutions between the individual sputum samples and pooled sputum samples.

Table 2. Comparison of cycle thresholds results for positive pooled versus individuals samples.

Sputum Pool	Number of Individual MTB Detected	Individual Sample CT Value	Pooled Sample CT Value
P 1	2	25.4, 16.5	23.8
P 6	1	16.8	17.4
P 7	1	23.7	24.8
P 11	1	23.4	23.7
P 19	3	24.7, 26, 26.5	24.8
P 22	3	15.5, 16.7, 17.3	16
P 23	3	9.7, 19.5, 18.8	16.8
P 28	2	16.4, 17.4	16.3
P 31	1	16.7	17.4
P 32	1	23.2	26.4
P 33	1	16.3	16.9
P 40	1	23.3	24.3
P 42	2	16.3, 16.8	17.3
P 44	1	24.7	27.8
P46	1	23.8	24.6
P 48	3	23.7, 24.6, 25.4	26.7
P 49	1	16.8	17.4
Total	**28**		

Table 3. Cycles threshold and dilution and effect observed after pooling samples.

Category	CT Range	Individual Sputum Samples	Pooled Sputum Samples
Very Low	<16	0	0
Low	16 - 22	13	7
Medium	23 - 28	13	10
High	>28	2	0
Total		**28**	**17**

3.5. Agreement of Detection between Individual and Pooled Xpert MTB/RIF Strategy Results

Pooling sputum Xpert MTB/RIF strategy detected all the positive MTB cases that were also detected from the individual Xpert MTB/RIF testing. There was a 100% agreement between results from pooling sputum Xpert MTB/RIF and individual Xpert MTB/RIF results. The sensitivity and specificity were 100% respectively.

3.6. Cost-Savings of Using Pooled Sputum for Xpert MTB/RIF Strategy

The strategy of pooling sputum, as was used in this scenario, has saved a total of 115 cartridges by using 135 cartridges instead of 250 that would have been used for individual MTB/RIF testing. This salvage is equivalent to 46.0%. Therefore considering that in year 2019 the Hospital performed 2803 Xpert MTB/RIF tests and using this strategy we could have saved 1290 cartridges.

4. Discussion

4.1. Pooled Sputum Strategy Diagnostic Accuracy

This study has shown a level of agreement of 100% between individual and pooled sputum on Xpert MTB/RIF testing with a sensitivity and specificity of 100% respectively. These results are in-line with two other studies conducted in Vietnam by Ho *et al.*, and Phuong *et al.*, by pooling 2 sputum samples per pool, where a sensitivity of 88% and 96% were detected respectively [9] [10]. Whereas studies that used 4 samples per pool in Nigeria (Abdurrahman *et al.*), Vietnam (Ho *et al.*) Cambodia (Chry *et al.*) and Brazil (Santoz *et al.*) found the sensitivity of 88%, 94%, 95% and 100% respectively [8] [9] [11] [12]. Our specificity of 100% is similar to the previous studies done by pooling 4 sputum samples per pool in Nigeria by Abdurrahman *et al.*, and in Cambodia by Chry *et al.*, and found the specificity of 99% and 100% respectively [8] [11].

Xpert MTB/RIF Pooled Sputum Strategy is expected to detect higher levels of TB cases in active case finding than in passive case finding, as in the active case finding there may be more risk missing. In our study we used passive case finding and got comparable results with those from studies that also utilized passive case finding from Nigeria and Vietnam [8] [10]. Surprisingly, an active case

finding study in Cambodia that employed the active case finding had comparable and very good results with that the studies utilized passive case finding. This could be attributed to the fact that in Cambodia study by Chry *et al.*, they used Xpert MTB/RIF Ultra [11]. These findings underpin the utility of Xpert MTB/RIF Pooled Sputum Strategy using ultra for screening of pulmonary tuberculosis in active case finding.

The concentration of MTB for positive samples was reduced in pooled sputum samples as compared to the individual samples; this was shown by the increase in cycle thresholds whereby pooled samples had slightly higher cycle thresholds than the original individual sample cycle threshold. Among all the positive individual sputum samples there was no one with a very low MTB detected as samples were pooled without the knowledge of the individual sample results. This scenario partly explains why we have a sensitivity of 100% apart from the dilution effect observed after pooling samples together. The dilution effect has also expressed itself in this study whereby there were two samples with high MTB detected but high TB was not detected in any of the pools. This effect was also observed in a study done by Abdurrahman *et al.*, [8] whereby samples with very low MTB detected turned negative when included in pools.

4.2. Cost-Savings of Xpert MTB/RIF Pooled Sputum Strategy

The strategy has proved to be cost-effective by saving 115 Xpert MTB/RIF test cartridges. This implies that if the technique is employed; 50 cartridges would be used to test the 50 pooled sputum samples and re-testing individual sputum samples making the 17 pools with detected MTB results (85 cartridges) making a total of 135 test cartridges. This salvage is equivalent to 46.0%. Therefore considering that in year 2019 the Hospital performed 2803 Xpert MTB/RIF tests and using this strategy we could save 1290 cartridges. Taking into consideration of the concession price of US$ 9.98 per cartridge [15] this strategy would have saved a total of US$ 12,874 for year 2019 expenditures on cartridges. Furthermore, this study has shown that performing fewer numbers of tests using this technique directly saves time as it has also been shown in previous studies in Nigeria, South Africa, Vietnam, Cambodia and Brazil [5] [7]-[12]. Studies were done in Nigeria by Abdurrahman *et al.*, and the one in Cambodia by Chry *et al.*, have also demonstrated the cartridge savings of 31% and 27% respectively [8] [11]. Of note, our cartridge savings of 46.0% is higher than those from these two previously reported studies due to the fact that we pooled 5 samples compared to previous studies that pooled 4 samples [8] [11]. Our study is limited by the fact that we did not establish the optimum pooling ratio.

5. Conclusion and Recommendations

This study has shown that the strategy can be used for screening and/or diagnosing MTB due to its high diagnostic accuracy but only when an optimum-pooling ratio that accommodates the dilution effect observed has been determined. It has in addition shown to save both cost and time. It is, therefore, high time to estab-

lish the optimum-pooling ratio in our settings to be able to consider using the technique.

Ethics Approval and Consent to Participate

The ethical approval to conduct this study was obtained from the joint Bugando Medical Centre/Catholic University of Health and Allied Sciences (BMC/CUHAS) ethics review board (CREC 672/2018). A waiver of informed consent was obtained from the ethics committee/institutional review board of Central Pathology Laboratory to use the samples for this study.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This study was funded by the Medical Mission Institute Wuerzburg, Rexroth Foundation and the DAHW Wuerzburg.

Author's Contributions

Edited and reviewed critically the manuscript: SH RVM BCO BRK GJ LGA CK. Conceived and designed the experiments: SH BRK CK. Performed the experiments: SH RVM BCO GJ LGA. Analyzed the data: SH BRK CK. Contributed reagents/materials/analysis tools: BRK CK. Wrote the paper: SH RVM BCO. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to acknowledge the technical support provided by the members of Bugando Hospital Tuberculosis Laboratory.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] WHO (2020) Global Tuberculosis Report. https://www.who.int/news-room/fact-sheets/detail/tuberculosis

[2] NTLP (2022) TB Prevalence in Tanzania—National Tuberculosis & Leprosy Programme. https://ntlp.go.tz/tuberculosis/tb-prevalence-in-tanzania/

[3] (2011) Policy Statement: Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System. World Health Organization, Geneva. http://www.ncbi.nlm.nih.gov/pubmed/26158191

[4] Steingart, K.R., Schiller, I., Horne, D.J., Pai, M., Boehme, C.C. and Dendukuri, N. (2014) Xpert® MTB/RIF Assay for Pulmonary Tuberculosis and Rifampicin Resistance in Adults. *The Cochrane Database of Systematic Reviews*, No. 1, CD009593.

https://doi.org/10.1002/14651858.CD009593.pub3

[5] Cuevas, L.E., Santos, V.S., Lima, S.V.M.A., Kontogianni, K., Bimba, J.S., Iem, V., Dominguez, J., Adams, E., Atienzar, A.C., Edwards, T., Squire, S.B., Hall, P.J. and Creswell, J. (2021) Systematic Review of Pooling Sputum as an Efficient Method for Xpert MTB/RIF Tuberculosis Testing during the COVID-19 Pandemic. *Emerging Infectious Diseases*, **27**, 719-727. https://doi.org/10.3201/eid2703.204090

[6] Ivan, T.B., Sherman, W., Repasky, M. and Beuming, T. (2013) Improved Docking of Polypeptides with Glide. *Journal of Chemical Information and Modeling*, **53**, 1689-1699. https://doi.org/10.1021/ci400128m

[7] Zishiri, V., Chihota, V., Mccarthy, K., Charalambous, S., Churchyard, G.J. and Mann, C.J. (2015) Pooling Sputum from Multiple Individuals for Xpert MTB/RIF Testing: A Strategy for Screening High-Risk Populations. *Internation Journal of Tuberculosis and Lung Disease*, **19**, 87-90. https://doi.org/10.5588/ijtld.14.0372

[8] Abdurrahman, S.T., Mbanaso, O., Lawson, L., Oladimeji, O., Blakiston, M., Obasanya, J. and Cuevas, L.E. (2015) Testing Pooled Sputum with Xpert MTB/RIF for the Diagnosis of Pulmonary Tuberculosis to Increase Affordability in Low Income Countries. *Journal of Clinical Microbiology*, **53**, 2502-2508. https://doi.org/10.1128/JCM.00864-15

[9] Ho, J., Jelfs, P., Nguyen, P.T., Sintchenko, V., Fox, G.J. and Marks, G.B. (2017) Pooling Sputum Samples to Improve the Feasibility of Xpert® MTB/RIF in Systematic Screening for Tuberculosis. *The International Journal of Tuberculosis and Lung Disease*, **21**, 503-508. https://doi.org/10.5588/ijtld.16.0846

[10] Phuong, N.T.B., Anh, N.T., Van Son, N., *et al.* (2019) Effect of Two Alternative Methods of Pooling Sputum Prior to Testing for Tuberculosis with Genexpert MTB/RIF. *BMC Infectious Diseases*, **19**, Article No. 347. https://doi.org/10.1186/s12879-019-3778-9

[11] Chry, M., Smelyanskaya, M., Ky, M., Codlin, A.J., Cazabon, D., Tan, E.M. and Creswell, J. (2020) Can the High Sensitivity of Xpert MTB/RIF Ultra Be Harnessed to Save Cartridge Costs? Results from a Pooled Sputum Evaluation in Cambodia. *Tropical Medicine and Infectious Disease*, **5**, Article 27. https://doi.org/10.3390/tropicalmed5010027

[12] Santos, P., Santos, A., Verma, R., Oliveira, R., Camioli, C., Lemos, E., *et al.* (2019) The Utility of Pooling Sputum Samples for Mass Screening for Tuberculosis in Prisons Using Xpert MTB/RIF Ultra. 50*th World Conference on International Union against Tuberculosis and Lung Disease*, Hyderabad, 30 October-2 November 2019, S110-S110. https://hyderabad.worldlunghealth.org/wp-content/uploads/2019/11/20191101_UNION2019_Abstracts_Final.pdf cited 2020 Mar 30

[13] Beyanga, M., Kidenya, B.R., Gerwing-Adima, L., *et al.* (2018) Investigation of Household Contacts of Pulmonary Tuberculosis Patients Increases Case Detection in Mwanza City, Tanzania. *BMC Infectious Diseases*, **18**, Article No. 110. https://doi.org/10.1186/s12879-018-3036-6

[14] GeneXpert Xpert MTB RIF User Manual. https://www.manualslib.com/manual/1197251/Genexpert-Xpert-Mtb-Rif.html#manual

[15] Piatek, A.S., Van Cleeff, M., Alexander, H., Coggin, W.L., Rehr, M., Van Kampen, S. and Mukadi, Y. (2013) GeneXpert for TB Diagnosis: Planned and Purposeful Implementation. *Global Health: Science and Practice*, **1**, 18-23. https://doi.org/10.9745/GHSP-D-12-00004

Abbreviations

BMC	Bugando Medical Centre
CREC	Catholic University of Health and Allied Sciences/Bugando Medical Centre Research and Ethics Committee
CUHAS	Catholic University of Health and Allied Sciences
DAHW	German Leprosy and Tuberculosis Relief Association (Deutsches Aussätzigen-Hilfswerk)
IQR	Interquartile Range
NTLP	National Tuberculosis and Leprosy Programme
TB	Tuberculosis
WHO	World Health Organization
Xpert MTB/RIF	Xpert *Mycobacterium tuberculosis*/Rifampicin