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Strengthening Mycobacterial Culture and Identification Capacity through a Multi-Year Proficiency Testing Program in a Regional TB Laboratory Network

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Abstract

Introduction

National TB Reference Laboratories, supported by the WHO Supranational Reference Laboratory Network (SRL), are central to TB diagnosis and quality assurance globally. Currently, low income settings are challenged with access to external quality assurance (EQA) programs. To assess the feasibility and performance, SRL-Uganda developed and implemented a multi-year ISO/IEC 17043:2023-aligned Mycobacterial culture and identification proficiency testing (PT) scheme to strengthen quality-assured culture and identification across regional laboratory networks

Methods

Mycobacterial culture proficiency testing materials were prepared at SRL-Uganda using well-characterized *M. tuberculosis* (H37Rv) and non-tuberculous mycobacteria reference strains under BSL-3 conditions. Materials were quality-checked, diluted, and assessed for stability over 21 days using Lowenstein–Jensen (LJ) medium and BACTEC MGIT 960 system. A pilot PT round in Uganda (2021) preceded three annual PT rounds distributed to 32 laboratories across 21 sub-Saharan African countries, with standardized scoring, consensus analysis, and performance classification aligned to ISO/IEC 17043:2023.

Results

The prepared mycobacterial culture and identification proficiency materials are stable up to 21 days at ambient temperature. Across three proficiency testing rounds, overall laboratory performance improved substantially, reaching 96.2% satisfactory results in the final round. Proficiency testing performance varied across rounds, declining in 2022 Round 2 but improving markedly by 2023, when over 96% of laboratories achieved satisfactory performance (≥ 80%), reflecting strengthened laboratory capacity over time.

Conclusion

Our findings demonstrate the feasibility of locally produced, stable PT materials and highlight the effectiveness of structured EQA in strengthening TB diagnostic capacity in resource-limited, high-burden settings.

Introduction

Globally, the National TB Reference Laboratories (NTRL) play an essential role in diagnosing Tuberculosis (TB) and monitoring its treatment[1]. It is tasked with developing the national plan for TB laboratory services, overseeing quality assurance (QA) for all procedures carried out in intermediate-level laboratories, and providing guidance to lower-tier TB laboratories[1]. Furthermore, the NTRL network receives support from the Supranational Reference Laboratory (SRL) it subscribes to. Founded in 1994, the WHO Supranational Reference Laboratory Network (SRLN) has steadily expanded and now supports countries by ensuring high-quality culture and drug susceptibility testing (DST), providing external quality assessment (EQA), and offering training in culture and DST, among other roles[2]. Since its designation in April 2013, the Uganda Supranational Reference Laboratory (SRL-Uganda), as a member of the SRLN, has been instrumental in strengthening and coordinating tuberculosis laboratory networks throughout Sub-Saharan Africa[3].This enhancement involves delivering standardized quality assurance for tuberculosis through the distribution of proficiency testing materials.

Mycobacterial culture in the TB testing arena remains the most sensitive approach for the detection of *M. tuberculosis* in clinical specimens which provides for all subsequent testing, including identification and DST as well as assisting in monitoring response to treatment[4]. Following decontamination by a preferred method, specimens may be cultured for *M. tuberculosis* complex and non-tuberculous mycobacteria (NTM) using solid media such as Lowenstein-Jensen (LJ) and Middlebrook 7H10 or 7H11 agars, as well as commercial semi-automated broth-based systems like the BACTEC MGIT 960 (Becton Dickinson, USA)[5]. According to the WHO consolidated TB treatment guidelines, monthly sputum culture is recommended to monitor patient response during MDR/Rifampicin Resistant (RR)-TB treatment [6].

Significant efforts have been directed toward innovating mycobacterial culture diagnostics however, less attention has been paid to ensuring their quality-assured use [7]. To ensure active TB is diagnosed accurately and promptly, laboratories should utilize comprehensive internal and external quality assurance (IQA and EQA) mechanisms[8]. Participation of TB diagnostic laboratories in EQA programs coordinated by international organizations and/or accredited providers has proven effective in maintaining and improving laboratory proficiency globally[9]. However, few quality assessment tools are available to validate results generated using *M. tuberculosis* complex culture methods in Sub-Saharan Africa, owing to limited access, high costs of procuring proficiency testing (PT) materials, suboptimal human resource capacity, customs and shipping expenses, and challenges in sustaining quality assurance systems[10][11]. In addition, most global *M. tuberculosis* culture proficiency testing (PT) schemes supply participants with culture isolates that are directly sub-cultured onto appropriate media without prior decontamination. This approach does not accurately reflect routine laboratory working *M. tuberculosis* culture processes, where specimens typically undergo decontamination before inoculation into TB culture media[12][13].

Building on the ISO 17043-accredited proficiency testing program at SRL-Uganda, we developed a PT scheme for Mycobacteria culture and identification to support quality-assured testing in Sub-Saharan African countries. This paper presents findings from a pilot and a four-year (four-round) Mycobacteria culture and identification proficiency testing (PT) program, detailing its development, implementation, experiences, and recommendations across 32 TB culture laboratories in 22 Sub-Saharan African countries and beyond.

Methodology Materials and Methods

Specimen Preparation

Mycobacteria PT culture materials were prepared at SRL Uganda using in-house standard operating procedures. In summary, three well-characterized reference strains representing the desired *Mycobacterium tuberculosis* (Mtb) identification profiles were used: one Mtb complex strain (H37Rv/ATCC 27294) and two non-tuberculous mycobacteria (NTM), *M. fortuitum* (ATCC 19542) and *M. kansasii* (ATCC 12478), all retrieved from permanent stocks maintained at − 80°C. The *M. tuberculosis* culturing and manipulation was performed in a Bio Safety Level-3 (BSL3) laboratory. The isolates were recovered by sub-culturing on selective Middlebrook 7H11 agar (Sigma-Aldrich® Germany), incubated at 37°C in a CO_2 incubator (Thermal Scientific, USA), and observing weekly for growth of the organisms for 3–4 weeks until they tested positive (without contamination). The plates were examined for growth to ensure that the morphology of the colonies is consistent with the chosen species of mycobacteria. Following sufficient growth, mycobacterial stock suspensions were prepared to a McFarland 1.0 standard (approximately 3.0×10^8 organisms/mL). The suspensions were then diluted to 1:50 for H37Rv and 1:50,000 for the non-tuberculous mycobacteria (NTM). For preparation of TB culture proficiency testing (PT) materials, equal volumes of diluted mycobacterial suspension and 7H9 Middlebrook broth (Becton Dickinson, USA)

were mixed, specifically 2 mL of mycobacterial suspension with 2 mL of 7H9 broth. Undiluted Middlebrook 7H9 broth was used to represent culture-negative PT materials. Purity of the mycobacterial stock suspensions was assessed using blood agar (Sigma-Aldrich®, Germany) to detect contamination, alongside SD TB MPT64 (SD Biosensor, South Korea) rapid testing and the GeneXpert (Cepheid®, USA) assay for identification of *M. tuberculosis* complex and non-tuberculous mycobacteria

Stability testing

To assess the stability of *M. tuberculosis* (Mtb) culture proficiency testing (PT) materials over time, a panel comprising Mtb complex H37Rv, *M. fortuitum*, and *M. kansasii* suspensions was prepared by dilution in equal volumes of Middlebrook broth as previously described and stored at 18–28°C. At SRL-Uganda, samples were analysed at baseline (day 0), day 7, and day 21. At each time point, mycobacterial culturing was performed on Lowenstein–Jensen (LJ) medium and using the BACTEC MGIT 960 system. For each isolate and time interval, three samples were processed and decontaminated using the standard N-acetyl-L-cysteine/sodium hydroxide (NALC/NaOH) method with a final NaOH concentration of 1.5%. An equal volume of NALC/NaOH solution was added to each specimen and incubated for 15–20 minutes. Following centrifugation at 3000 × g for 15 minutes, the resulting pellet was suspended in 3–5 mL of sterile phosphate-buffered saline (pH 7.4). The sediment was then inoculated on the LJ and BACTEC MGIT media and incubated at 37° C till positive. For positive culture, *Mycobacterium tuberculosis* complex and NTM confirmatory tests were performed as described by Global Laboratory initiative[14]. We collected data on time to positivity and *Mtb* outcomes.

The pilot study (dup: abstract ?)

The pilot study
The initial round of proficiency testing (PT) was conducted as a pilot exercise between April and August 2021 and involved three tuberculosis culture laboratories in Uganda. Each participating laboratory received a panel of ten samples, comprising two culture-negative samples, four non-tuberculous mycobacteria (NTM) samples, and four *Mycobacterium tuberculosis* complex samples, prepared as previously described. The panels were packaged and transported at ambient temperature and accompanied by standardized testing instructions.

Participating laboratories were instructed to analyze the PT materials using their routine mycobacteria culture procedures. Upon completion of testing, results were recorded on standardized reporting forms and categorized as *M. tuberculosis* complex, no growth, NTM/Mycobacteria Other Than Tuberculosis (MOTT), no result, or contaminated. Completed forms were manually filled, scanned, and submitted electronically to the Uganda Supranational Reference Laboratory (SRL-Uganda). In addition to test outcomes, data were collected on laboratory performance indicators, specimen transit time, decontamination methods, and mycobacteria culture methodologies used.

PT program: three rounds

As part of the ongoing culture-based proficiency testing (PT) program, laboratories within the Uganda Supranational Reference Laboratory (SRL) network receive *Mycobacterium tuberculosis* (Mtb) culture and identification PT panels annually. Since the program's inception in 2021, four PT rounds have been organized and

distributed to laboratories in 21 countries across sub-Saharan Africa, with complete cycles between 2021 and 2023. A total of 32 participants were enrolled across the three annual rounds shipped each August of the year.

Similar to the pilot phase, each round comprised ten PT samples representing different categories, including *Mycobacterium tuberculosis* complex (MTBC), non-tuberculous mycobacteria (NTM/MOTT), and no growth. The panels were prepared, packaged, and distributed at ambient temperature. Participants manually recorded their results on standardized reporting forms, which were scanned and submitted to the Uganda SRL for analysis.

Data collected included PT material performance, shipment transit time, decontamination procedures, and *Mtb* culture methods used. Analysis was conducted using a semi-automated Microsoft Excel-based workbook with pre-set formulas aligned to expected result indicators.

Participant performance was scored by assigning 10 points for each correctly identified sample and 0 points for discrepant results. Final scores were expressed as the percentage of correctly identified MTBC, NTM/MOTT, and no-growth samples. Reports also included consensus results aggregated across all participating laboratories. Consensus for each PT sample was defined as ≥ 80% agreement between participant results and the expected outcome. Overall laboratory performance was classified qualitatively, with a minimum threshold of 80% correct results considered acceptable proficiency

Uganda SRL M.tb culture PT network

The Uganda National TB Reference Laboratory became WHO SRL in April 2013 with defined key roles[3][2]. One of the core functions of the Uganda Supranational Reference Laboratory (SRL-Uganda) is the provision of external quality assurance (EQA) for tuberculosis (TB) reference laboratories through the supply of proficiency testing (PT) materials covering all TB diagnostic assays performed by National Reference Laboratories (NRLs). In addition, Uganda's strategic geographic location and advanced laboratory capacity position SRL-Uganda as a regional hub for public health laboratory functions in sub-Saharan Africa. SRL-Uganda has established an international network of laboratories to facilitate centralized evaluation and comparison of TB diagnostic testing across the *Mycobacterium tuberculosis* culture laboratory network. The laboratories currently participating in this network as shown Fig. 1 are located in Angola, Botswana, Burundi, Eritrea, Eswatini, Ethiopia, Kenya, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Namibia, Pakistan, Rwanda, Somalia, Somaliland, Tanzania, Uganda, Zambia, and Zimbabwe.

Results

Stability study

Time to positivity increased progressively with storage duration across all organisms and culture methods. Overall, BACTEC MGIT 960 consistently demonstrated shorter times to positivity than Löwenstein–Jensen (LJ) media at baseline, 7 days, and 21 days. *M. fortuitum* showed the shortest time to positivity, followed by *M. kansasii*, while *M. tuberculosis* H37Rv exhibited the longest detection times, particularly on LJ. Delayed processing was associated with increased time to positivity for all species, with the most pronounced delays observed after 21 days of storage, especially on solid media. These findings in Fig. 2 confirm the superior sensitivity and faster growth detection of liquid culture systems and underscore the impact of storage duration on mycobacterial viability.

The pilot study

All three participating sites in Table 1 tested the full pilot panel of ten specimens and demonstrated complete concordance with expected results. Each site correctly identified all *Mycobacterium tuberculosis* complex (n = 4), NTM/MOTT (n = 4), and no-growth (n = 2) samples, achieving 100% accuracy. These findings indicate a high level of baseline technical competence across sites and confirm the suitability of the pilot panel for evaluating laboratory performance in mycobacterial culture and identification.

Table 1
Baseline laboratory performance in the pilot round of the culture and identification PT program

Parameter	Participant 1	Participant 2	Participant 3
Total panels tested	10	10	10
M. tuberculosis complex	4	4	4
NTM/MOTT	4	4	4
No growth	2	2	2
Correct identifications, n (%)	10 (100%)	10 (100%)	10 (100%)

Proficiency testing program: three rounds

Across the three proficiency testing rounds in Table 2, assay performance improved over time. In Round 1 (2021), all laboratories using LJ achieved satisfactory results (100%), whereas MGIT showed a lower pass rate (73%). Combined use of LJ and MGIT yielded an 80% pass rate. In Round 2 (2022), performance for LJ declined to 67%, while MGIT remained stable (73%); however, laboratories using both assays achieved 100% satisfactory performance. By Round 3 (2023), performance improved substantially, with MGIT and the combined LJ and MGIT approach achieving universal satisfactory results (100%), and LJ alone demonstrating a high pass rate (87%). Overall, combined assay use was associated with consistently superior performance.

Table 2
Performance of LJ and MGIT culture assays across three proficiency testing rounds, 2022–2023

Round	Assay	Pass, n (%)	Fail, n (%)
2021 (R1)	LJ	8 (100)	0 (0)
	MGIT	8 (73)	3 (27)
	LJ + MGIT	4 (80)	1 (20)
2022 (R2)	LJ	7 (67)	2 (33)
	MGIT	7 (73)	2 (22)
	LJ + MGIT	8 (100)	0 (0)
2023 (R3)	LJ	7 (87)	1 (13)
	MGIT	9 (100)	0 (0)
	LJ + MGIT	9 (100)	0 (0)

***Abbreviations**: LJ, Löwenstein–Jensen medium; MGIT, Mycobacteria Growth Indicator Tube, (LJ + MGIT) = Participants that reported both test methods

Comparison of specimen decontamination method performance across PT rounds (2021–2023)

Performance of specimen decontamination methods varied across rounds as seen in Table 3, with overall improvement over time. In Round 1 (2021), high satisfactory rates were observed for 1.5% NaOH (92%) and BD BBL™ MycoPrep™ Kit[6], 4% NaOH, and 2% NaOH (all 100%), while Petroff's method showed lower performance (83%). In Round 2 (2022), 1.5% NaOH maintained consistent performance (92%), whereas BD BBL™ MycoPrep™ Kit demonstrated unsatisfactory performance in the single participating laboratory. All other methods achieved 100% satisfactory results. By Round 3 (2023), all methods except 4% NaOH demonstrated universal satisfactory performance, with 1.5% NaOH and Petroff's reaching 100%. Overall, standardized decontamination methods were associated with improved and consistent performance across rounds.

Table 3
Performance of different specimen decontamination methods across three proficiency testing rounds, 2021–2023

Round	1.5% NaOH		Petroff's		BD BBL™ MycoPrep™ Kit		4% NaOH		2% NaOH		Modified Petroff's	
	Pass, n (%)	Fail n %)	Pass n (%)	Fail n (%)	Pass n (%)	Fail n (%)	Pass n (%)	Fail n (%)	Pass n (%)	Fail n (%)	Pass n (%)	Fail n (%)
2021 (R1)	12 (92)	1 (8)	6 (83)	1 (17)	2 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	–	–
2022 (R2)	11 (92)	1 (8)	3 (75)	1 (25)	0 (0)	1 (100)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)	0 (0)
2023 (R3)	18 (100)	0 (0)	3 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	3 (100)	0 (0)	–	–
*Abbreviations: NaOH, sodium hydroxide												
*Legend: Pass = Expected results consistently observed, fail= Expected result not observed												
Notes: – indicates method not used in that proficiency testing round. Percentages are calculated per method within each round												

Overall proficiency testing performance over successive PT rounds

The proficiency testing performance across three rounds (2022–2023) is summarized in Fig. 3, in Round 1 (2022), 82.6% of participating laboratories met the satisfactory performance criteria, while 17.4% did not. Performance declined in Round 2 (2022), with only 60.9% achieving satisfactory results and 39.1% falling below proficiency standards. However, performance improved markedly in Round 3 (2023), with 96.2% of laboratories demonstrating satisfactory proficiency and only 3.8% underperforming.

Shipping and reporting turnaround times across PT rounds

The average turnaround times (TAT) for proficiency testing (PT) across three rounds (2021–2023) are summarized in Fig. 4, The average shipping TAT to report PT results improved notably, decreasing from 18.09 days in Round 1 (2021) to 14.96 days in Round 2 (2022) and 14.8 days in Round 3 (2023). In contrast, the average results reporting TAT showed minimal overall change, remaining relatively stable at 79.61 days in Round 1, 74.87 days in Round 2, and 82.58 days in Round 3. This pattern indicates a sustained improvement in laboratory analytical and reporting efficiency, while logistics for sample shipping were consistent across the observation period.

Discussion

This study demonstrated strong baseline laboratory capacity, with 100% concordance in the pilot round for MTBC, NTM/MOTT, and negative samples. Proficiency testing performance declined from 82.6% to 60.9% before improving markedly to 96.2% in Round 3. Combined LJ and BACTEC MGIT 960 use yielded higher pass rates. Extended storage increased time to positivity, with MGIT consistently outperforming LJ. Rapid growers were detected earlier than *M. tuberculosis*. Decontamination practices improved over time. Shipping turnaround improved modestly, though reporting delays persisted.

Across three PT rounds, data in Fig. 3, reveal significant variability in laboratory performance. The PT performance initially declined from 82.6% satisfactory in Round 1 to 60.9% in Round 2, then improved substantially to 96.2% in Round 3, indicating strong recovery and quality improvement. Similar performance has been reported in China by Jian Du and colleagues in China[15]. Strengthening mycobacteria culture and identification proficiency testing contributes to improved diagnostic reliability and supports regional efforts toward TB elimination targets.

In the pilot round, all three laboratories demonstrated complete (100%) concordance in identifying MTBC, NTM/MOTT, and negative specimens, reflecting strong baseline technical performance and confirming the suitability of the prepared PT materials. For this PT scheme, we selected *M. tuberculosis* (H37Rv ATCC 27294; final dilution 1:500), *M. kansasii* (ATCC 12478; final dilution 1:50,000), and *M. fortuitum* (ATCC 6841; final dilution 1:5,000), based on BACTEC MGIT 960 medium quality control validation procedures described by Siddiq and colleagues at FIND[16]. This pilot study was limited by the relatively small number of participating laboratories and may not fully represent national-level performance variability.

As shown in Fig. 2 above, Time to positivity increased progressively with extended storage across all organisms and culture systems, demonstrating the impact of pre-analytical delays on mycobacterial viability. The BACTEC MGIT 960 consistently detected growth faster than Löwenstein–Jensen (LJ) media at baseline, 7 days, and 21 days, confirming the superior sensitivity of liquid culture. Among species tested, *M. fortuitum* showed the shortest detection times, followed by *M. kansasii*, while *M. tuberculosis* H37Rv had the longest, particularly on LJ. The consistently shorter detection times observed with BACTEC MGIT 960 compared with Löwenstein–Jensen media align with previous studies [17][18]. This demonstrates the superior sensitivity and faster growth detection of liquid culture systems, particularly under suboptimal specimen conditions. Species-specific differences were evident, with rapidly growing non-tuberculous mycobacteria such as *M. fortuitum* detected earlier than *M. kansasii* exhibited the longest detection times, especially on solid media. The pronounced delays observed after prolonged storage, notably at 21 days, suggest declining bacillary viability and reduced recovery efficiency, disproportionately affecting solid media. These findings prompted us to use *M. fortuitum* for NTM/MOTT for subsequent PT rounds.

Over three years, sputum decontamination performance improved and became more standardized as shown in Table 3, The modified Petroff's method, employing 2–4% sodium hydroxide (NaOH) in combination with N-acetyl-L-cysteine (NALC), is widely used in developing countries for sputum processing prior to culture.[19]. High initial success with 1.5% NaOH and other methods reflects robustness, while Petroff's method showed variability due to procedural complexity. Iterative EQA feedback led to near-universal satisfactory performance by Round 3, supporting standardized protocols and ongoing EQA participation to maintain consistent culture outcomes in TB laboratories. No decontamination method is capable of eliminating contaminating flora without adversely affecting mycobacteria during sputum processing. Therefore, sputum decontamination represents a balance between minimizing mycobacterial loss while effectively suppressing contaminating organisms. The isolated unsatisfactory result observed with BD BBL™ MycoPrep™ Kit in Round 2 should be interpreted cautiously given the small number of participating laboratories, but emphasizes the need for strict adherence to manufacturer instructions. By Round 3, near-universal satisfactory performance across methods indicates improved harmonization of decontamination practices through iterative EQA feedback. These findings support the adoption of standardized decontamination protocols and continued participation in EQA programs to ensure consistent culture performance within TB laboratory networks

The proficiency testing turnaround time (TAT) analysis in Fig. 4 reveals a key operational insight. On average, the shipping time ranged between 14 to 18 days and the results reporting TAT showed no sustained improvement,

persisting around 75–83 days. The delays in distributing the PT samples may be strongly attributed to customs clearance and logistical resources. Unlike earlier studies [20][21] reporting that the LJ method may require 42–56 days to recover Mycobacteria or confirm negative samples, participants took prolonged days to report PT results

Overall, these results indicate that the PT materials were stable and suitable for use as quality assurance tools in resource-limited settings. Approximately 96% of participating laboratories achieved results meeting the standards for certified mycobacterial culture and identification. The notable improvement across successive PT rounds emphasizes the value of structured EQA programs in enhancing diagnostic capacity. Continuous PT participation, alongside targeted corrective actions and adherence to standardized procedures, is crucial for maintaining reliable mycobacterial detection and supporting TB control in high-burden regions

Challenges and limitations

The mycobacterial culture and identification PT program, while invaluable, faces inherent challenges. Logistical constraints, including the need for strict cold-chain maintenance, biosafety-compliant packaging, and timely shipping, can affect sample integrity and limit participation, particularly in remote or resource-limited laboratories. Technical variability, such as maintaining the viability and stability of live, infectious *Mycobacterium tuberculosis* (MTB) and non-tuberculous mycobacteria (NTM) strains for shipment is complex and poses biosafety risks. The program's educational value can be limited if laboratories use atypical, non-routine methods exclusively for PT samples. Furthermore, the high cost and technical demand of culture-based PT can restrict participation, particularly in resource-limited settings where quality assurance is most needed. Programmatic limitations, including variable staff training, delayed result reporting, and difficulties implementing corrective actions, further affect program effectiveness. Finally, PT assesses a single time point and may not detect intermittent errors or systemic issues that occur outside the testing window, potentially offering a performance snapshot that is not fully representative of routine diagnostic quality.

Conclusions

This study demonstrates that *mycobacteria* culture and identification proficiency testing programs is feasible and a practical approach to strengthening diagnostic capacity in low resource Laboratories. Across three proficiency testing rounds, laboratory performance improved markedly, with combined use of MGIT and LJ yielding the most consistent satisfactory outcomes. Standardized specimen decontamination methods were associated with enhanced and more reliable performance over time. Improvements in shipping turnaround times further reflect strengthened operational efficiency, underscoring the value of standardized protocols, combined culture approaches, and continuous proficiency testing to improve mycobacterial culture performance.

Declarations

Ethics approval

This study evaluated the implementation and performance of a tuberculosis culture and identification proficiency testing (PT) programme across participating laboratories. The PT panels were prepared using well-characterized mycobacterial reference strains; *Mycobacterium tuberculosis* complex strain (H37Rv/ATCC 27294) and two non-tuberculous mycobacteria (NTM), *M. fortuitum* (ATCC 19542) and *M. kansasii* (ATCC 12478), and did not involve human participants, patient recruitment, or identifiable patient data. As the work constituted external quality assessment (EQA) and laboratory systems strengthening activities without the use of human subjects, formal

ethical approval was not required in accordance with institutional and national guidelines. Administrative clearance to carry out this study was obtained from the National Health Laboratory Diagnostic Services under the National Tuberculosis Reference Laboratory/WHO Supranational Reference Laboratory in Kampala, Uganda. Additionally, all participating sites were required to sign a Confidentiality Waiver and a Participant Biosafety Compliance Agreement prior to involvement in the PT scheme.

Clinical trial registration: Not applicable. This study did not involve prospective assignment of participants to interventions and therefore does not meet the definition of a clinical trial

Consent for publication

Not applicable

Availability of data and materials

All relevant data are within the paper and its Supporting Information files.

Competing of Interest

None to declare

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Authors' contributions:

PL HB HSN KM and MLJ: Contributed to the conception of the study;

PL HB HSN KM OG AN CM and MLJ: Contributed to the design of the work;

PL HB PU IA HSN JN JMK AN CM GWK OG and KF: Contributed to the acquisition and analysis of data;

PL HB PU IA HSN JN JMK AN CM GWK OG and KF: Contributed to the interpretation of data;

PL HB IA HSN JN JMK AN CM GWK OG and KF MLJ: Drafted the work and substantively revised it;

PL HB PU IA HSN JN JMK AN CM GWK OG KF KM and MLJ: Approved the submitted version;

PL HB PU IA HNS JN JMK AN CM GWK OG KF KM and MLJ: Agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, appropriately investigated, resolved, and the resolution documented in the literature.

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References

1. Global Laboratory Initiative (GLI). Practical manual on tuberculosis laboratory strengthening, 2022 update, 2022. https://www.who.int/publications/i/item/9789240061507 (accessed Jun. 19, 2024).
2. World Health Organization (WHO), Network factsheet SRL. 2022. https://www.who.int/publications/m/item/srl-network-factsheet (accessed Jun. 19, 2024).
3. SRL-Uganda, Home Main - National TB Reference Laboratory, 2024. https://www.ntrl.or.ug/ (accessed Jun. 19, 2024).
4. Global Laboratory Initiative (GLI.), global laboratory initiative advancing TB diagnosis A publication of the Global Laboratory Initiative a Working Group of the Stop TB Partnership Mycobacteriology Laboratory Manual.
5. Caulfield AJ, Wengenack NL. Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. J Clin Tuberc Other Mycobact Dis. Aug. 2016;4:33–43. 10.1016/J.JCTUBE.2016.05.005.
6. WHO. Consolidated Guidelines on Tuberculosis Treatment Module 4: Treatment and care, *Who*, p. 99, 2025, Accessed: Feb. 10, 2026. [Online]. Available: https://iris.who.int/server/api/core/bitstreams/f663f086-5d97-4355-beea-0635d47f1227/content
7. Carter JY. External quality assessment in resource–limited countries. Biochem Med. Feb. 2017;27(1):97–109. 10.11613/BM.2017.013/FULLARTICLE.
8. World Health Organization (WHO). Content Sheet 10 – 1. Overview of External Quality Assessment (EQA); 2011.
9. Badrick T. Integrating quality control and external quality assurance. Clin Biochem. Sep. 2021;95:15–27. 10.1016/J.CLINBIOCHEM.2021.05.003.
10. Urdea M et al. Requirements for high impact diagnostics in the developing world., *Nature*, vol. 444 Suppl 1, pp. 73–79, 2006, 10.1038/NATURE05448
11. Schroeder LF, Amukele T. Medical Laboratories in Sub-Saharan Africa That Meet International Quality Standards, *Am. J. Clin. Pathol.*, vol. 141, no. 6, pp. 791–795, Jun. 2014, 10.1309/AJCPQ5KTKAGSSCFN
12. Van Deun A, Wright A, Zignol M, Weyer K, Rieder HL. Drug susceptibility testing proficiency in the network of supranational tuberculosis reference laboratories.
13. Global Laboratory Initiative (GLI). global laboratory initiative advancing TB diagnosis, Accessed: Sep. 29, 2025. [Online]. Available: https://challengetb.org/publications/tools/lab/Guide_for_Providing_Technical_Support.pdf
14. Global Laboratory Initiative (GLI). global laboratory initiative advancing TB diagnosis A publication of the Global Laboratory Initiative a Working Group of the Stop TB Partnership Mycobacteriology Laboratory Manual, 2014, Accessed: Jun. 28, 2024. [Online]. Available: https://stoptb.org/wg/gli/assets/documents/gli_mycobacteriology_lab_manual_web.pdf
15. Du J et al. Development and validation of external quality assessment panels for mycobacterial culture testing to diagnose tuberculosis in China, *Eur. J. Clin. Microbiol. Infect. Dis.* 2019 3810, vol. 38, no. 10, pp. 1961–1968,

Jul. 2019. 10.1007/s10096-019-03634-8

16. Siddiqi SH, Rüsch-Gerdes S. For BACTEC™ MGIT 960™ TB System (Also applicable for Manual MGIT) Mycobacteria Growth Indicator Tube (MGIT) Culture and Drug Susceptibility Demonstration Projects, 2006, Accessed: Feb. 13, 2026. [Online]. Available: https://www.finddx.org/wp-content/uploads/2023/02/20061101_rep_mgit_manual_FV_EN.pdf
17. Diriba G, et al. Performance of Mycobacterium Growth Indicator Tube BACTEC 960 with Lowenstein-Jensen method for diagnosis of Mycobacterium tuberculosis at Ethiopian National Tuberculosis Reference Laboratory, Addis Ababa, Ethiopia. BMC Res Notes. May 2017;10(1). 10.1186/s13104-017-2497-9.
18. Chien HP, Yu MC, Wu MH, Lin TP, Luh KT. Comparison of the BACTEC MGIT 960 with Löwenstein-Jensen medium for recovery of mycobacteria from clinical specimens.
19. Sharma A, Agarwal A. Evaluation of the performance of a novel sputum processing ReaSLR methodology for culture of sputum samples in solid and liquid media in comparison with modified Petroff's method. Indian J Tuberc. Apr. 2021;68(2):255–60. 10.1016/j.ijtb.2020.09.015.
20. Kathleen Eisenach A, Demers A-M, Jones F. Mycobacteriology Sourcebook for TB Clinical Trials Mycobacteriology Laboratory Sourcebook For Harmonization and Support of TB Clinical Trials. Mycobacteriology Sourceb TB Clin Trials, 2018.
21. Levidiotou S, et al. Detection of mycobacteria in clinical specimen using the mycobacteria growth indicator tube (MGIT) and the Lowenstein Jensen medium. Microbiol Res. Sep. 1999;154(2):151–5. 10.1016/S0944-5013(99)80008-1.

Figures

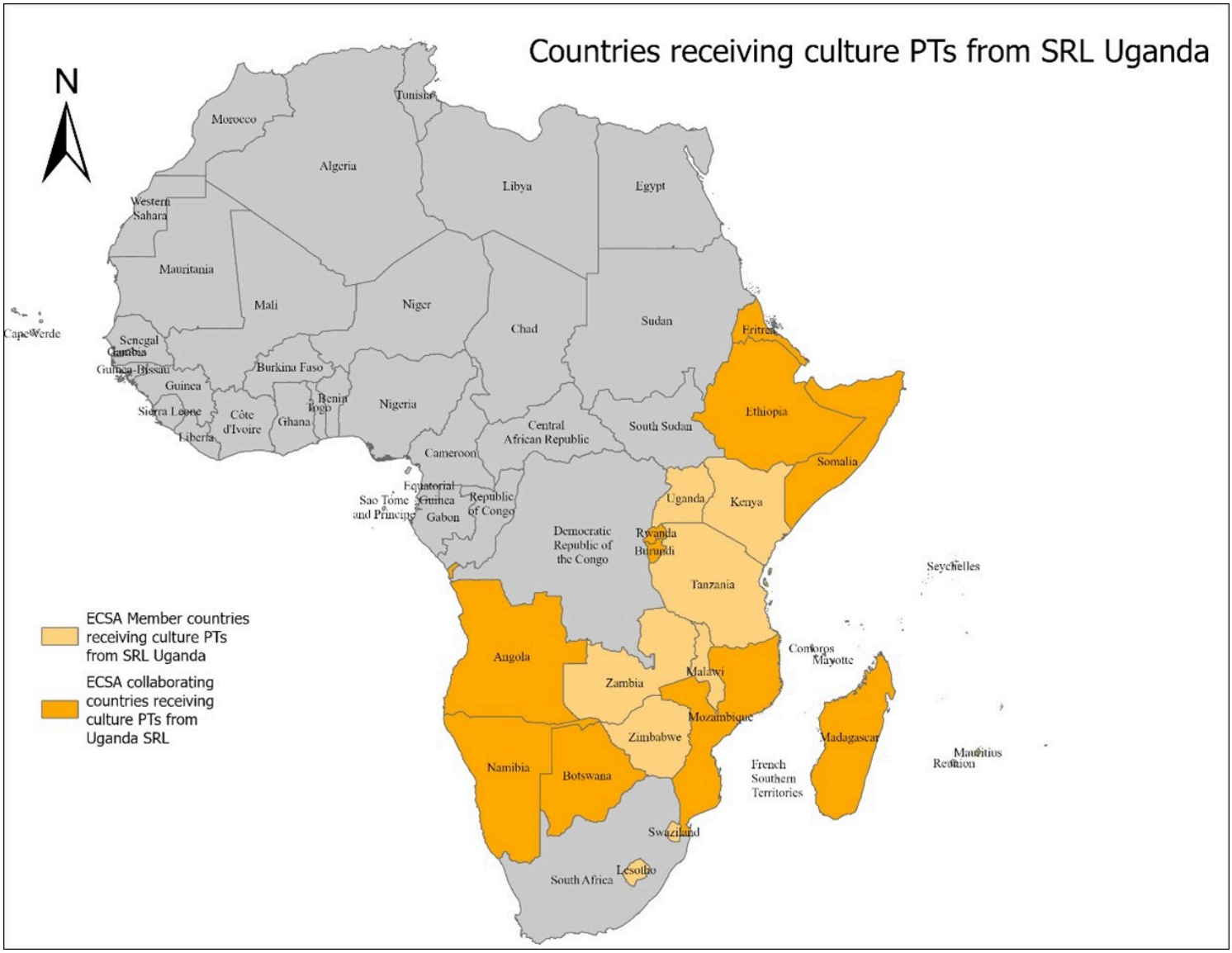


Figure 1

Countries receiving mycobacteria culture and identification Proficiency Testing panel

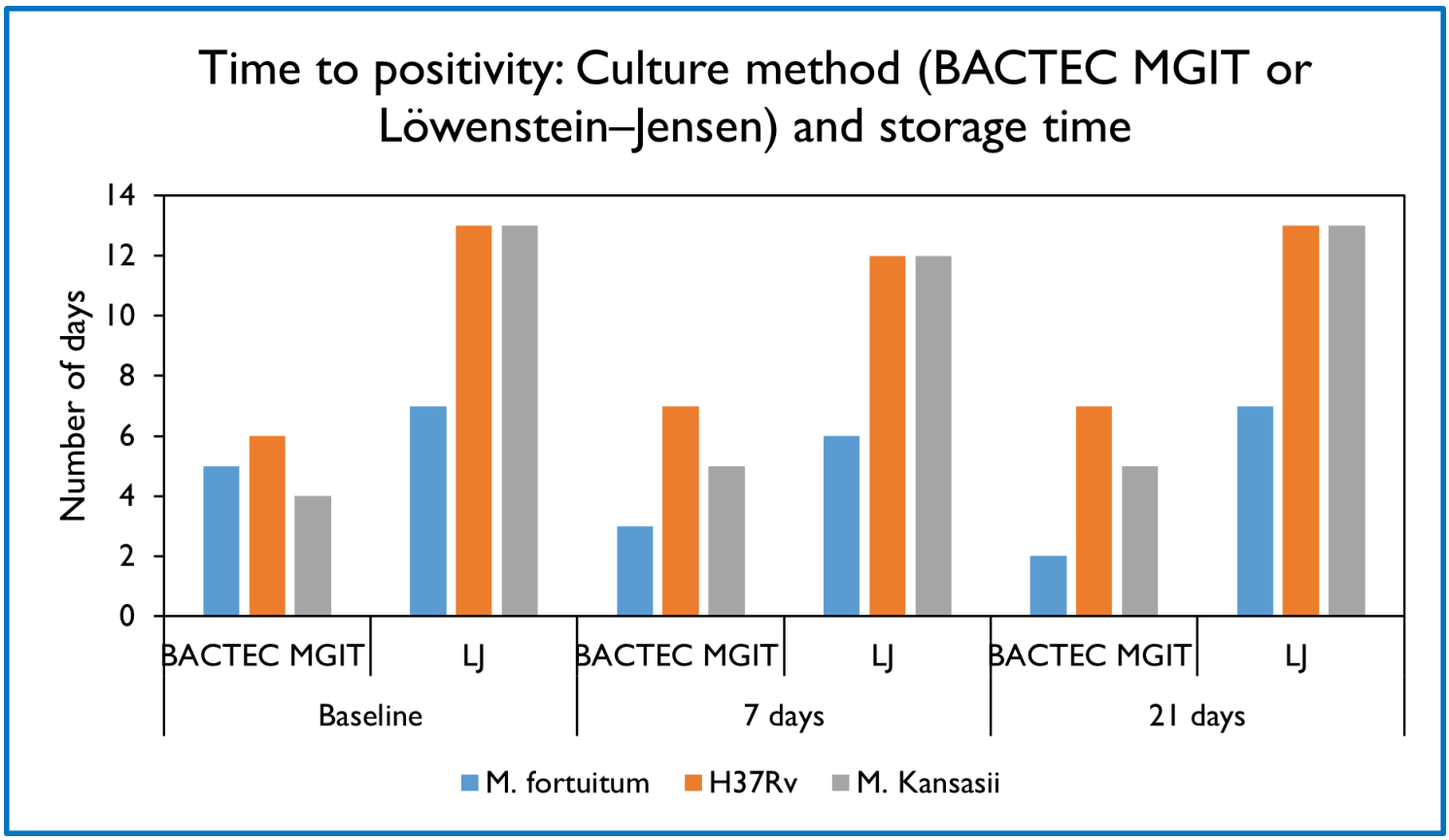


Figure 2

Effect of Storage Duration on Time to Positivity in MGIT and LJ Culture Systems

***Abbreviations:** LJ, Löwenstein–Jensen medium; MGIT, Mycobacteria Growth Indicator Tube

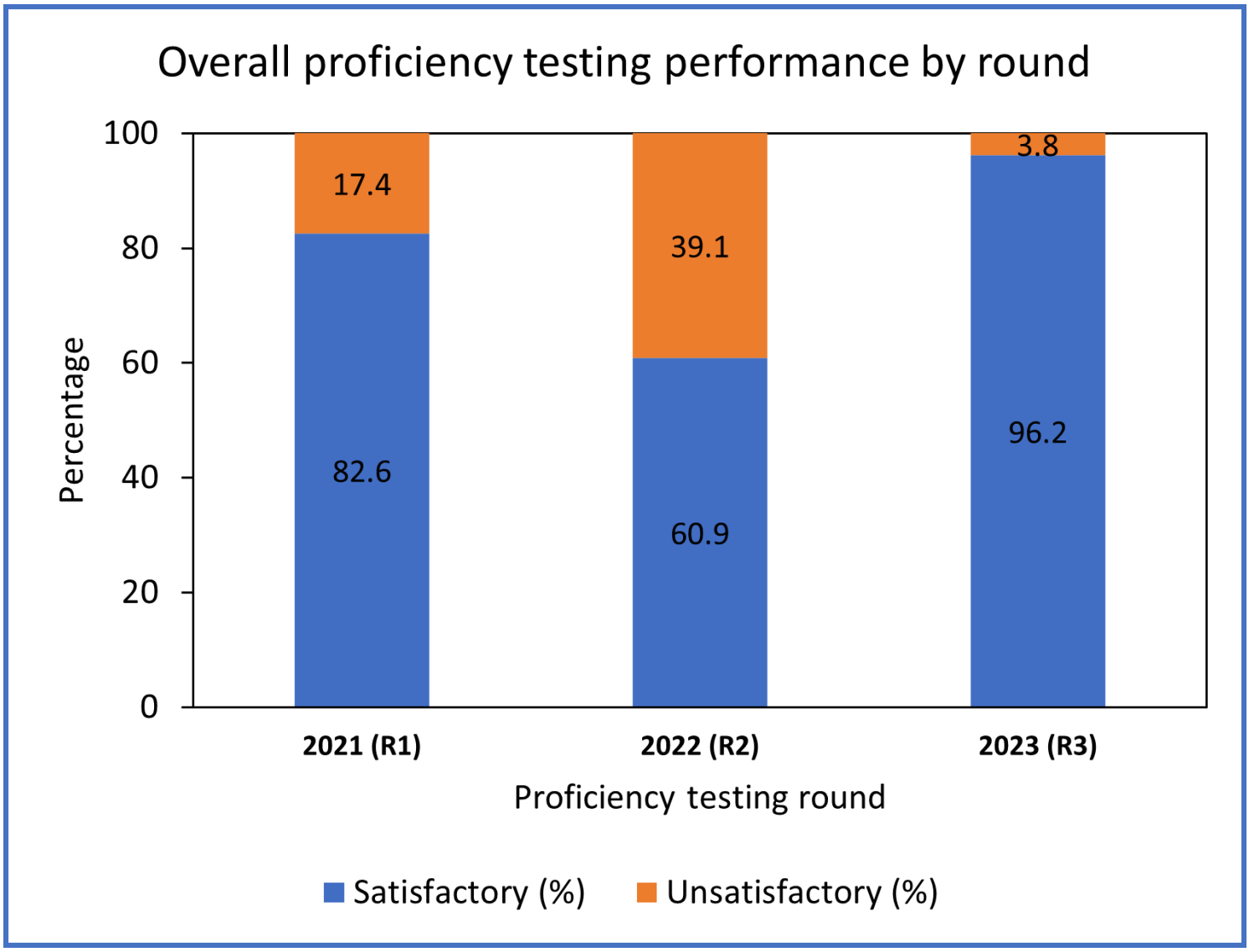


Figure 3

Overall proficiency testing performance by round. Stacked bars show the proportion of satisfactory and unsatisfactory results across proficiency testing rounds.

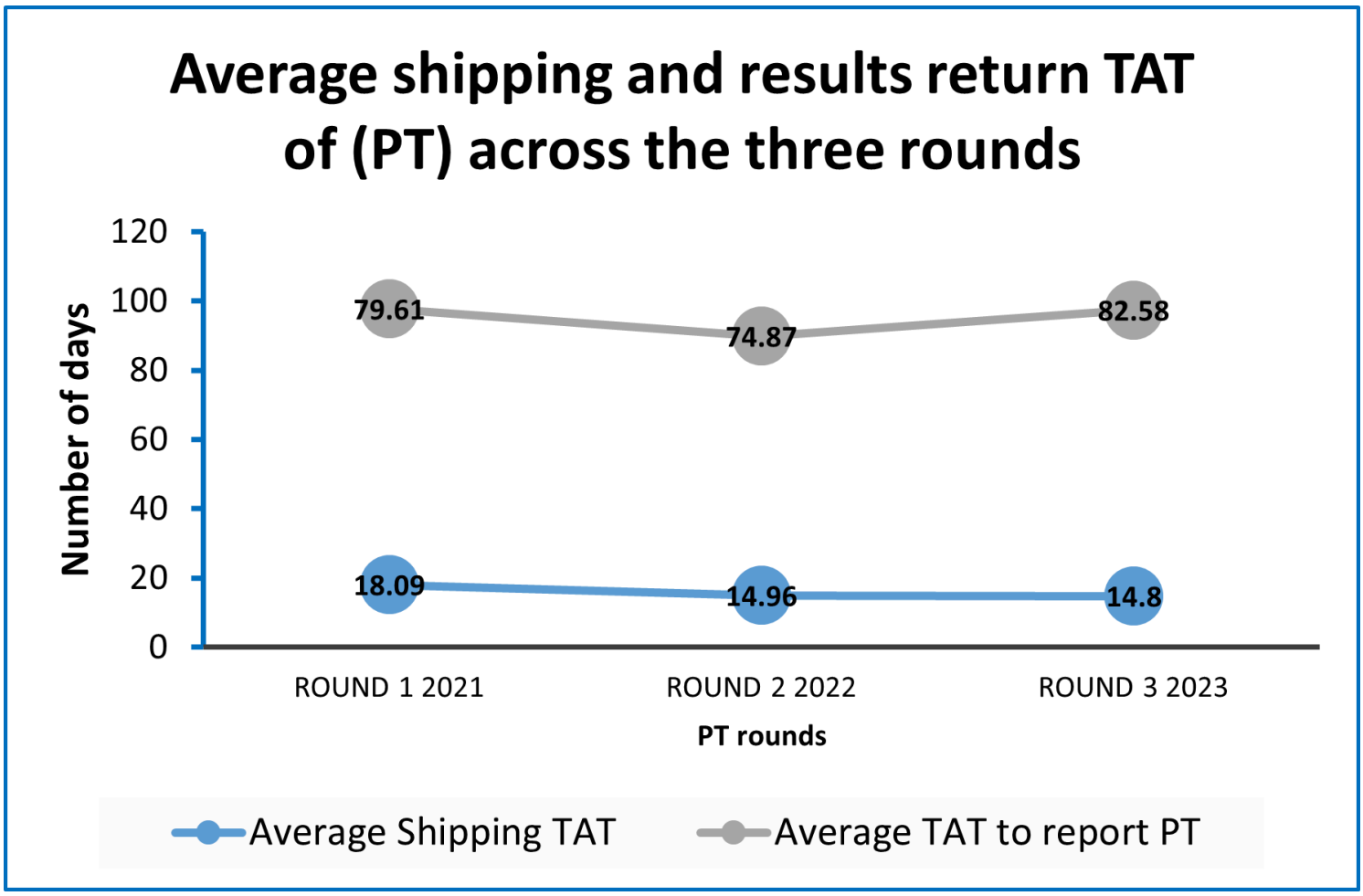


Figure 4

Average shipping and reporting turnaround times for proficiency testing panels across three rounds.