



RESEARCH LETTER

DISCORDANCE BETWEEN XPERT MTB/RIF ASSAY AND LINE PROBE ASSAY FOR DETECTION OF RIFAMPICIN RESISTANCE: HOW TO INTERPRET?

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A 9-year-old girl presented in March 2024 with fever, night sweats, and a left cervical lymphadenopathy for 1 month. Investigations are shown in Table 1. Fine needle aspiration cytology of the cervical lymph node showed necrotizing granulomatous lymphadenitis. Gastric lavage and stool Xpert MTB/Rif was negative. Chest X-ray was normal. She was started on first-line antitubercular therapy with isoniazid(H), rifampicin(R), pyrazinamide(Z), and ethambutol(E) in March 2024 in view of histopathological findings. In April 2024, she underwent left cervical lymph node biopsy which detected rifampicin-sensitive *Mycobacterium tuberculosis* on Xpert MTB/Rif. Neck ultrasound in May 2024 showed left submandibular, supraclavicular, and posterior triangle lymphadenopathy (largest: 10x8 mm) with loss of hilar reflectivity, and a cold abscess in the left posterior triangle measuring 7x3 mm. Neck ultrasound repeated in August 2024 showed reduction in the size of previously enlarged nodes. The patient was shifted onto continuation phase with HRE. In August 2024, pus *Mycobacteria* Growth Indicator

Tube (MGIT) was positive and phenotypic three-drug susceptibility testing revealed pyrazinamide resistance. First-line (FL) and second-line (SL) line probe assay (LPA) performed in August 2024 revealed resistance to rifampicin and fluoroquinolones. Her regimen was not changed. Neck ultrasound in January 2025 showed a complete resolution of the previously enlarged lymph nodes. ATT was stopped in January 2025 after 10 months and she was asked to follow-up in 3 months with a neck ultrasound.

The reason attributed for this discordance is cross-contamination in LPA. In LPA, the sequential steps of sample decontamination and nucleic acid extraction, PCR amplification, and hybridization, are performed in three different rooms. This open system predisposes the LPA to microbial contamination.^{1,2,3} MGIT, is a liquid-culture based technique, and thus is also predisposed to contamination at rates as high as 30%.⁴ LPA was performed on the MGIT culture isolate 3 months after the initial specimen was collected and thus any contamination during the 6-week processing of MGIT

Table 1. Investigations of the patient.

Parameters	March 2024	May 2024	August 2024	October 2024	November 2024	January 2025	Reference Ranges
Hemoglobin (gm/dL)	10.7	12.8	11.9	12.2	12.4	-	11.5-15.5
White blood cell count (cells/cumm)	10,060	5200	8000	5700	8450	-	5000-13,000
Absolute neutrophil count (cells/cumm)	7143	2236	6720	2394	4470	-	2000-8000
Absolute lymphocyte count (cells/cumm)	2113	2600	800	2850	3842	-	1000-5000
Platelets (10 ⁵ cells/cumm)	3.03	3.42	2.36	3.71	3.45	-	1.50-4.50
ESR (mm/hr)	70	35	25	60	15	22	0-10
ALT (IU/L)	11.1	23.1	-	-	-	19	<41

Note : ESR- Erythrocyte sedimentation rate, ALT- Alanine aminotransferase.

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may result in false-positive results on LPA.³ On the other hand, Xpert MTB/Rif is performed in a closed system in an automated fashion, and it has a lower risk of cross-contamination.³

Other causes of discordance proposed include mixed infections/heteroresistance.^{1,2} Reports have been found that show rifampicin resistant strains not being detected and resulting in falsely sensitive results on Xpert MTB/Rif assays in patients with mixed infections.¹ Heteroresistance, from the presence of both susceptible and resistant MTB populations, or from the endogenous development of two sub-populations of MTB during treatment, may result in the resistant strain being detected on LPA and the sensitive strain being detected on Xpert MTB/Rif. Since our patient had received 1 month of first-line ATT prior to sample collection and testing with Xpert MTB/Rif and LPA, it is possible that a heteroresistant strain emerged during this period.²

Since the discordant LPA results were obtained after 5 months of first-line ATT and after shifting to continuation phase based on clinico-radiological improvement, we attributed it to contamination in LPA and not to mixed infections/heteroresistance. However, close follow-up with neck ultrasound was advised to monitor for recurrence after treatment completion.

Compliance with Ethical Standards

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Conflict of Interest None

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