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Comparative Analysis of Genexpert MTB/Rif version 4 and Genexpert MTB/Rif Ultra on Frozen Sputum

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Abstract:

Background:*The Genexpert MTB/Rif version 4(Xpert) and Genexpert MTB/Rif Ultra(Ultra) are cartridges used to detect mycobacterium (MTB) using the same machine.Ultra has lower limit of detection allowing detection of "traces" of MTB.*

The aim of this study was to carry out a comparative analysis using Ultra on clinical samples of frozen sputum previously analysed on Xpert and mycobacterium growth indicator tube (MGIT) culture.

Materials and Methods:*During the study 109 de-identified frozen sputum collected from 16/10/2012 to 23/08/2013 during a study on presumptive drug resistant TB patients (DRTB) patients, were analyzed on Ultra in 2019according to manufacturer's instructions.*

Results:*There was 96/109 (88%) concordance and 10/109 (9.2%) discordance between Xpert and Ultra. Sputum frozen for 7 years then analyzed on Ultra performed better than 7 years previously when it was analyzed as raw sputum onXpert using MGIT as gold standard(95.6% and 93.2% respectively).The results were comparable and statistically significant, kappa analysis between Xpert and frozen sputum Ultra was not statisticallysignificant showing a slight level of agreement of 0.147 (p value =0.079), 95% CI (-0.1078, 0.4018).*

Conclusion: *Results of Ultra on frozen sputum correlate reasonably well.The study findings show that TB positive frozen sputum from presumptive DRTB, run on Ultra after 7 years freezing can perform better than previously run Xpertresults compared to MGIT as gold standard.In light of the research findings, potential implication are that to save on cost, comparison of performance of newly diagnosed tests can be done using frozensputum, pre run on panel of TB tests.*

Key Word: *Sputum, Mycobacteria Tuberculosis; Frozen, Xpert; Ultra*

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I. Introduction

The in country TB program has to be agile and make it feasible for new TB tests to be added for routine use .On introduction of a new TB test, performance comparison studies have to be carried out between at least 2 tests using a reference specimen and one reference method. Such an exercise of new TB test method verification has cost implications. The TB program in Zimbabwe introduced the Genexpert MTB/Rif Ultra (Ultra) cartridges into routine use in 2019.Genexpert MTB/Rif version 4(Xpert) and Genexpert MTB/Rif Ultra(Ultra) are cartridges used to detect mycobacterium (MTB) using the same machine.Ultra is an alternative to Xpert & has improved sensitivity due to:

Targeting the multicopy IS6110 and IS1081 genes for the detection of MTB rifampicin resistance pattern, leading to Improved rifampicin resistance detection ([1,2])

More rapid thermal cycling with fully nested nucleic acid amplification,

Improved fluidics and enzymes

Larger DNA reaction chamber than the Xpert

Xpert has lowest limit of detection of 112.6 colony forming units per ml (cfu/ml). The limit of detection for Ultra is 15.6 cfu/ml, plustheadditional semi quantitative result of MTB detected Trace. ([1])Xpert sensitivity is 88% in smear positive and 22-66% sensitivity in smear negative, while Ultra has 5% extra increase in sensitivity across the board. Xpert specificity is 98%, while Ultra has decreased specificity by minus

3% ([3,4]) The statement of the problem is there is need for cost effective methods of in country evaluation of new TB tests.The study hypothesiswas that freezing sputum has no effect on comparative analysis of Xpert and Ultra. The aim of the current study was to carry out a comparative perfomanceof Ultra on clinical samples of frozen sputum previously analysed on Xpert and cultured on mycobacterium growth indicator tube (MGIT).The specific objectives were to ascertain percentage concordance or discordance between Xpert and Ultra, as wellas to establish effect of freezing sputum on Ultra.The justification for the study was the need to ascertain whether previously analysed frozen sputum can be used, for comparing with thawed and retested results from newly availed TB tests.There is need to cut on cost of performing panel of TB tests each time a new TB diagnostic test is availed for use.

II. Material And Methods

This was a retrospective study, nested in a prospective pre-freezing survey(primary study). Theprospective part of this study was carried out from 16/10/2012 to 23/08/2013 at Biomedical Research and Training Institute (BRTI,) Harare Zimbabwe. The retrospective part of this comparative study was carried out using the frozen sputum stored at BRTI. The retrospective analysis using Ultra was carried out in October 2019 at the Mutare Provincial Hospital Laboratory, Manicaland province, Zimbabwe.

Study Design:The study employed both retrospective and prospective cross sectional design.The study design was selected as the most appropriate to answer the research question and the specific objectives

Study Location:The frozen sputum analysis was carried out at Mutare Provincial Hospital Manicaland Zimbabwe, as a study in the Collage of Health Agriculture and Natural Sciences of Africa University, Manicaland, Zimbabwe.

Study Duration: The study from which the sputum specimen was initially analyzed as fresh sputum, then frozen was from 16/10/2012 to 23/08/2013 during a study on presumptive DRTB patients. This current study re analyzing the 7 year frozen sputum was from February 2019 to October 2019.

Sample Size: A total of 109 frozen sputum were analyzed using Ultra.

Subjects & Selection Method: In the primary study, subjects were presumptive pulmonary DRTB patients. The subjects were selected from the outpatients departments of health facilities around Harare, Zimbabwe ([6]).The subjects in the nested study were TB culture positive frozen sputum samples from the primary study.

Study Population: The study population of the nested study was the primary study frozen sputum samples.

Inclusion criteria:

The primary study inclusion criteria was being a presumptive pulmonary DRTB patient presenting at outpatient department of the following facilities around Harare, Zimbabwe: Epworth, southern high-density suburbs health clinics and 2 infectious disease hospital clinics in Harare ([6]).

The inclusion criteria for the nested study was being among the frozen sputum samplesof study Medical Research Authority of Zimbabwe (MRCZ) ref MRCZ A/1552, being TB positive on at least one of the previously run panel of testsand having sufficient frozen sputum for Ultra analysis.

Exclusion criteria:

Sputum samples not among the frozen sputum samples of study MRCZ ref MRCZ A/1552.

Having insufficient frozen sputum for Ultra analysis

Procedure methodology

Data collection procedureAfter obtaining approval from the Africa University Research Ethics Committee (AUREC) and from the MRCZ- De-identified frozen sputum samples collected from 16/10/2012 to 23/8/2013 during a study on presumptive DRTB patients, were analyzed using Ultra in 2019. The frozen sputum was thawed to reach room temperature before being mixed with sample processing buffer at a ratio of 1 to 2.After 10 minute incubation at room temperature and second mixing step followed by a further 5 minute incubation, analysis on Ultra was performed according to manufacturer's instructions. The Excel sheet with data on MGIT results, Microscopy results and Xpert results from the primary study was availed from the author of the primary study.

Ultra result print was transcribed to the Excel sheet containing Xpert results data from primary study.Rationale for choosing means of collecting data was that it was most appropriate as it built on the Excel sheet generated in previous study,only adding the other variables from Ultra results.One thing that could have been done differently were it not for insufficient volumes of frozen sputum, would have been to also repeat analysis using Xpert, to establish what effect storage had on Xpert test

Statistical analysis

Data cleaning was carried out before importing the Excel data to Epi Info for analysis.There was generation of frequency tables. The p value of less than 0.05 was considered as statistically significant. Cohen's kappa coefficient analysis was carried out to measure the level of agreement.

III. Results

Comparative Analysis of Ultra versus Xpert Frequencies:

There was 88.1% concordance between Xpert results run 7 years previously and Ultra results analyzed on same sputum which had been frozen for up to 7 years (Table 1).

Table1 Frequency of Xpert versus Ultra Concordance

ULTRA Versus Conventional Genexpert cartridge	Frequency	Percentage
Concordant	96	88.1%
Discordant	10	9.2%
Error Ultra	1	0.9%
No Xpert result	2	1.8%

Of the 109 sputum 10 (9.2%) were discordant between Xpert and Ultra. Of the 10 discordant, 5 discordant were due to Xpert result being negative where Ultraresult was positive. 5 out of these 10 Xpert negative Ultra positive, were all culture (MGIT) positive. The remaining 5 of the 10 discordant were discordant in that Xpert result was positive where Ultra result was negative. 2 of the 5 were MGIT negative. 3 of the 5 were MGIT positive. 2/109 (1.8%) had no previous Xpert result although they were MGIT positive. 1/109(0.9%) had an Ultra result of error.

Comparative analysis of mgit versus xpert results:

Of the 88 sputum that was MGIT positive 93, 2% (from78.4% and 13.8%) was Xpert positive (Table 2).

Table 2 Results of MGIT versus Xpert

Ultra MTB Result	MGIT Contaminated	MGIT Missing	MGIT Negative	MGIT Positive
	N=9	N=1	N=11	N=88
	n(%)	n(%)	n(%)	n(%)
Xpert Positive RR	1	1	3	13
	(11.1%)	(100%)	(27.3%)	(13.8%)
Xpert Positive RS	8	0	4	69
	(88.9%)	(0.0%)	(36.4%)	(78.4%)
Xpert Negative	0	0	4	4
	(0.0%)	(0.0%)	(36.4%)	(4.5%)
Xpert Missing	0	0	0	2
	(0.0%)	(0.0%)	(0.0%)	(2.3%)

Of the 11sputum that was MGIT negative, 7/11 (63.7%) were Xpert positive (27.3% & 36.4%).

Comparative Analysis of MGIT versus Ultra Results:

It was noted that of the 88 sputum that were MGIT positive, 85/88(95.6%) produced Ultra positive results and 3/88(4.4%) produced Ultra negative results (Table 3).

Table 3Results of MGIT versus Ultra

Ultra MTB Result	MGIT Contaminated	MGIT Missing	MGIT Negative	MGIT Positive
	N=9	N=1	N=11	N=88
	n(%)	n(%)	n(%)	n(%)
Ultra Positive	8	1	7	85
	(88.9%)	(100%)	(63.6%)	(95.6%)
Ultra Negative	1	0	4	3
	(11.1%)	(0.0.%)	(36.4%)	(4.4%)

From table no 2 and table no. 3 it is shown that sputum frozen for 7 years then analyzed on Ultra performed better than raw sputum Xpert using MGIT as gold standard(95.6% and 93.2% (from 78.4% plus13.8%) respectively.

Measure of Agreement Between Xpert and MGIT Results:

The interrater reliability for the Xpert code versus MGIT code (Table 4) was found to yield aCohen's kappa coefficient of 0.186 (p =0.004), 95% CI (0.01784, 0.38984). This measure of agreement, while statistically significant, is only a slight agreement according to kappa agreement interpretation([13]).

Table 4 Kappa Measure of Agreement Between Xpert and MGIT Results

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.186	.104	2.883	.004
N of Valid Cases		109			

Measure of Agreement Between Ultra and MGIT Results:

In the interrater reliability for the Ultra code and MGIT code cross tabulation (Table 5) raters were found to have kappa value of 0.235 (p value <0.001), 95% CI (0.03116, 0.43884). This measure of agreement, while statistically significant, is only a fair agreement according to Kappa agreement interpretation([13])

Table 5 Kappa Measure of Agreement Between Ultra and MGIT Results:

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.235	.104	3.637	.000
N of Valid Cases		109			

Measure of agreement between xpert and ultra results:

Table 6 Kappa Measure of Agreement Between Xpert and Ultra Results

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.147	.130	1.756	.079
N of Valid Cases		109			

The interrater reliability for theUltra code versus Xpert code raters(Table 6) showed kappa of 0.147 (p value =0.079), 95% CI (-0.1078, 0.4018). This measure of agreement, while not statistically significant, is only a slight agreement that can be due to chance according to Kappa agreement interpretation ([13]).

Tables 4, 5 and 6 show that compared to Xpert versus MGIT as well as Ultra versus MGIT and Xpert versus Ultra, the interrater reliability were found to be all the same range of fair agreement (kappa 0.186,kappa of 0.235& kappa 0.147) respectively.

IV. Discussion

Chakravorty S et al reported in their findings that Ultra performed better than Xpert on sputum spiked with known quantity of MTB strain. Bisogninet al found that after freezing sputum for 4 years, some Xpert negative sputumtested Ultra positive.The finding that was predicted by literature is Ultra picking positive where Xpert could not pick positive, due to the explained difference in limit of detection, for the 5 discordant due to Xpert negative([1, 4]).

The current study adds to literature in that TB positive frozen sputum from presumptive DRTB, run on Ultra after 7 years freezing performed better than Xpert compared to MGIT as gold standard.

The findings that differ from expectations were 5 discordant due to Ultra negative. 2 of the 5 were MGIT negative. 3 of the 5 were MGIT positive. Possible explanations could be due to additional factors that could not be conclusively resolved in this study.

Literature states that Ultra ISO probe makes Ultra more specific for rifampicin resistance (RR) resulting in higher likelihood of picking rifampicin resistance if it's there, which was not among the findings of the research.

Sputum frozen for 7 years then analyzed on Ultra performed better than raw sputum Xpert using MGIT as gold standard(95.6% and 93.2% respectively).

Effect of freezing sputum on Ultra explains the 5% increase in sensitivity not being demonstrated..

The current study ascertained 88% concordance between Xpert and frozen sputum analyzed on Ultra.

Results of Ultra on frozen sputum correlate reasonably well, considering possible deterioration due to long term storage.

V. Conclusion

The implications of findings in light of the research objectives are that frozen sputum can be used for economic evaluation of newly discovered TB diagnosis tests as there was concordance. That means comparison of performance of one newly diagnosed test can be done on frozen sputum , pre run on panel of TB tests .To save on cost, in country method verification of new TB diagnostic tests can be carried out on frozen sputum , pre run on panel ofTB tests.Frozen sputum can also be used to assess epidemiological pattern of TB in population.

VI. Recommendations

To save on in country test evaluation budget of newly introduced TB tests, consider use of frozen sputum and ride on previous test results of reference method.

Recommendations for future research with large volumes sputum to enable repeat of both tests on day 1 and year TB studies to capture all parameters for any TB test run even those of no interest in current study to enhance frozen sputum raw data base, e.g. this study needed the Xpert Cycle Thresh hold values of Xpert tests analysed in primary study.

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