Chronicles of Biomedical Sciences
ISSN:3065-923X
Homepage: https://cbsciences.us/index.php/cbs

Optimizing Diagnosis of Pediatric Tuberculosis: The Value of Stool Samples for GeneXpert Testing

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REVIEW ARTICLE

Received on: February 05, 2026.
Accepted on: March 10, 2026.
Published on: March 15, 2026.
Keywords: GeneXpert; Global Burden; Pediatric; Smear-Negative Tuberculosis.

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ABSTRACT

Tuberculosis (TB) remains a leading infectious cause of morbidity and mortality worldwide, afflicting an estimated 10.7 million people in 2024 and causing approximately 1.23 million deaths, despite decades of public health efforts. Of these cases, children under 15 accounted for around 1.2 million, underscoring a persistent global challenge in pediatric TB diagnosis and care. TB disproportionately affects low- and middle-income countries, with eight countries including India, Indonesia, the Philippines, China, and Pakistan—bearing two-thirds of the global burden. Pakistan alone contributed an estimated 6.3 % of total global cases in 2024, ranking among the top five high-burden countries. Pediatric TB is largely underdiagnosed due to nonspecific symptoms and challenges inherent in specimen collection from young children, who often cannot produce sputum. Conventional tools such as sputum smear microscopy fail to detect many pediatric cases due to paucibacillary disease. The GeneXpert MTB/RIF and Xpert Ultra assays have transformed TB diagnostics by enabling rapid nucleic acid amplification with high specificity, significantly improving case detection, especially in smear-negative disease. Because children commonly swallow respiratory secretions containing *Mycobacterium tuberculosis*, stool samples have emerged as a feasible, non-invasive specimen for GeneXpert testing. Evidence from multiple studies demonstrates that stool GeneXpert yields moderate sensitivity and high specificity, expanding diagnostic opportunities where respiratory specimens are difficult to obtain. This review synthesizes global and national epidemiology, diagnostic performance, operational considerations, and research advances, culminating in recommendations for enhanced implementation and future research to close the pediatric TB detection gap.

Citation: Rehman S, Khan S, Munir MA. Optimizing diagnosis of pediatric tuberculosis: the value of stool samples for GeneXpert testing. Chron Biomed Sci. 2026;3(1):69. Available from: https://cbsciences.us/index.php/cbs/article/view/69.

Introduction

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis*, primarily affecting the lungs but capable of causing extrapulmonary disease. Despite global progress in control, TB remains one of the top 10 causes of death globally and the leading cause from a single infectious agent. In 2024, an estimated 10.7 million people developed active TB disease worldwide, including 1.2 million children, demonstrating persistent transmission and major gaps in case detection and treatment coverage [1][2].

While TB affects all regions, the burden is concentrated in certain countries. Pakistan is among the top five high-burden nations, contributing roughly 6.3 % of all global cases (~670,000 cases in 2024), highlighting its critical role in global TB control [3]. Although treatment

success for drug-susceptible TB is high (~88%), national programs face challenges including diagnostic gaps, particularly for pediatric cases and drug-resistant TB [1]. Childhood TB continues to cause significant mortality and morbidity worldwide, especially in high-burden regions. According to the WHO, over a million children develop TB annually, but a substantial proportion remain undiagnosed or unreported due to diagnostic challenges unique to this age group. Young children often cannot expectorate sputum, and alternative respiratory specimens such as gastric aspirates (GA) or induced sputum—require trained personnel and infrastructure, limiting diagnostic access in low-resource settings [1][2].

The GeneXpert MTB/RIF assay and its advanced version, Xpert Ultra (Cepheid), are rapid molecular nucleic acid amplification tests (NAATs) designed for the detection of MTB and rifampicin resistance. These assays offer results in under two hours with minimal biosafety requirements and have transformed TB diagnosis globally. However, their performance depends on the quality and type of specimen provided [4].

MTB bacilli expelled from infected lung sites may be swallowed with sputum or nasopharyngeal secretions, traversing the gastrointestinal tract relatively intact [5]. These bacilli or their DNA fragments can be detected in fecal samples using NAAT platforms such as GeneXpert. The detection likelihood varies with bacillary load, age, nutritional status, and disease severity. Pediatric TB is difficult to diagnose: symptoms are nonspecific, children produce low bacillary loads ("paucibacillary") and often cannot provide sputum samples. Children frequently require invasive procedures like gastric aspirates or induced sputum, which are resource-intensive and poorly accessible in many settings [6]. These limitations have contributed to underdiagnoses and delayed treatment, with many childhood cases being reported only clinically without bacteriological confirmation.

Stool offers a non-invasive, readily obtainable specimen particularly valuable in children who cannot expectorate sputum. The concept is based on physiological swallowing of respiratory secretions containing MTB organisms, which subsequently pass through the gastrointestinal tract and can be detected in faeces by sensitive molecular assays. The inherent simplicity of stool collection makes it an attractive alternative or supplementary specimen for TB diagnosis, especially in children under 5 years who account for the largest gaps in case detection [7]

Considering above narration the aim of this review is to evaluate the role of stool samples as a non-invasive specimen for GeneXpert MTB/RIF and Xpert Ultra testing in the diagnosis of pediatric tuberculosis, particularly in settings where respiratory specimen collection is difficult. Further to discuss the operational advantages and limitations of stool-based molecular testing in routine clinical and programmatic settings.

Diagnostic Challenges in Paediatric Tuberculosis

Paucibacillary Disease and Specimen Collection: In children, particularly under age five, TB typically presents with low bacillary loads. This paucibacillary nature lowers the sensitivity of conventional diagnostics like smear microscopy and even culture, which require larger numbers of organisms to detect infection. The inability of young children to expectorate sputum compounds this challenge, forcing reliance on invasive sampling or clinical diagnosis [8].

Smear-Negative TB and GeneXpert: Smear microscopy, long a staple of TB diagnosis, exhibits limited sensitivity in both adult and pediatric settings, particularly in smear-negative patients. Smear-negative TB is common in children due to the paucibacillary nature of disease. Molecular diagnostics such as the GeneXpert MTB/RIF assay have enhanced performance in such cases, detecting *M. tuberculosis* DNA and rifampicin resistance with greater sensitivity than microscopy. Research from Pakistan showed that GeneXpert detected 54 additional cases missed by smear microscopy, demonstrating added value in smear-negative populations [9]. Pediatric TB diagnosis is challenging due to nonspecific symptoms and difficulty obtaining respiratory samples. In this study of 187 children, gastric aspirate GeneXpert showed higher diagnostic accuracy (86% sensitivity, 92% specificity) compared with stool GeneXpert (63% sensitivity, 76.7% specificity). Although less sensitive, stool testing provides a practical non-invasive alternative for TB diagnosis in resource-limited settings where gastric aspirate collection is difficult. The Xpert Ultra assay, the next-generation platform with lower limits of detection, further improves sensitivity, especially in paucibacillary specimens, making molecular diagnostics indispensable for modern TB programs [10].

Advantages of Stool GeneXpert Testing

Non-Invasive and Accessible: Stool collection is simple, painless, and non-invasive, making it particularly suitable for children who cannot produce sputum. Unlike

gastric aspirate procedures, stool collection does not require specialized equipment, trained personnel, or hospital admission, allowing specimen collection even in community or primary healthcare settings. This improves access to molecular TB testing in resource-limited areas and reduces the burden on tertiary care facilities [11].

Enhances Case Detection: Stool-based GeneXpert testing helps identify tuberculosis cases among children who are unable to expectorate sputum, a common limitation in pediatric TB diagnosis. Since children often swallow respiratory secretions containing Mycobacterium tuberculosis, these bacilli can pass through the gastrointestinal tract and be detected in stool samples. Studies have shown that stool GeneXpert can detect TB in cases where respiratory specimens are unavailable, thereby improving bacteriological confirmation and reducing underdiagnoses in high-burden settings [12].

High Specificity: Evidence from multiple studies indicates that stool GeneXpert testing demonstrates consistently high specificity, often exceeding 95%, meaning that false-positive results are rare. High specificity is crucial in TB diagnostics because it ensures that children identified as positive are very likely to have true infection, thereby supporting timely treatment initiation and strengthening clinician confidence in the test results. This reliability makes stool-based molecular testing a valuable adjunct diagnostic tool in pediatric TB programs [13].

Performance of Stool GeneXpert in Pediatric TB

A meta-analysis incorporating 12 pediatric studies (2 177 children) found that Xpert MTB/RIF on stool specimens had a pooled sensitivity of 50 % (95 % CI: 44–56 %) against bacteriologically confirmed TB via respiratory specimens, with high pooled specificity of 99 % (95 % CI: 98–99 %). Despite heterogeneity, this supports stool as a supplementary diagnostic specimen in children when standard respiratory samples are unavailable [14]

A cohort study in Pakistan reported overall stool Xpert-Ultra sensitivity of 56 % (CI 43–67 %) against a composite reference standard for microbiologically confirmed TB, with specificity up to 100 %. Sensitivity was higher in older children (≥10 years) and severely malnourished children, while specificity remained consistently high [7]. In a study from Bangladesh of 447 children, Xpert Ultra on stool had sensitivity of 58.6 % and specificity of 88.1 % compared to induced sputum bacteriologic confirmation, suggesting superior sensitivity of Ultra over standard Xpert in pediatric stool [15]. Studies in resource-limited settings (e.g., South Africa) found that stool Xpert had specificity >99 %, albeit modest sensitivity (~32 %). Severe disease features (e.g., cavitary lung lesions) increased stool positivity [16].

Diagnostic Findings of Key Studies

A body of studies demonstrates that GeneXpert and Xpert Ultra on stool samples yield moderate sensitivity but high specificity; In a study of Pakistani children, stool GeneXpert showed lower sensitivity compared with gastric aspirate GeneXpert % vs. 86 %), though the stool method remained a feasible non-invasive alternative [10] Prospective hospital studies report sensitivities ranging from approximately 32 % to 83 % in various cohorts, with high specificity [17]. In Dushanbe, Tajikistan, Xpert Ultra on stool achieved ≈69 % sensitivity and ~99 % specificity, underscoring the utility even in lower burden populations [18]. National scale-up programs in Bangladesh reported substantial testing volumes and positivity rates, validating operational scalability [15]. Studies consistently show that sensitivity increases with bacillary load and age, while specificity remains high, making stool GeneXpert an important supplementary diagnostic tool.

Table 1: Diagnostic findings from key studies

Study	Setting	Age	Specimen	Sens. (%)	Spec. (%)	Comments
Khan et al. (2025)	Pakistan tertiary	2–12	Stool vs Gastric GeneXpert	63 vs 86	76.7 vs 92	Stool non-invasive, lower sensitivity [10]
Dushanbe et al. (2023)	Tajikistan	≤15	Stool Xpert Ultra	68.8	98.7	High specificity, feasible implementation [18]
Muni et al. (2025)	India tertiary	≤15	Stool Xpert	32–83	High	Comparable performance trends [17]
Bangladesh scale-up (2024)	National program	<15	Stool Ultra	~5.3 % positivity (~Trace)	—	Large field implementation [15]

Limitations and Challenges

Moderate Sensitivity: Although stool GeneXpert testing provides a useful diagnostic option, its sensitivity is generally moderate compared with respiratory specimens such as gastric aspirate or induced sputum. This limitation is mainly due to the paucibacillary nature of pediatric TB, where the bacterial load is low, particularly in younger children. As a result, some true TB cases may remain undetected if stool testing is used alone. Therefore, stool GeneXpert is often recommended as a complementary test rather than a standalone diagnostic tool, especially in high-burden settings [11]

Sample Processing Variability: The diagnostic performance of stool GeneXpert can vary depending on sample preparation and processing techniques. Stool contains substances such as bile salts, complex organic material, and PCR inhibitors that may interfere with molecular assays. Currently, different laboratories use varying preprocessing methods (e.g., dilution, filtration, centrifugation), which can lead to differences in test sensitivity and reproducibility. Standardized protocols are therefore needed to improve test reliability and allow better comparison of results across studies and clinical settings [12].

Resource Implications: Although stool collection itself is simple and non-invasive, the GeneXpert testing platform still requires laboratory infrastructure, trained personnel, electricity supply, and consistent availability of cartridges. These requirements can limit widespread implementation in decentralized or rural healthcare facilities in low- and middle-income countries. Additionally, cartridge costs and supply chain interruptions may affect program sustainability. Strengthening laboratory networks and ensuring reliable supply systems are essential for effective integration of stool-based GeneXpert testing into routine paediatric TB diagnostic services [13].

Discussion

Paediatric TB remains a significant global health challenge, particularly in high-burden countries such as Pakistan. Although advances in diagnostic technology have improved TB detection overall, diagnosing TB in children continues to be difficult due to the paucibacillary nature of the disease and challenges in obtaining suitable respiratory specimens. This review highlights the emerging role of stool specimens for molecular detection of *Mycobacterium tuberculosis* using GeneXpert assays and their potential contribution to improving pediatric TB diagnosis [17]

A key obstacle in pediatric TB diagnosis is the inability of young children to expectorate sputum. Conventional diagnostic procedures such as gastric aspiration or induced sputum collection require specialized infrastructure and trained healthcare personnel, which may not be readily available in many low- and middle-income countries. Consequently, a large proportion of childhood TB cases are diagnosed clinically without bacteriological confirmation. Stool samples represent a practical alternative specimen because they can be collected easily and non-invasively in both hospital and community settings [18].

The biological basis for stool-based testing lies in the natural swallowing of respiratory secretions by children. Secretions originating from infected pulmonary sites may contain *M. tuberculosis* bacilli that pass through the gastrointestinal tract. These organisms or their genetic material can subsequently be detected in fecal samples using molecular diagnostic platforms. This concept has gained increasing attention as researchers seek alternative specimens that can enhance microbiological confirmation of TB in children [19].

The development of molecular nucleic acid amplification technologies, particularly the GeneXpert MTB/RIF assay and the more sensitive Xpert MTB/RIF Ultra, has significantly transformed TB diagnostics worldwide. These assays allow rapid detection of *M. tuberculosis* DNA and rifampicin resistance within a short time frame and demonstrate greater sensitivity than conventional smear microscopy. Their ability to detect TB in smear-negative and paucibacillary specimens has made them particularly valuable in pediatric populations [20][21].

Evidence from multiple studies indicates that GeneXpert testing on stool specimens demonstrates moderate sensitivity but consistently high specificity. A large meta-analysis including more than two thousand children reported pooled sensitivity of approximately 50% and specificity approaching 99% when compared with bacteriologically confirmed TB using respiratory samples. These findings suggest that while stool testing may not detect all cases, a positive result provides strong evidence of infection and can facilitate early initiation of treatment [10][15].

Research conducted in high-burden settings has produced similar results. Studies in South Asia have reported stool GeneXpert sensitivities ranging from approximately 32% to 70%, depending on disease severity, age group, and laboratory methods used. Importantly, specificity in these studies has remained

consistently high, often close to 100% [18]. In Pakistan, investigations comparing stool GeneXpert with gastric aspirate testing demonstrated lower sensitivity for stool specimens but confirmed their value as a feasible non-invasive diagnostic option when respiratory sampling is not possible [17].

Another important implication of stool-based testing is its potential role in strengthening TB surveillance and case detection. Childhood TB is widely recognized to be underdiagnosed and underreported globally. The availability of a simple and non-invasive specimen may increase microbiological confirmation rates and support more accurate epidemiological monitoring. This is particularly relevant in countries with high TB burden, where delayed diagnosis contributes to disease progression and continued community transmission [7].

Despite these advantages, several challenges must be considered before widespread implementation. The moderate sensitivity observed in many studies remains the most important limitation. Pediatric TB often involves low bacterial loads, which reduces the probability of detecting *M. tuberculosis* DNA in stool samples. Additionally, variability in stool processing techniques may influence test performance. Differences in sample preparation methods, including dilution, filtration, and centrifugation protocols, can affect assay sensitivity and create inconsistencies across laboratories [11][12].

Operational factors also influence the feasibility of stool GeneXpert testing. Although specimen collection itself is simple, molecular testing still requires specialized equipment, stable electricity supply, and consistent availability of cartridges. These requirements may limit expansion in resource-constrained settings. Nevertheless, ongoing international efforts to scale up molecular TB diagnostics are gradually improving access to GeneXpert platforms in many high-burden regions.

Overall, the available evidence supports the role of stool GeneXpert testing as a complementary diagnostic approach rather than a replacement for respiratory specimen analysis. When integrated with existing diagnostic strategies, stool testing can increase the likelihood of bacteriological confirmation in children who cannot provide sputum samples. The introduction of more sensitive assays such as Xpert Ultra may further improve detection rates, particularly in paucibacillary disease.

Future research should focus on optimizing stool sample processing techniques and establishing standardized laboratory protocols to improve diagnostic accuracy. Large-scale implementation studies are also needed to evaluate the cost-effectiveness and operational impact of stool-based testing within national TB control programs [20].

In conclusion, stool specimens analyzed with GeneXpert assays provide a valuable non-invasive option for improving the diagnosis of pediatric tuberculosis. Although sensitivity remains variable, the consistently high specificity and ease of specimen collection make stool GeneXpert an important supplementary diagnostic tool. Incorporating this approach into TB diagnostic algorithms may help close the persistent diagnostic gap in childhood TB and contribute to earlier detection and improved treatment outcomes.

Future Directions

Further research should focus on:

- Standardizing stool processing and sample handling.
- Enhancing molecular assay sensitivity for pediatric specimens.
- Evaluating operational implementation and cost-effectiveness in routine care.

Integrating stool GeneXpert into national diagnostic algorithms with quality assurance frameworks.

Authors' contributions

ICMJE criteria	Details	Author(s)
1. Substantial contributions	Conception, OR Design of the work, OR Data acquisition, analysis, or interpretation	1 2 3
2. Drafting or reviewing	Draft the work, OR Review critically for important intellectual content	1,2 3
3. Final approval	Approve the version to be published	1,2,3
4. Accountable	Agree to be accountable for all aspects of the work	1,2,3

Acknowledgement

None

Funding

This research study received no specific grant from any funding agency.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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