



## Rifampicin susceptibility discordance between Xpert MTB/RIF G4 and Xpert Ultra before MDRT-TB treatment initiation: A case report from Uganda

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### ABSTRACT

Tuberculosis (TB) resistance to rifampicin, the most powerful drug leads to increase in mortality. Globally, half a million new patients develop such resistant TB each year, coupled with both inappropriate diagnosis and treatment initiation.

We report a case of rifampicin resistant *Mycobacterium tuberculosis* whose rifampicin resistance was missed by Xpert MTB/RIF Assay G4 but detected by the Xpert MTB/RIF Ultra assay at different time points leading to increased delays for MDR-TB treatment initiation at Mulago Hospital, Kampala, Uganda. Our case report compels greater urgency in accelerating the transition to the newer assay, Ultra, to benefit from higher sensitivity of rifampicin resistance detection.

### 1. Introduction

Treatment of tuberculosis (TB) becomes challenged when the disease causing agent, *M. tuberculosis*, becomes resistant to rifampicin. Worldwide, half a million new patients develop rifampicin-resistant (RR-)TB each year, of whom only a minority are recognized and treated appropriately[1]. In the last decade, the GeneXpert® MTB/RIF assay (Xpert; Cepheid, Sunnyvale, CA, USA), a rapid qPCR-based assay, has revolutionized the diagnosis of TB and its resistance to rifampicin[2]. In Uganda, Xpert has been gradually rolled-out since 2011, allowing nationwide access for TB diagnosed among presumptive TB patients. Since 2017, Uganda started a phased replacement of Xpert to the next generation XpertMTB/RIF Ultra (Ultra; Cepheid, Sunnyvale, CA, USA). Patients who are resistant to rifampicin are initiated on multi-drug resistant TB (MDR-TB) treatment as they wait for full drug susceptibility testing results. Here we present a discordant RR-TB case with associated delay in appropriate MDR-TB treatment initiation cause by

discordance between two versions of Xpert.

### 2. Case report

A 40-year old, HIV seronegative male presented to Mulago national referral hospital, Kampala, Uganda in September 2019 with a five-month history of cough, weight loss, poor appetite and a month's history of haemoptysis. He had no prior history of exposure to anti-TB medication and no known TB contact. The patient was referred from the general health facility in Kampala with rifampicin resistance (RR) detected on 15th August 2019 using Xpert MTB/RIF Ultra assay (Ultra; Cepheid, Sunnyvale, CA, USA). Upon arrival at the Multi-drug resistant TB (MDR-TB) treatment unit sputum sample was requested for repeat testing to estimate the bacterial load before MDR-TB treatment initiation basing on cycle-threshold (Ct) values as a requirement for clinical trial eligibility. The Xpert testing was repeated from the trial laboratory, the College of American Pathologist Accredited Mycobacteriology (BSL-3)

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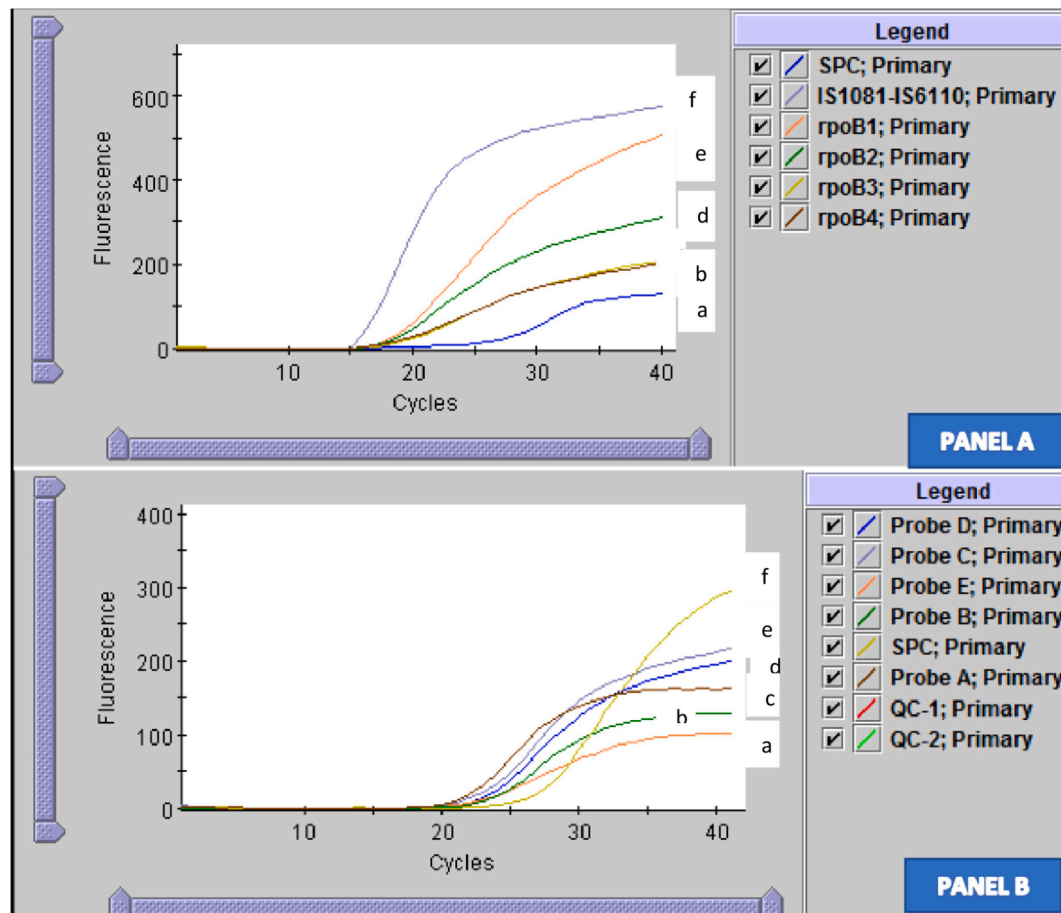
E-mail address: [willyssengooba@gmail.com](mailto:willyssengooba@gmail.com) (W. Ssengooba).

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**Table 1**  
Sample flow and results.

Sputum samples	Collection date	Xpert MTB/RIF Ultra (MDR-TB clinic)	Xpert MTB/RIF Ultra (Makerere)	Xpert MTB/RIF Assay G4 (Makerere)	Xpert MTB/RIF Ultra (NRL)	Smear result	LPA	pDST on isolate (ITM)	LPA on isolate (ITM)	Xpert MTB/RIF G4 on isolate (ITM)	Xpert Ultra on isolate (ITM)	rpoB sequencing on isolate
Sample 1	15/08/2019	MTB detected High, Rifampicin resistance Detected	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sample 2	19/08/2019	ND	ND	MTB detected High, Rifampicin resistance NOT detected	ND	3+	RIF-S	ND	ND	ND	ND	ND
Sample 3	22/08/2019	MTB detected High, Rifampicin resistance Detected	ND	MTB detected High, Rifampicin resistance NOT detected	ND	ND	ND	ND	ND	ND	ND	ND
Sample 4 Screening	23/08/2019	ND	ND	MTB detected Medium, Rifampicin resistance NOT detected	ND	ND	ND	ND	RIF-S Weak MUT3	MTB detected Medium, Rifampicin resistance not detected	MTB detected High, Rifampicin resistance not detected	WT
Sample 5	27/08/2019	ND	ND	ND	MTB detected, Rifampicin resistance Detected	ND	ND	ND	ND	ND	ND	ND
Enrolment	29/08/2019	ND	ND	ND	ND	ND	ND	RIF-R	RIF-S Weak MUT3	MTB detected High, Rifampicin resistance not detected	MTB detected High, Rifampicin resistance not detected	ND
Sample 6	03/09/2019	ND	MTB detected, Medium, Rifampicin resistance Detected	ND	ND	ND	RIF-R					

RIF-R = Rifampicin Resistant, RIF-S = Rifampicin Susceptible, ND = Not Done, pDST = Phenotypic Drug Susceptibility Testing using Lowenstein Jensen (LJ), MTB = M. tuberculosis.



**Fig. 1.** Patterns of probes hybridization in both, Xpert MTB/RIF Ultra Assay (panel A) and Xpert MTB/RIF Assay G4 (panel B). Key: PANEL A = The probe for rifampicin resistance Detected in Xpert MTB/RIF Ultra assay, a = SPC, b = rpoB4, c = rpoB3, d = rpoB2, e = rpoB1, and f = IS1081-IS6110. PANEL B = The probes amplified with rifampicin resistance NOT detected. a = probeE, b = Probe B, c = probe A, d = probe D, e = probe C and f = SPC.

laboratory at Makerere University, Kampala, Uganda. The trial laboratory used Xpert MTB/RIF Assay G4, as it was always the test used for repeat testing of all potential trial participants.

The repeat test with Xpert MTB-RIF Assay G4 was done on 19th August 2019 on a second smear positive (3+) sample and turned out to be “MTB detected high RR NOT detected” (Table 1). This created a discordance and led to repeat testing at the MDR-TB clinic on 22 August 2019 by Ultra assay and the results remained “MTB detected high, RR Detected”. The trial laboratory could not believe the results and requested for the same sample to be tested again at the trial laboratory on 23 August 2019 and results did not change “MTB detected High, RR NOT detected”. The trial laboratory requested for a fresh sample which was tested again on 23 August 2019 and the results were “MTB detected Medium, RR NOT detected”. On 27th August 2019, a new/5th sample was sent to the National TB Reference Laboratory (NTRL) as a tie breaker, where Ultra was used and results turned out to be “MTB detected, RR Detected”. On 03rd September 2019, the trial laboratory repeated the testing on a new/6th sample with Ultra Assay and results were “MTB detected, Medium, RR Detected”. At this point we confirmed that the discordance was between the two versions of GeneXpert with Ultra Assay consistently detecting RR in this particular patient whereas Xpert MTB/RIF Assay G4 was consistently missing it (Table 1). The Fig. 1 shows the patterns of probes hybridization in both, Ultra Assay (panel A) and Xpert MTB/RIF Assay G4 (panel B). The melting peak temperature for the Ultra-probes were *rpoB1* 69.3, *rpoB2* 73.1, *rpoB3* 75.1 and *rpoB4* 65.1, whereas for *rpoB4* mutant was 73.6.

On the other investigation, line probe assay (LPA) MTBDR<sub>plus</sub> and MTBDR<sub>sl</sub> assays were done on 20th August 2019 on the same/2nd

sample to assess the patient for resistance to isoniazid and second line anti TB medicines; aminoglycosides and fluoroquinolones. Results for LPA were; sensitive to all drugs. Repeat LPA testing was done on 29th August 2019 on a 6th sample and results were rifampicin (WT8 and MUT3) and isoniazid resistant (*KatG* MUT1), but susceptible to aminoglycosides and fluoroquinolones. After culture, DST was set for Rifampicin (1ug/mL) and isoniazid (0.2ug/mL) using both mycobacterial growth indicator tube (MGIT 960) and Agar proportional method (APM). Results of MGIT DST reported on 21/November 2019 were sensitive to both drugs whereas the isolate was resistant on APM for both drugs.

The patient was examined again and he was in fair general condition, wasted, temperature was 37 °C. No jaundice, no lymphadenopathy, no digital clubbing. The systemic examination was unremarkable. The patient was subjected to chest radiograph which showed bilateral upper and lower lung field infiltrates, with cavitation on the left (Fig. 2). Other laboratory testing revealed a normal complete blood count, normal renal & liver function tests, except for low serum Albumin of 29 g/L. He was initiated on second line Anti TB medicines on 29th August 2019 including: Kanamycin (for 4-months), together with Isoniazid (400 mg daily), Prothionamide, Clofazimine, Ethambutol, Levofloxacin and Pyrazinamide, to make a total of 10 months of treatment. The screening sample 4 (23/08/2019) and the treatment initiation sample (enrolment, 29/08/2019) were shipped to Institute of Tropical Medicine (ITM) in Antwerp, Belgium to perform Phenotypic Drug Susceptibility Testing using eggs-based medium Lowenstein Jensen (pDST) on the enrolment sample, Xpert G4 and Ultra on both samples and *rpoB* gene sequencing on the screening *M. tuberculosis* isolate. Results from ITM varied between

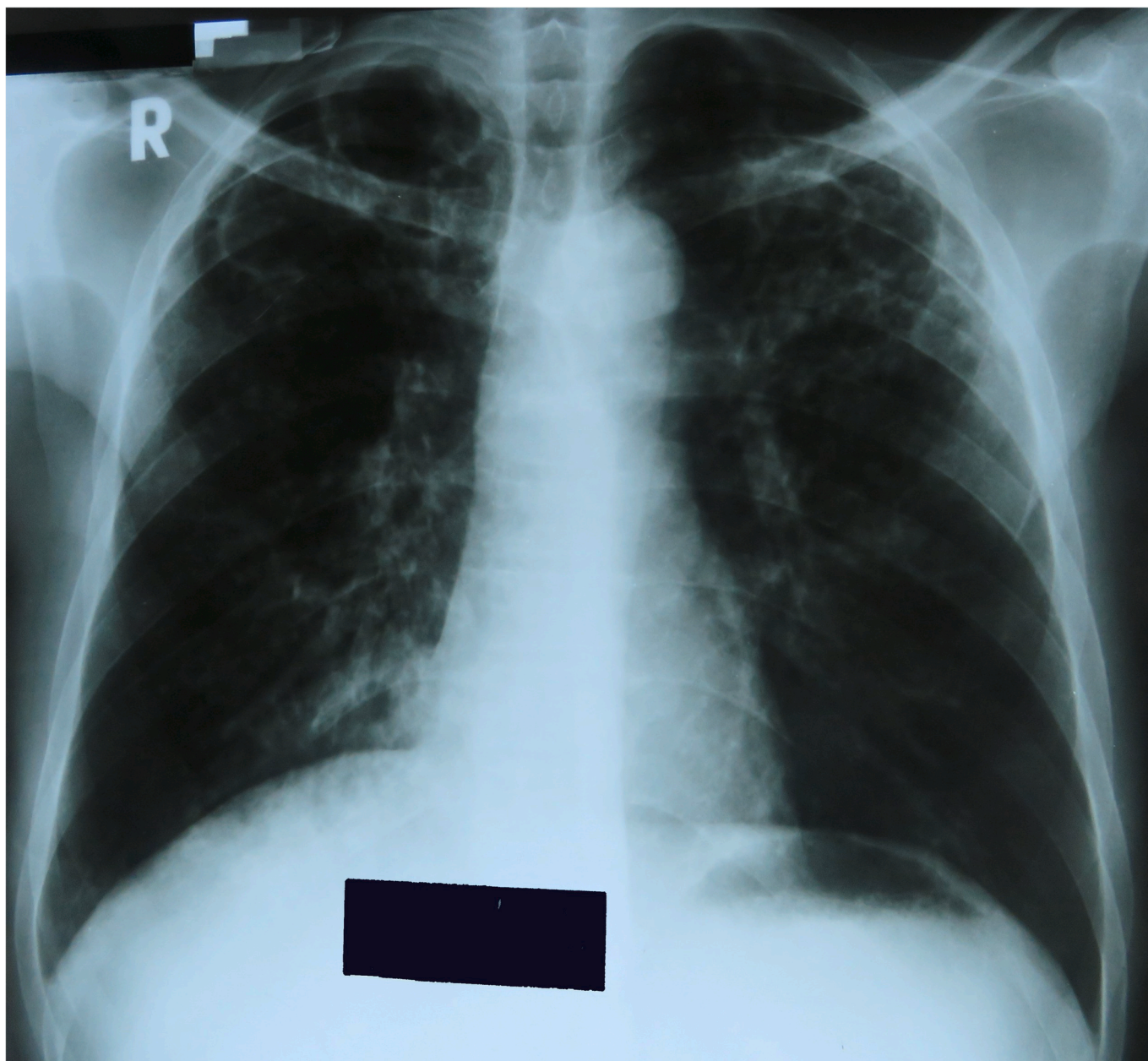


Fig. 2. Chest radiograph. Chest radiograph which shows bilateral upper and lower lung field infiltrates, with cavitation on the left.

tests and samples, with LPA suggestive of hetero-resistance in both samples, pDST resistant to rifampicin, Ultra and G4 assays on the screening and enrolment isolates showed resistance to rifampicin not detected and *rpoB* Sanger sequencing revealed wild type (WT) on the screening isolate [Table 1](#).

### 3. Discussion

We present a case report concerning an MDR-TB patient with discordant *Mycobacterium tuberculosis* results for RR between the Ultra and the Xpert MTB/RIF Assay G4 and the associated delays towards treatment initiation. Ultra assay was endorsed in March 2017 by the World Health Organization (WHO) to replace Xpert MTB/RIF Assay G4 for diagnosis of *Mycobacterium tuberculosis* and detection of rifampicin resistance [\[3,4\]](#). This was based on the improved sensitivity and specificity for both diagnosis and detection for rifampicin resistance. Apart from modifications in the design of the sloppy molecular beacon probes there are mainly two modifications made on the cartridge toward Ultra

including a larger DNA amplification chamber of 50  $\mu$ l which is double the amount allowable for the Xpert MTB/RIF Assay G4, and two molecular targets for *Mycobacterium tuberculosis* i.e. IS1081 and IS6110.

To date, several high TB burden countries have been slow toward its implementation and most have not yet fully transitioned to Ultra. The discordance between the 1st and 2nd LPA for resistance determination could have been a technical challenge since resistance to both rifampicin and isoniazid were missed in the first test. Discordance was not only between Xpert assays but also between the pDST methods. Such discordance usually require sequencing of the *rpoB* gene to be resolved to investigate the presence of mutations reported to be missed by MGIT-DST and some other pDST methods[\[5\]](#). Sequencing of the *rpoB* gene of the screening isolate at ITM revealed wild type strain for rifampicin highlighting more diagnostic challenges associated with hetero-resistance strains, yet Sanger sequencing is less sensitive to detect particular strains than the pDST or LPA. Previous studies have found mixed strains of *M. tuberculosis* to lead to false-negative results for rifampicin resistance on Xpert MTB/RIF Assay G4 which is also

associated with poor clinical outcome[6]. A recent study in Rwanda found out that reducing diagnostic and treatment delays reduces rifampicin-resistant tuberculosis mortality[7]. Deeplex® MycTB or Whole Genome Sequencing are more sensitive than Sanger sequencing to detect hetero-resistance or mixed infections[8].

Our report presents a case that uncovers diagnostic challenges towards confirming MDR-TB as well as the associated delays in resolving the discordances before MDR-TB treatment initiation. It also confirms that Ultra can detect RR better than the Xpert MTB/RIF G4 Assay. In our case, with access to other standard facilities such as the Uganda NTRL for results comparison, it took ~20 days (from 15th August to 03rd September 2019) to resolve discordance. Ultra has been found to unambiguously identify a wide range of RRDR mutations [9], referred to as “disputed mutations” which had been reported to be missed by the pDST [10–12], but none have been reported between two versions of GeneXpert assays currently in use. This is because the nature of the discordance is different, Xpert detects disputed mutations which are missed by the phenotypic drug susceptibility testing. Another study of follow-up GeneXpert testing found the RR detection discordance (Xpert MTB/RIF G4 false resistance) to be associated with Xpert probe delay and mainly probe B [13], with 22% of the discordance associated with delayed probe E (Ct-values 16.5, 17.9, 17.2, 17.8 and 19.0 for probes A, B, C, D and E). This discordance reported in the literature is due to false resistance of Xpert G4[14], whereas in the case presented here the “resistant result” was obtained with Ultra. Ultra has higher sensitivity and lower specificity to detect MTB but with higher sensitivity and specificity for RR detection [15]. Contrary to the previous study’s association of GeneXpert discordance among sample with MTB detected, very low and RR detected and recommendation for repeat testing for such cases[16], our case indicated persistence of the discordance for RR in presence of high levels of MTB detected due to hetero-resistance. It is worth noting that many low income settings have a low coverage for drug susceptibility testing[17]. For example, Uganda with approximately 1700 TB diagnostic health facilities, only 264 (15.5%) currently have Xpert machines, and all transitioned to Ultra. This makes access another contributing factor for delay in appropriate treatment initiation.

#### 4. Conclusions

The source of discordance may not differ much from the previous comparisons between tests and in samples with suspected hetero-resistance, but our findings present a real life implication of diagnostic discordances towards early MDR-TB treatment initiation. Such discordance should be resolved by Deeplex® MycTB or Whole Genome Sequencing methods. This dilemma further highlights that the implementation of Xpert MTB/RIF Ultra, which seems to detect hetero-resistance better than G4, should be highly recommended.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### References

- [1] World Health Organization. Global tuberculosis report 2018. *World Health Organization Document* 2018; WHO/CDS/TB/2018.20: 1-231.
- [2] Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010; 363(11):1005–15.
- [3] Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, et al. The New Xpert MTB/RIF Ultra: Improving Detection of Mycobacterium tuberculosis and Resistance to Rifampin in an Assay Suitable for Point-of-Care Testing. *mBio* 2017;8(4). <https://doi.org/10.1128/mBio.00812-17>.
- [4] WHO. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/MTM/TB/2017.04). Licence: CC BY-NC-SA 3.0 IGO. URL: <https://www.who.int/tb/publications/2017/XpertUltra/en/> Accessed 09 July 2019. 2017.
- [5] Rigouts L, Gumusboga M, de Rijk WB, Nduwamahoro E, Uwizeye C, de Jong B, et al. Rifampin Resistance Missed in Automated Liquid Culture System for Mycobacterium tuberculosis Isolates with Specific rpoB Mutations. *J Clin Microbiol* 2013;51(8):2641–5.
- [6] Zetola NM, Shin SS, Tumedji KA, Moeti K, Ncube R, Nicol M, et al. Mixed Mycobacterium tuberculosis complex infections and false-negative results for rifampin resistance by GeneXpert Ultra are associated with poor clinical outcomes. *J Clin Microbiol* 2014;52(7):2422–9.
- [7] Ngabonziza J-C, S, Habimana YM, Decroo T, Migambi P, Dushime A, Mazarati JB, et al. Reduction of diagnostic and treatment delays reduces rifampicin-resistant tuberculosis mortality in Rwanda. *Int J Tuberc Lung Dis* 2020;24(3):329–39.
- [8] Ng KCS, Supply P, Cobelens FGJ, Gaudin C, Gonzalez-Martin J, de Jong BC, et al. How Well Do Routine Molecular Diagnostics Detect Rifampin Heteroresistance in Mycobacterium tuberculosis? *J Clin Microbiol* 2019;57(11). <https://doi.org/10.1128/JCM.00717-19>.
- [9] Ng KCS, van Deun A, Meehan CJ, Torrea G, Driesen M, Gabriëls S, et al. Xpert Ultra Can Unambiguously Identify Specific Rifampin Resistance-Confering Mutations. *J Clin Microbiol* 2018;56(9). <https://doi.org/10.1128/JCM.00686-18>.
- [10] Mwanza W, Milimo D, Chilufya MM, Kasese N, Lengwe MC, Munkondya S, et al. Diagnosis of rifampicin-resistant tuberculosis: Discordant results by diagnostic methods. *Afr J Lab Med* 2018;7(2). <https://doi.org/10.4102/ajlm.v7i2.806>.
- [11] Ajbani K, Kazi M, Tornheim J, et al. Pyrosequencing to resolve discrepant Xpert MTB/RIF and Mycobacterial Growth Indicator Tube 960. *Lung India* 2018;35(2): 168–70.
- [12] Torrea G, Ng KCS, Van Deun A, André E, Kaisergruber J, Sengooba W, et al. Variable ability of rapid tests to detect Mycobacterium tuberculosis rpoB mutations conferring phenotypically occult rifampicin resistance. *Sci Rep* 2019;9(1). <https://doi.org/10.1038/s41598-019-48401-z>.
- [13] Berhanu RH, Schnippel K, Kularatne R, et al. Discordant rifampicin susceptibility results are associated with Xpert(R) MTB/RIF probe B and probe binding delay. *Int J Tuberc Lung Dis* 2019;23(3):358–62.
- [14] Ngabonziza JCS, Decroo T, Migambi P, Habimana YM, Van Deun A, Meehan CJ, et al. Prevalence and drivers of false-positive rifampicin-resistant Xpert MTB/RIF results: a prospective observational study in Rwanda. 2020 *Lancet. Microbe* 2020;1(2):e74–83.
- [15] Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, 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(1):76–84.
- [16] Sahrin M, Rahman A, Uddin MKM, et al. Discordance in Xpert(R) MTB/RIF assay results among low bacterial load clinical specimens in Bangladesh. *Int J Tuberc Lung Dis* 2018;22(9):1056–62.
- [17] Ismail F, Omar SV, Blows L, Gardee Y, Koornhof H, et al. Drug resistant tuberculosis in Africa: Current status, gaps and opportunities. *Afr J Lab Med* 2018;7(2). <https://doi.org/10.4102/ajlm.v7i2.781>.