Open Science Journal of Clinical Medicine
2014; 2(2): 59-62
Published online MM DD 2014 (http://www.openscienceonline.com/journal/osjcm)

Non-conforming rifampicin susceptibility test reports from Xpert MTB/RIF assay: The national reference laboratory experience in Nigeria

Nkiru N. Nwokoye[1], Chizoba C. Onubogu[1], Okechukwu P. Nwadike[3], Abigail T. Abiodun[1], Dan Onwujekwe[2], Mustapha Gidado[3], Olawale O. Olubi[4], Oni E. Idigbe[1]

[1]National TB Reference Laboratory, Microbiology Division, Nigerian Institute of Medical Research, Lagos, Nigeria
[2]Clinical Sciences Division, Nigerian Institute of Medical Research, Lagos, Nigeria
[3]KNCV/TB CARE1 project, Federal Capital Territory, Abuja, Nigeria
[4]Department of Ear Nose and throat, Lagos State University teaching Hospital, Ikeja, Lagos, Nigeria

Email address

Nkirunwokoye@ymail.com (N. N. Nwokoye), cathyonubogu@yahoo.co.uk (C. C. Onubogu), peter.nwadike@kncvtbc.org (O. P. Nwadike), topilep01@yahoo.com (A.T. Abiodun), dan.onwujekwe@yahoo.com (D. Onwujekwe), mustapha.gidado@kncvtbc.org (M. Gidado), waleolubi@yahoo.com (O. O. Olubi), oniidigbe@yahoo.com (O. E. Idigbe)

To cite this article

Nkiru N. Nwokoye, Chizoba C. Onubogu, Okechukwu P. Nwadike, Tope A. Abiodun, Dan Onwujekwe, Mustapha Gidado, Olawale O. Olubi, Oni E. Idigbe. Non-Conforming Rifampicin Susceptibility Test Reports from Xpert MTB/RIF Assay: The National Reference Laboratory Experience in Nigeria, *Open Science Journal of Clinical Medicine.* Vol. 2, No. 2, 2014, pp. 59-62

Abstract

GeneXpert MTB/RIF assay is a technology that allows for the simultaneous detection of *Mycobacterium tuberculosis* complex and rifampicin resistance in a single reaction. Detection of rifampicin resistance and indeterminate results is based on a predetermined standard parameters derived from the cycle threshold values of the probes. Test reports that fail to conform to the set standard are generally non-reliable. This study is aimed at highlighting the rifampicin resistance and indeterminate results that failed to conform to the standard parameter values stated by the assay manufacturers. A retrospective review of archived Xpert results was carried out to scrutinize for discrepancies between the assay report and the standard parameter values. Discrepant test reports were selected and analysed. Out of 1,046 test reports reviewed, 5 failed to conform to the standard parameter values. Three of the non-conformed results were rifampicin resistance while two were associated with rifampicin indeterminate results. Since initiation of drug-resistant tuberculosis treatment in Nigeria, is based on the result of Xpert assay, withholding treatment for patient with non-conforming rifampicin susceptibility result until phenotypic drug susceptibility test report is out is highly recommended. This is to curtail the adverse effects of misdiagnosis on not just the patient, but on the TB program and the community at large.

Keywords

Mycobacterium Tuberculosis, Rifampicin Resistance, Xpert MTB/RIF, Cycle Threshold, Hybridization Probe

1. Introduction

GeneXpert (Xpert) MTB/RIF assay is a technology that allows for the simultaneous detection of *Mycobacterium tuberculosis* (MTB) complex and rifampicin (rif) resistance in a single reaction [1]-[3]. It is based on the principle of a hemi-nested real-time PCR and molecular beacon technology that targets the *rpoB* gene [4], [5]. Five differently coloured hybridization probes are used, each covering a separate nucleic acid sequence within the amplified *rpoB* gene [6]. The probes screen for mutation in the core region of the gene [7], [8]. In the absence of mutation, the probe binds to their complementary DNA targets resulting in the onset of fluorescence [9]. On the other hand, a mutation in the core region of the *rpoB* gene results in either delayed onset (partial inhibition) or complete suppression of fluorescence of the corresponding

probe [8], [10].

MTB is identified when at least two of the five probes give positive signals with cycle threshold (C_T) of ≤38 cycles and that differ by no more than a pre-specified number of cycles [8].The basis of detection of rif resistance is the difference between the first and the last C_T of the MTB probes (ΔC_T). For xpert version G3, any deviation from the wildtype sequence resulting in a delay in the appearance of the signal exceeding a predetermined ΔC_T value of 5, is reported as rif resistance [6], [11]. The assay terminates after 38 cycles thus was considered indeterminate for rif resistance if the first probe C_T is >34.5 cycles and the last probe has a C_T of >38 cycles [1], [8].

Few studies have reported the effect of parameter values (in the xpert system) on the determination of rif resistance. To fill this gap, this study is aimed at highlighting the discrepant rif resistance and indeterminate results encountered in the course of running the Xpert assay in a reference laboratory.

2. Methodology

This was a retrospective study conducted at the National TB Reference Laboratory, Nigerian Institute of Medical Research, Lagos, Nigeria between January 2012 and December 2013. A total of 1,046 archived test reports were retrieved and reviewed for discrepancies. Assay Reports that demonstrated ''MTB detected'' were further reviewed to determine their rif susceptibility status using the cycle threshold values of the probes. Tests with ΔC_T *of* <5 which were reported as rif resistance were considered as non-conformance. Also those with first and last probe C_T readings of <34.5 and <38 respectively which were reported as rif indeterminate were equally considered as unusual. Such tests were included in the study for review.

To ensure high quality test results, assays were run by skilled personnel who had earlier been trained on the technique, and who has over time demonstrated high level of proficiency via panel testing. Cartridges used for the assay were stored in the refrigerator (between 4 ^{O}C and 8^{O}C). Assays were run with Xpert MTB/RIF version G3 under temperature controlled environment (temperature range of 18 - 21 ^{O}C). Fresh spot sputum specimens were used for the assay and were processed within 4 hours of collection. Calibration of Xpert machine was done after one year of usage/installation.

3. Results

Out of 1,046 test results reviewed, 778 were reported as MTB negative, 135 as rif sensitive MTB, 80 reported as rif resistant MTB, 5 were rif indeterminate MTB while 48 were recorded as errors and invalid results. In all, 5 of the test results did not conform to the predetermined parameter values described by the manufactures.

A deviation in the basis of detecting rif resistance using C_T values of the probes was observed in 3 samples. Samples 1 and 2 were reported as rif resistance with a ΔC_T max values of 4.4 each and a delayed hybridization of probes A (C_T value=28.7) and E (C_T value=33.7) respectively. Sample 3 which was also rif resistant equally showed a ΔC_T max value of 4.9 and delayed hybridization of probe E (C_T value= 3.4) Table 1. In general, the delayed hybridization of probe was observed for probe E and A with E occurring more frequently (4 out of 5 samples).

Table 2 depicts some inconsistency in C_T values among the rif sensitive and indeterminate results. The C_T values for the first and last probes were within the interpretable parameter values (33.1 and 36.1 respectively) yet the assay reported sample 3 as rif indeterminate. On the contrary, sample 4 with first and last probes outside the parameter values (37.2 and 38.6 respectively) has a determinate rif sensitive result.

4. Discussion

Several prior studies had shown that Xpert assay is a promising tool for the detection of TB and drug resistant (DR) TB. It has the advantages of being rapid [12], simple [13], specific [14], and sensitive [15], [16]. However, our findings revealed some non-conformances (against a pre-determined parameter values) in the reporting of rif resistance and indeterminate test results.

Table 1. *Non conforming rifampicin resistant results.*

Sampe	MTB detected, concentration	Rifampicin resistance detection	C_T values						
			Probe D	Probe C	Probe E	Probe B	SPC	Probe A	ΔC_T max value
1	Detected, low	Detected	24.7	25.1	25.7	24.3	26.8	28.7	4.4
2	Detected, very low	Detected	29.3	29.5	33.7	29.5	25.6	29.5	4.4
3	Detected, low	Detected	27.5	27.1	31.4	27.4	34.3	26.5	4.9

All ΔC_T max value were <5.0 yet assays were reported as rif resistance

Table 2. *Inconsistency in* C_T *cut-off values among the rifampicin indeterminate results.*

Sample	MTB detected, concentration	Rifampicin resistance detection	C_T values					
			Probe D	Probe C	Probe E	Probe B	SPC	Probe A
4	Detected, very low	Indeterminate	35.0	33.2	36.1	33.1	26.0	34.1`
5	Detected, very low	Not detected	38.6	37.3	38.6	38.2	34.5	37.2

C_T values for 1st and last probes (sample 4) were <34.5 and 38 respectively yet assay reported indeterminate; CT value for1st and last probes (sample 5)were >34.5 and 38 respectively, assay was able to determine rif resistance.

The report showed cases of partial probe inhibition that resulted in delay hybridization in all the samples studied. None of the samples illustrated complete blockage (that results in dropout of probe) of the molecular beacon. The delay hybridization of probe was observed for probe E and A with E occurring more frequently. This may be cases of false positive rif resistant results as some studies have reported same with geneXpert MTB/RIF assay [2], [15], [17]. The country's diagnostic algorithm recognizes geneXpert MTB/RIF as an entry point for presumptive drug resistant (DR) cases and TB diagnosis among presumptive TB cases for people living with HIV [18]. Rif resistant cases are referred to the DR treatment centers where other samples are taken for conventional TB culture and DST (which may take 8 to 12 weeks to produce result) and 2nd line anti-TB drugs are administered. In this scenario, patient with false positive rif resistant result from xpert are compelled to undergo treatment with 2nd line anti-TB drug for as long as it takes the laboratory to produce the DST result. Owing to the toxic effect of the drugs on the patient and the cost implication on the national TB programme, we strongly recommend withholding treatment for patients with non-conforming rif susceptibility results until DST result is out.

On the other hand, since no further investigations such as sequencing and phenotypic drug resistance testing was done to confirm the rif resistance, it may also be a case of heterogenous MTB population containing both wildtype and mutant strains. Probe E encompasses codon 533 and screens for mutation Leu533pro [11]. An earlier study indicated that Xpert MTB/RIF cannot detect LEU533pro unless 100% of the DNA population are mutants [4]. This confirmed the already established fact that mutations that effectively block the hybridization of the assay's molecular beacon to the mutant *rpoB* sequence appear to be easily visible than those that merely inhibit probe hybridization [4].

We observed that all the samples with non-conforming rif susceptibility reports had low or very low MTB concentrations implying that bacterial load has a role to play in the interpretation of rif resistant and indeterminate strains. "Reference [11] reported a similar case on the effect of variable bacterial load on rif susceptibility status". It could be that low bacterial load limits the ability of the assay to correctly identify mutated and wildtype sequence in the core region of the *rpoB* gene.

According to [19], rif indeterminate result occurs when MTB concentration is very low and resistance could not be determined [19]. Although the MTB concentration was very low in sample 4, the C_T values of the probes were within rif susceptibility detection limits (1st and last probes had C_T values of 33.1 and 36.1 respectively) yet the assay could not determine rif susceptibility status. It is worthy to note that many samples that demonstrated very low MTB concentrations but probes hybridizing within the C_T detection limits (as evident in sample 4) had a rif determined susceptibility status. Conversely, sample 5 which ideally should read rif indeterminate because the first and last probes had C_T values exceeding the predetermined limits was equally reported as rif sensitive. This calls for review of assay parameters especially the C_T cut offs for establishing rif resistance and indeterminate status for Xpert version G3 machine.

Report has shown that considerable less hybridization of probe E can affect rif susceptibility status [11]. Another study identified the bead manufacturing scale-up and annealing temperature requirements of probe B as potential causes of false positive rif resistant result [2]. We recognized less hybridization of both probes E and B as possible causes of non conformance in the reporting of rif susceptibility status using C_T values. We therefore recommend revision of sequence length, the hybridization and software detection parameters for not just for probe E but also for A. This approach will decrease cases of misdiagnosis and increase the positive predictive value of Xpert MTB/RIF assay for detecting rif resistance.

5. Conclusion

Although the number of non-conformed specimens was low, it is important to have a good understanding of assay performance characteristic and always confirm rifampicin resistance and indeterminate results that failed to conform to the standard parameter values with phenotypic drug susceptibility testing before initiation of treatment. This is to curtail the adverse effects of misdiagnosis on not just the patient, but on the TB program and the community at large.

Acknowledgement

We gratefully acknowledge the National Tuberculosis and Leprosy Control program of Nigeria and KNCV/TB

CARE1 for supplying the geneXpert machine and the cartridges used for this study.

References

[1] Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 2010;48:229-237

[2] Van Rie A, Page-Shipp L, Scott L, et al. Xpert MTB/RIF for point-of-care diagnosis of TB in high HIV burden, resource limited countries: hype or hope? Expert Rev Mol Diagn 2010;10(7):937-946

[3] Ioannidis P, Papaventsis D, Karabela S, et al. Cepheid GeneXpert MTB/RIF assay for Mycobacterium tuberculosis detection and rifampin resistance identification in patients with substantial clinical indications of tuberculosis and smear-negative microscopy results. J.Clin. Microbiol. 2011; 49(8): 3068-3070

[4] Blakemore R, Story E, Helb D, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol 2010;48(7):2495-501

[5] Van Der Zanden AG, Te Koppele-Vije EM, Vijaya Bhanu N, et al. Use of DNA extracts from Ziehl- Neelsen-stained slides for molecular detection of rifampin resistance and spoligotyping of Mycobacterium tuberculosis. J Clin Microbiol 2003;41:1101-1108

[6] Steingart KR, Sohn H, Schiller I, et al. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database of Systematic Reviews 2013;Issue 1

[7] Piatek AS, Telenti A, Murray MR, et al. Genotypic analysis of Mycobacterium tuberculosis in two distinct populations using molecular beacons: implications for rapid susceptibility testing. Antimicrob Agents Chemother 2000;44(1):103-10

[8] Lawn SD, Nicol MP. Xpert®MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol 2011;6:1067-1082

[9] Tyagi S, Kramer FR. Molecular beacons: probes that fluoresce upon hybridization. Nat Biotechnol 1996;14:303–308

[10] Piatek AS, Tyagi S, Pol AC, et al. Molecular beacon sequence analysis for detecting drug resistance in Mycobacterium tuberculosis. Nat Biotechnol 1998;16:359–363

[11] Somoskovi A, Deggim V, Ciardo D, et al. Diagnostic implicationsof inconsistent results obtained with Xpert MTB/RIF assay in detection of Mycobacterium tuberculosis isolates with an *rpoB* mutation associated with low-level rifampin resistance. J Clin Microbiol 2013;51(9):3127-3129

[12] Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 2011;377:1495-1505

[13] Moure R, Munoz L, Torres M, et al. Rapid detection of Mycobacterium tuberculosis complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. J Clin Microbiol 2011; 49:1137-39

[14] Nicol MP, Workman L, Isaacs W, et al. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. Lancet Infect Dis. 2011;11:819-24

[15] Marlowe EM, Novak-Weekley SM, Cumpio J, et al. Evaluation of the cepheid Xpert MTB/RIF assay for direct detection of Mycobacterium tuberculosis complex in respiratory specimens. J Clin Microbiol 2011;49:1621-1623

[16] Armand S, Vanhuls P, Delcroix G, et al. Comparison of the Xpert MTB/RIF test with an IS6110-TaqMan real-time PCR assay for direct detection of Mycobacterium tuberculosis in respiratory and non respiratory specimens. J Clin Microbiol 2011; 49:1772-1776

[17] Theron G, Peter J, van Zyl-Smit R, et al. Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. Am J Respir Crit Care Med 2011;184:132-140

[18] Federal Ministry of Health, Department of Public Health, National tuberculosis and leprosy control programme. Guidelines for the clinical management and control of drug resistant tuberculosis in Nigeria. Federal Republic of Nigeria, July 2011 ; Pg 32-58

[19] Cepheid (2009). Cepheid brochure: Xpert®Mtb/RIF. Two-hour detection of MTB and resistance to rifampicin. Available: Http://www.cepheid.com/