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ENSURING QUALITY OF **TUBERCULOSIS TEST RESULTS**

USAID's Infectious Disease Detection and Surveillance (IDDS) project improves the detection of diseases of public health importance and identification of antimicrobial resistance (AMR) in priority pathogens. IDDS works with more than 20 host countries across Asia and Africa.

Oem Leakhena, midwife and laboratory technician, loads a cartridge into a Trueprep instrument at Prek Dambok Health Center, Srey Santhor Operational District, Kampong Cham province, Cambodia. Photo by Cindy Liu for IDDS

BACKGROUND

Laboratory diagnostics are a primary tool in early detection of *Mycobacterium tuberculosis* (MTB). The quality of laboratory tests is integral to the accuracy of results delivered to the patient. Internal quality control is designed to detect, prevent, and minimize erroneous results by standardizing a laboratory's internal processes. External quality assessment (EQA), also known as a proficiency testing, is used to compare a laboratory's testing to a source outside the laboratory that offers a statement of quality to the laboratory.[1] This brief focuses on EQA and its role in improving test results for MTB that are obtained using rapid molecular diagnostics.

EQA is recognized as a World Health Organization (WHO) quality system essential.[2] Participation is required for a laboratory to achieve accreditation from the International Organization for Standardization (ISO). Regular participation provides opportunities to assess the performance of diagnostic tools and can help identify gaps in the pre-analytical, analytical, and post-analytical stages of testing that can be corrected to ensure continued quality improvement. Common errors identified by EQA include mishandling and mislabeling samples, improper storage, instrument performance, cross-contamination, and misreporting results. Further, EQA participation ensures that:

- all testing is done in compliance with national testing algorithms and standard operating procedures (SOPs)
- a cadre of competent users are available to perform the tests
- testing sites are monitored and can provide uninterrupted diagnostic services

- testing services are unaffected by stock-outs and module failures
- good quality samples are collected, quality testing is done in a timely manner, and the diagnostic test results are reported without delay
- underperforming laboratories are identified, along with their needs for technical assistance, guidance, and on-site supportive supervision
- the TB diagnostic network is monitored, using electronic systems where possible, and the collected data are analyzed, evaluated, and used to inform decision-making

In short, EQA participation helps ensure the test system is managed to ensure quality and accuracy of the laboratory's results.[3]

INTRODUCTION OF EQA FOR TRUENAT MTB AND MTB/RIF TESTING

The USAID Infectious Disease Detection and Surveillance (IDDS) project, in collaboration with the Stop TB Partnership implementing the introducing New Tools Project (iNTP), supported the introduction of Truenat instruments and tests for nearly 585,000 people in 9 high-TB burden countries in Africa and Southeast Asia to improve access and timeliness of TB and drug-resistant TB detection.

To ensure that Truenat MTB and MTB RIF testing was quality assured when first introduced, IDDS partnered with SmartSpot Quality (https://www.smartspotq.com/about), an accredited manufacturer of validated Truenat MTB/RIF EQA panels, to provide each site with 3 cycles of EQA for 225 Truenat sites in Bangladesh, Cambodia, DRC, Philippines, Kenya, Uganda, and Zimbabwe as part of iNTP. These panels were provided for centralized distribution, at no charge to the local project, the national TB programs, or the Missions.

The EQA panels are composed of 4 inactivated Dry Culture Spot (DCS) that do not require refrigeration and are stable at ambient temperatures for up to 24 months. The DCS panels were inactivated to be non-infectious so they could avoid being shipped as hazardous materials. Each site was enrolled, the DCS panels were dispatched in a single shipment, and panels were distributed using the national sample transport networks. Virtual and in-person training, facilitated by IDDS, was provided to the end users and other stakeholders for EQA sample processing and electronic reporting of results. In addition, IDDS and SmartSpot produced and distributed short training video clips that could be disseminated to the Truenat sites via WhatsApp for those who could not attend the virtual trainings. Laboratories were expected to report results to SmartSpot Quality Monitor within 30 days of the start of each cycle. All enrolled laboratories and designated Ministry of Health staff (NTP and NTRL) were to have access to the performance reports. These panels were meant to serve as an interim EQA measure in fiscal years 2022 and 2023 until national laboratories and others can build up their own capacity to produce EQA panels validated for Truenat.

RESULTS

In 2022, IDDS distributed 2,384 EQA panels to the 7 participating countries. On average, across three cycles, 89.6 percent of the controls were tested and had their results submitted to SmartSpot for analysis. Many testing sites encountered difficulties in reporting through the website, due to lack of internet access and laboratory technicians' unfamiliarity with the EQA process. IDDS saw an improvement in the reporting rates from cycle 1 (85.3 percent) to cycle 2 (93.3 percent) and cycle 3 (91.2 percent). The improvements were

made possible with technical assistance from "super-users," who are national and sub-national laboratory staff trained to support Truenat implementation, and program staff to support the reporting process.

All countries passed the 80 percent benchmark in their average scores across three cycles. Half of the participating countries achieved "acceptable" performance (meaning their scores exceeded 90 percent) across three cycles. Given the novelty of the Truenat assay, the performance was positive. However, more training and technical assistance are still needed for all participating countries and sites to reach passing performance.

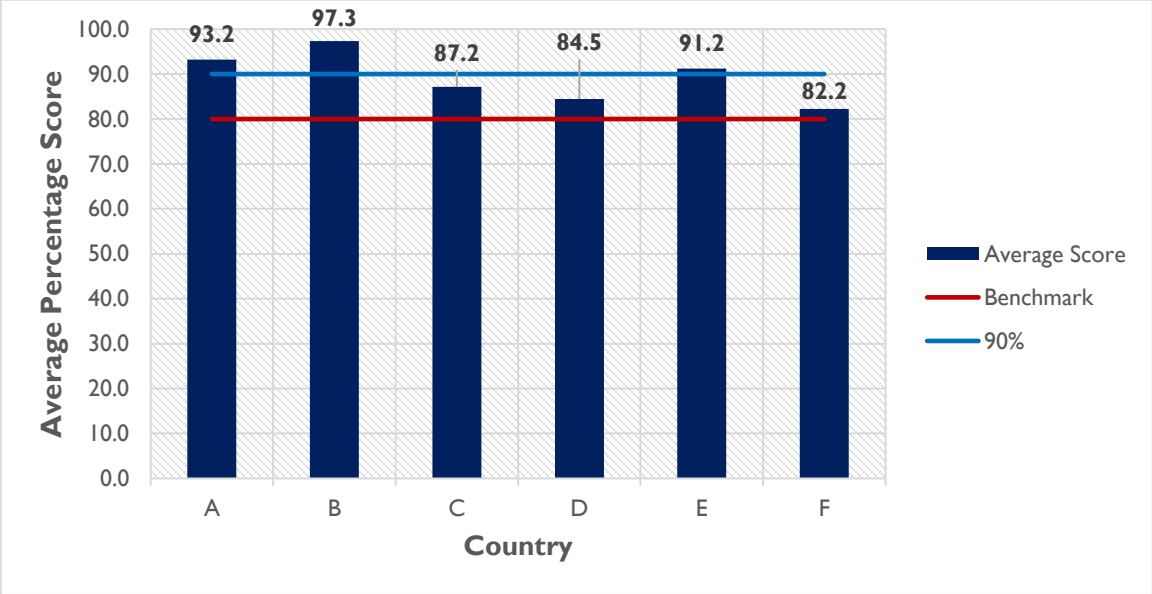

Figure 1. Average scores across 2022 cycles. The graph included six countries that had reported results for all three cycles.

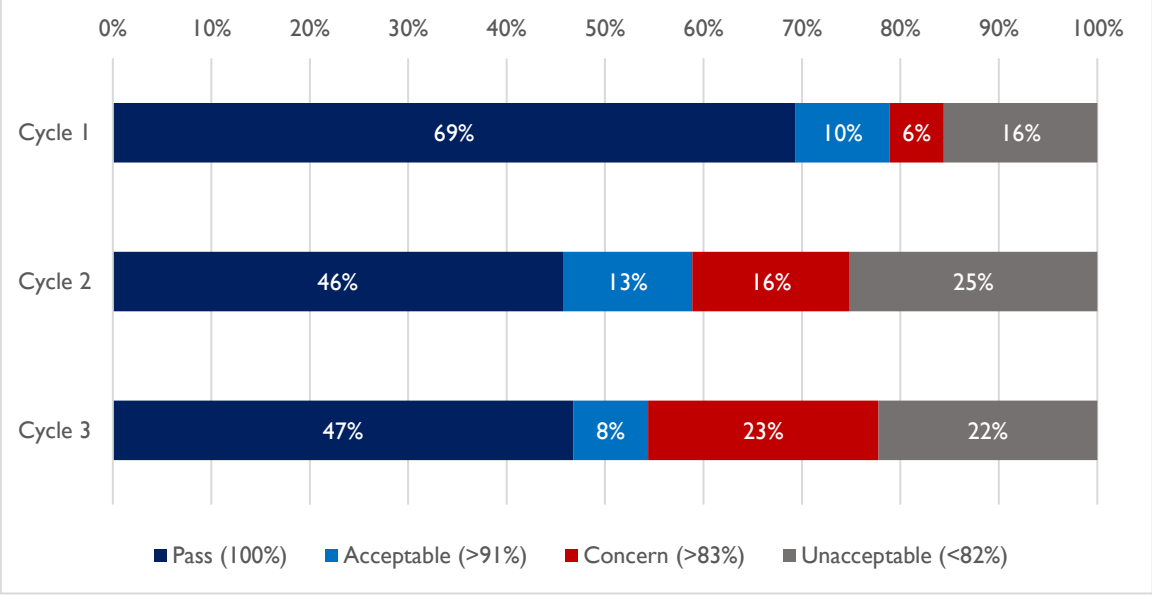

Figure 2. Percentage of sites by outcome for Truenat EQA 2022. This graph excludes sites that did not participate or did not submit any results.

Averaged across three cycles, the most common incorrect results were rifampicin-resistance "indeterminate" results (7 percent of controls), false negative for rifampicin detection (3 percent), invalid for MTB detection (2 percent) and no reflex RIF testing for positive MTB results (2 percent). Some possible causes for these incorrect results include:

- End users did not adhere to SOP, including insufficient incubation time (many sites did not have a laboratory timer), leading to incomplete liquefication of the samples
- End-users did not follow the diagnostics algorithm, such as not conducting RIF testing after a positive MTB result
- Other end-user errors such as sample switching, incorrect sample ID inputs, or pipetting errors
- Lower efficiency of the Truenat MTB/RIF assay in detecting H526Y *rpoB* RIF mutation

CHALLENGES

Each of the countries participating in the Truenat EQA program faced similar challenges. In most countries supported by IDDS, Truenat MTB/RIF testing was implemented at peripheral level health facilities. Many sites lacked internet access, making it difficult for laboratorians to attend virtual EQA training sessions, receive registration details, report results via the web-based portal, and receive remote support for troubleshooting. Most facilities were staffed with microscopists that had little or no experience with molecular diagnostic testing or an EQA program; they were primarily performing proficiency testing by sample rechecking, were not sensitized to molecular EQA process, and lacked experience pipetting. Timely delivery of panels to remote sites proved challenging and countries relied on the established sample referral systems. In some cases, laboratorians did not strictly adhere to the SOP due to lack of laboratory equipment (e.g., timers), heavy workload, and lack of a QR code reader on the Truenat instrument (meaning that end users needed to enter the sample numbers manually, which increased errors). Other challenges included incompatibility of the Truenat results file with the iOS system for exporting results, lack of personal protective equipment and hypochlorite to minimize risk of cross-contamination, and the need for strengthening good laboratory biosafety practices.

LESSONS LEARNED

The rollout of the Truenat EQA program has been successful despite these key challenges. In Bangladesh and Cambodia, all 53 of the Truenat laboratories submitted their EQA test results. Overall, nearly 90 percent of the laboratories in the 6 countries participating in 2022 successfully submitted results across all 3 EQA cycles. The lessons learned from the Truenat EQA program include the following:

- Early engagement and close coordination with national TB program staff were important to build support for the EQA program.
- EQA cycle reports were essential to inform national program staff of site-specific performance and target technical assistance by super-users for continual quality improvement.
- Following the SOP with proper incubation times is critical to ensure sufficient elution of mycobacteria from the DCS and the diagnostic algorithm needs to be strictly followed to minimize incorrect results.
- In-person EQA training of end users and super-users improved participation and performance.

- Collaborating with the EQA provider to produce short training video clips and translating instructions for use to the local language improved the end users' understanding of the EQA processes.
- Exporting results to CSV provided additional technical details—including crossing threshold values—which are valuable for results analysis and troubleshooting.
- Debriefing with the national TB reference laboratory and super-users after each cycle is important. The EQA results were used as a reference point for super-users to provide additional mentorship to underperforming sites.
- Provision of certificates as an acknowledgement of participation and performance motivates the end users to improve performance.
- Creating user groups on social media that include end users, a local Molbio agent, and super-users was an effective means to communicate with the end users and facilitated timely response for deadline reminders, troubleshooting, and assistance with reporting results. Further, it was an effective tool to disseminate short training video clips (e.g., sample processing and exporting results) to sites lacking internet access.

Photo by IDDS

- The EQA results are not fully representative of the performance and competency of the operators; routine collection and analysis of key performance indicators and on-site supervision is still required.
- The sustainability of the Truenat EQA program is feasible and requires capacity building at the national level to be able to provide panels produced in each country.

HOW IDDS RESPONDED TO CHALLENGES

Since this was the first time participating in a molecular EQA program for most of the Truenat users, IDDS developed training materials for sensitization to the EQA program and good biosafety practices, developed job aids on proper preparation of hypochlorite solution to minimize contamination risk, and incorporated these materials into the end user and super-user training packages that were delivered in person in each participating country. To overcome the lack of internet connection or availability of staff for training, IDDS collaborated with SmartSpot Quality to produce short EQA training video clips that could be disseminated to end user groups via WhatsApp. These included short clips on how to process the DCS panels, exporting results from the instrument, and uploading to the reporting website or the mobile application. Furthermore, IDDS worked with SmartSpot Quality to translate the instructions for use into the local language so instructions could be better understood. To improve motivation of the end users, IDDS created certificates for each site to acknowledge the participation and performance after each cycle, a practice which was piloted in Zimbabwe. To ensure continual quality improvement, IDDS debriefed with national TB program staff, country teams, and super-users after each cycle to review performance and assist with root cause analysis to guide timely corrective actions.

CONCLUSIONS

Across all cycles, 89.6 percent of the distributed EQA panels were tested and submitted to SmartSpot for assessment. The successful submission rate improved over three cycles with super-users' help throughout the EQA results reporting. All countries met the minimal performance benchmark of 80 percent scores. Half met achieved at least a 91 percent ("acceptable") score for EQA results across three cycles. The most common incorrect results were RIF "indeterminate" and false negative on the RIF test. After each cycle, IDDS debriefed with countries and superusers utilized the EQA results to follow up with underperforming sites and increased mentorship, which improved performance.

REFERENCE LIST

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