



**USAID**  
FROM THE AMERICAN PEOPLE

# INTRODUCTION OF STOOL-BASED TESTING FOR TB DIAGNOSIS IN CHILDREN CHALLENGES AND LESSONS LEARNED



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. Tuberculosis is a focus for USAID and IDDS because it remains one of the world's leading infectious disease killers.

Pediatric TB testing training in the Democratic Republic of the Congo. Photo by IDDS

## INTRODUCTION

Tuberculosis (TB) is a communicable disease that is a major cause of ill health and one of the leading causes of death worldwide. The World Health Organization (WHO) estimates that 1.25 million children and young adolescents (ages 0 to 14 years) fell ill with TB in 2022 (World Health Organization, 2023). Of those who became ill, an estimated 214,000 children died of TB in 2022 (Dodd et al., 2017). Most of these deaths occur in children who were not diagnosed or treated.

Examining sputum specimens is the traditional method for confirmation of pulmonary TB, but children, especially young children, generally find it difficult to expectorate sputum spontaneously for TB testing. Obtaining other recommended specimens for confirming pulmonary TB in children requires invasive procedures (such as gastric aspiration or sputum induction), skilled clinical staff, and the availability of consumables that are not readily available in many settings.

Since 2021, WHO has recommended using Xpert MTB/RIF Ultra in stool specimens to diagnose pulmonary TB in children younger than 10 years old (World Health Organization, 2021). Of the many documented stool processing methods, the Global Laboratory Initiative manual describes the most used techniques in detail (World Health Organization, 2022). These are the optimized sucrose flotation (OSF) method developed by TB-Speed (Lounnas M et al., 2020), and the simple one-step (SOS) method developed by the KNCV Tuberculosis Foundation (KNCV) (de Haas et al., 2021).

## IDDS COUNTRY EXPERIENCES

The United States Agency for International Development (USAID) Infectious Disease Detection and Surveillance (IDDS) project collaborated with USAID and other technical experts to develop a generic protocol to assist countries in introducing and scaling up the SOS stool processing method. The SOS method is cost-effective and easy to implement at lower-level laboratories (Jusumbak et al., 2021). The protocol outlines the three implementation phases: introduce the stool-based approach for childhood TB testing into a country, scale up operations as part of routine testing, and integrate it into the TB diagnostic network (Table 1). An important aspect of the protocol is that it guides countries to select an appropriate phase of implementation based on the country's priorities for TB control and existing TB strategic plans.

**Table 1: Outline of phases in preparing for routine implementation of stool-based Xpert testing**

	<b>PHASE 1: RAPID VALIDATION</b>	<b>PHASE 2: LABORATORY PRACTICES</b>	<b>PHASE 3: OPERATIONAL ASPECTS</b>
<b>PURPOSE</b>	Provide data to support use of stool-based testing as an alternative to sputum testing	Define laboratory procedures and build skills and confidence of laboratory staff to test stool	Define operational needs for routine use of stool-based testing throughout the health system
<b>SETTING</b>	National Tuberculosis Reference Laboratory (NTRL) or another central laboratory with Xpert	Hospitals and primary care facilities in defined subnational area(s)	Variety of routine health care and geographic settings
<b>SAMPLE</b>	25 to 50 individuals with bacteriologically confirmed TB	300 to 500 children with presumptive TB	At least 1,500 children with presumptive TB
<b>ANTICIPATED OUTCOME</b>	Study results confirm validity of the method in the setting, standard operating procedures (SOPs) drafted for routine laboratory procedures	Revised SOPs for laboratory procedures and standardized training materials for phase 3	Revised pediatric TB diagnosis algorithm and operational guidance on how to implement stool-based Xpert in routine health care settings
<b>WHEN NEEDED?</b>	Clear need for validation of stool-based Xpert in country context	No need for in-country validation; NTP and stakeholders are ready to start introduction	Country is ready to implement or expand stool-based testing into routine settings



IDDS collaborated with national TB programs (NTPs) to adapt the protocol to the local context and implement it per country requirements in Burma (2022), Cambodia (2022), the Democratic Republic of the Congo (DRC) (2021), and Malawi (2022). IDDS-trained laboratory personnel implemented the protocol using KNCV’s standardized SOS training package. IDDS trainings also included sessions for clinicians on strengthening the clinical diagnosis of TB. Involving clinicians is a critical aspect of the approach as it creates a demand for childhood TB testing, which might not otherwise exist. Depending on the country context, IDDS provided needed supplies (Xpert MTB/RIF Ultra testing cartridges and wooden applicators) and support in adapting or developing data management tools. In DRC, IDDS also worked to adapt DHIS2 modules to support electronic data entry for childhood TB indicators from subnational levels. IDDS conducted weekly bi-weekly meetings to provide mentorship and guidance on implementation as NTPs moved through the various implementation phases. To ensure that countries followed established SOPs, enrolled eligible children, and accurately collected and reported data, IDDS conducted early monitoring visits to supported countries, which provided an additional opportunity to build in-country capacity on the approach.

**DEMOCRATIC REPUBLIC OF THE CONGO (DRC)**

To improve pulmonary TB detection among children, the DRC NTP piloted stool-based testing on the GeneXpert platform at 25 health facilities in Kinshasa. To facilitate data collection and reporting, IDDS developed a customizable tracker application (Figure 2) for the existing health management information system platform (DHIS2) and collaborated with the DRC DHIS2 team to incorporate the tracker into the in-country server for use during the pilot phase. From June to November 2022, the pilot sites enrolled and tested 815 children (48 percent were children aged 0 to 4 years) for TB using Xpert MTB/RIF Ultra assay. Of the 775 stool specimens with interpretable results, 134 (17.3 percent) had detected *Mycobacterium tuberculosis* (MTB). Three of the specimens had rifampicin (RIF) resistance. The results demonstrate how incorporating stool testing in TB diagnosis can significantly improve the diagnosis of TB in children. The NTP plans to scale up and integrate the technique into the TB diagnostic network across DRC.

**MALAWI**

Through the Malawi National Tuberculosis and Leprosy Elimination Program (NTLEP), IDDS and its implementing partners assessed the feasibility of integrating stool-based testing to diagnose pulmonary TB within routine pediatric TB services in eight hospitals. During the pilot period from November 2022 to May 2023, 578 children ages 0 to 14 presumed to have TB were enrolled and produced samples for testing. Of the 536 with valid results, 31 tested positive for TB. As part of the IDDS assessment of the pilot, staff carried out appraisal interviews, and community beneficiaries and clinicians said that “stool testing saves our children.” They stressed how much relief this technique brings to parents who have a child with presumptive TB. Health care workers trained by IDDS recognized the enormous opportunity that stool testing brings to pediatric TB diagnostics and, therefore, accepted its introduction to TB diagnostics. Samples can be collected in a health

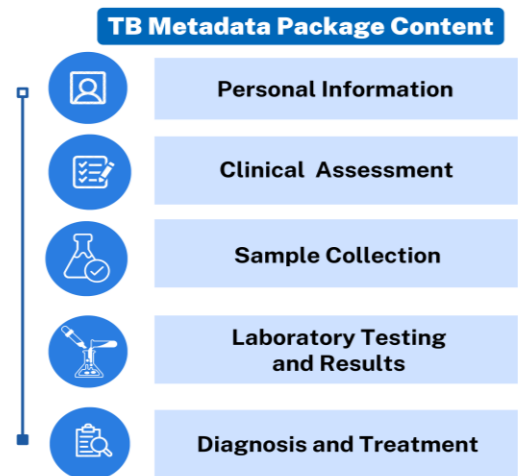


Figure 1. TB Stoolbox metadata package content

“I’m very satisfied with the pilot phase of the introduction of stool Xpert study for pediatric TB diagnosis.”  
–Professor Michel Kaswa Kayomo, DRC NTP Director

“Both clinical and laboratory staff are very appreciative of this innovation as it accords us with critical information to provide correct treatment.”

–Grace Mabaso, laboratory technician,  
Mzuzu Central Hospital, Malawi

care facility or at a child’s home and processed by any laboratory technician familiar with the GeneXpert® instrument. The NTLEP used the pilot implementation experience to plan to integrate stool-based testing into the routine of TB diagnostics across Malawi.

**BURMA**

In a collaborative effort with IDDS, the NTP in Burma initiated the implementation of stool Xpert MTB/RIF Ultra testing at two regional-level TB laboratories in the Yangon Region in January 2024. This joint endeavor was aimed at enhancing bacteriologically confirmed TB case detection in children in Burma. IDDS played a crucial role by providing technical support, including developing an implementation plan, SOPs, job aids, and stool specimen collection pamphlets. Additionally, IDDS held a stakeholder advocacy meeting and conducted comprehensive training sessions for laboratory and health care staff on stool specimen collection, storage, transportation, and testing. IDDS provided the necessary equipment and materials for implementation, such as GeneXpert 10-color instruments, Xpert MTB/RIF Ultra cartridges, stool specimen containers, and other accessories. From January 22 to February 29, 2024, 192 children ages 0-8 years had their stool tested for TB; 6 tested positive for MTB. All diagnosed children promptly began TB treatment. Though the implementation time was limited, the findings suggest the need for a child-friendly test. The positive impact of stool testing on prompt intervention and treatment initiation for MTB-positive cases underscores the importance of this diagnostic approach.

In a collaborative effort with IDDS, the NTP in Burma initiated the implementation of stool Xpert MTB/RIF Ultra testing at two regional-level TB laboratories in the Yangon Region in January 2024.

**CAMBODIA**

IDDS provided technical support to the National Tuberculosis Program of Cambodia (CENAT) in revising its diagnostic algorithm to include stool testing for childhood TB. IDDS trained 10 NTP staff, staff from 36 hospitals and 111 health centers from selected operational districts, and district staff on stool collection and processing. IDDS collaborated with CENAT to implement stool-based testing at selected 24 pilot health sites in 6 provinces. As part of implementation support, IDDS procured and distributed the stool collection kits and conducted supervision visits to monitor the field health staff. During the two-month implementation period, facilities tested 30 stool specimens for TB, and only 1 had an invalid result. Despite the short implementation time, laboratory technicians found stool processing simple and easy to adopt, which will help the CENAT scale up stool testing elsewhere in the country.



Figure 2. Training session in Cambodia. Photo by IDDS

**BANGLADESH, MOZAMBIQUE, AND TANZANIA**

IDDS developed SOPs for stool processing in Bangladesh and trained 40 regional TB reference laboratory technicians. In Mozambique, IDDS developed a national implementation plan for pediatric TB diagnosis. The project also trained two master trainers (a pediatrician from the NTP and a laboratory technician from the NTRL) and reviewed the training package to align with the local context. Additionally, IDDS trained 73 health

professionals from four provinces. In Tanzania, IDDS collaborated with the National TB and Leprosy Program technical working group to review existing TB diagnostic guidelines and incorporate stool testing for children.

## VIETNAM

IDDS collaborated with Vietnam’s National Tuberculosis Control Program to improve pediatric TB diagnostic techniques and tools. To ease specimen collection and acceptability among children and caregivers, IDDS implemented pediatric stool Xpert testing with the NTP in five provinces in Vietnam. IDDS developed a package of SOPs and conducted essential training on stool sample collection and stool Xpert testing in children. Following a successful pilot in the Nghe An province, IDDS established 13 GeneXpert sites to test stool specimens and supported the development of a network of 117 health facilities that supported childhood TB testing. IDDS also trained 462 health workers. A review of 2021 data showed that stool-based tests accounted for 37 percent of children’s total TB diagnostic tests in the 5 provinces, and 1 percent of the tested children had TB and started treatment immediately.

## ZIMBABWE

In Zimbabwe, IDDS collaborated with the National TB Control Program to scale stool-based testing for children’s TB diagnosis. IDDS trained 24 laboratory staff working at the provincial level to serve as national and provincial trainers. IDDS also trained 129 health care workers in 24 facilities in Harare city. To ensure that all childhood TB stakeholders embrace the scale-up of stool-based testing, IDDS organized the first

“If stool GeneXpert testing is scaled up, I believe that the diagnosis of TB in children will be improved significantly [which would] reduce the under-diagnosis due to sample collection difficulties.”

–Dr. Dau Minh Quang, Director of Nghe An Hospital

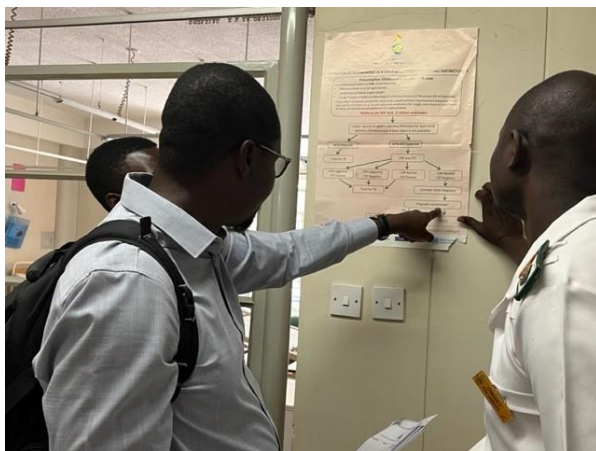


Figure 3. Mentorship support and supervision in Zimbabwe. Photo by IDDS

National Childhood TB Stakeholder Meeting, attended by 25 participants from the provinces and the central NTP. IDDS provided technical support to draft SOPs and job aids for the SOS stool processing method for childhood TB. Additionally, IDDS offered technical support to the NTP to cascade training on strengthening pediatric TB detection, diagnosis, and treatment. Following facilities’ training, IDDS collaborated with the NTP to conduct supportive supervision and mentorship visits to 62 facilities in 10 provinces and mentored 211 providers during the visits. Due to these visits, the NTP addressed the challenges identified and resolved some bottlenecks that facilities were facing.

## REGIONAL SUPPORT

IDDS identified the need to provide sustainable technical assistance for countries seeking to adopt stool-based testing methods. To accelerate the introduction and scale-up of stool-based testing techniques beyond supported countries, IDDS trained six master trainers from the Uganda Supranational TB Reference Laboratory (SRL) in Kampala. The Uganda SRL helps countries in the East and Southern Africa regions build robust and resilient TB laboratory systems, including providing support to introduce new technologies and



approaches. As a result of the training, the SRL supported an additional 10 countries in introducing stool testing using Xpert MTB/RIF and supported timely childhood TB diagnosis and detection of RIF resistance. The partnership enabled the Uganda SRL to train 29 participants from the supported countries, including additional participants from IDDS-supported countries (Cambodia, Mozambique, and the Philippines).



Figure 4. A group of SOS Master Trainers in Bulawayo, Zimbabwe. Photo by IDDS

## VALIDATING STOOL-BASED TESTING ON THE TRUENAT PLATFORM

To expand access to stool testing using other mWRDs, IDDS supported activities to establish the protocol for stool processing and testing on the Truenat platform using both the Truenat MTB Plus and Truenat MTB-RIF diagnostic assays. IDDS also supported activities to determine the protocol’s sensitivity and specificity against the SOS stool method on Xpert MTB/RIF Ultra.

Truenat is a near-point-of-care test that could expand access to stool-based testing. It has some advantages over Xpert in that it is a mobile technology that does not rely on a consistent supply of electricity, does not require extensive training to operate, and can be used to identify RIF resistance.

Since Truenat is a newer technology, a strong evidence base is needed to determine whether Truenat is suitable for stool testing. Molbio, the Truenat manufacturer, demonstrated the viability of stool testing using specimens spiked with TB bacilli, but did not conduct further evaluations.

Implementation was in two phases: the proof-of-concept phase (Phase 1) to establish SOPs for processing stool using the Truenat platform and the validation and pilot implementation phase (Phase 2) to pilot the implementation of the SOPs in routine settings. Building on the findings from background research conducted in Phase 1a, the team collectively developed a draft protocol and SOPs for validating samples from patients with confirmed TB (Phase 1b) and implemented it in routine settings (Phase 2).

Preliminary findings show that a defined stool protocol on Truenat works and can successfully detect MTB in stool.



## CHALLENGES AND LESSONS LEARNED

IDDS encountered common challenges in the project's efforts to scale SOS stool testing across supported countries. In facilities with multiple entry points, not all children visiting the facility were screened for TB, leading to missed opportunities to detect TB early. Collecting stool samples at the point of care can also be challenging and requires a caregiver to return the specimen at another time, which can result in missing cases among those who do not return. In some instances, there were delays in testing samples because trained laboratory technicians had other assignments or were not available.

IDDS experiences led to several important lessons for countries seeking to introduce this approach. These include:

- Engage stakeholders at all levels of the diagnostic network (national, regional, and facility levels) to create awareness and ensure the successful implementation of pediatric TB interventions.
- Train clinical staff alongside laboratory technicians to ensure that all providers can identify children presumed to have presumptive TB. Recommend appropriate laboratory tests, give appropriate treatment and referrals, and follow up with caregivers to come back as needed.
- Ensure the availability of updated diagnostic algorithms, SOPs, and job aids at the point of care to guide providers' implementation and decision-making.
- Plan and account for needed procurements to support stool-based testing, including Xpert cartridges and consumables, especially once the approach is integrated into the TB diagnostic algorithm.
- Plan for face-to-face targeted mentorship and supervision, which is vital to bridging knowledge gaps between trained and non-trained providers, supporting guidance of additional providers, and resolving bottlenecks.



## CONCLUSION

Stool-based testing offers a unique opportunity for children to access much-needed laboratory confirmation. Collecting stool samples is easy and acceptable to providers and caregivers. By supporting countries implementing stool-based testing, IDDS contributed to improving TB detection in children in supported countries, providing a feasible approach to closing testing gaps for this demographic group.



## REFERENCE LIST

1. Dodd, P. J., Yuen, C. M., Sismanidis, C., Seddon, J. A., & Jenkins, H. E. (2017). The global burden of tuberculosis mortality in children: A mathematical modelling study. *The Lancet Global Health*, 5(9), e898–e906. [https://doi.org/10.1016/S2214-109X\(17\)30289-9](https://doi.org/10.1016/S2214-109X(17)30289-9)
2. de Haas P, Yenew B, Mengesha E, Slyzkyi A, Gashu Z, Lounnas M et al. The simple one-step (SOS) stool processing method for use with the Xpert MTB/RIF assay for a child-friendly diagnosis of tuberculosis closer to the point of care. . *Journal of Clinical Microbiology*, 59(8), e0040621. <https://doi.org/10.1128/JCM.00406-21>
3. Jasumback, C. L., Dlamini, Q., Kahari, J., Maphalala, G., Dlamini, M. G., Dube, G. S., DiNardo, A., Kirchner, H. L., Mandalakas, A., & Kay, A. W. (2021). Laboratory comparison of stool processing methods for Xpert® Ultra. *Public Health Action*, 11(2), 55–57. <https://doi.org/10.5588/pha.20.0079>
4. Lounnas, M., Diack, A., Nicol, M. P., Eyangoh, S., Wobudeya, E., Marcy, O., Godreuil, S., & Bonnet, M. (2020). Laboratory development of a simple stool sample processing method diagnosis of pediatric tuberculosis using Xpert Ultra. *Tuberculosis*, 125, 102002. <https://doi.org/10.1016/j.tube.2020.102002>
5. World Health Organization (2021a). *WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection (2021 update)*. Geneva: World Health Organization. <https://www.who.int/publications/i/item/9789240030589>
6. World Health Organization (2021b). *WHO consolidated guidelines on tuberculosis: Module 3: Diagnosis: Rapid diagnostics for tuberculosis detection (2021 update)*. Geneva: World Health Organization. <https://apps.who.int/iris/handle/10665/342331>
7. World Health Organization (2022). *Practical manual of processing stool samples for diagnosis of childhood TB*. Geneva: World Health Organization. <https://www.who.int/publications/i/item/9789240042650>
8. World Health Organization (2023). *Roadmap towards ending TB in children and adolescents (3rd edition)*. Geneva: World Health Organization. <https://www.who.int/publications/i/item/9789240084254>