Review Article

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Current Applications of Non-invasive, Non-Sputum-Based Diagnostic Tests for Pulmonary Tuberculosis

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Abstract:

Tuberculosis (TB) remains one of the leading infectious causes of death worldwide. Sputum is the primary specimen for pulmonary TB (PTB) diagnosis through culture, smear microscopy, and nucleic acid amplification tests (NAATs). However, sputum collection is often difficult in high-risk groups such as children, the elderly, and immunocompromised patients. Also, sputum induction poses biosafety risks and performs poorly in paucibacillary disease. To address these limitations, alternative non-invasive specimens, including saliva, oral swabs, urine, and stool, have been investigated over the past decade for PTB diagnosis. This review synthesises evidence on diagnostic accuracy, target populations, and assay platforms. Saliva and oral swabs are easy to collect and well accepted by patients, with sensitivities ranging from 30–100% and specificity from 80–100%, though yield depends on collection method and bacillary load. Urine lipoarabinomannan (LAM) assays provide another option: the AlereLAM test is endorsed by the World Health Organization (WHO) for people living with HIV with CD4 counts ≤100 cells/µL, with pooled sensitivities of 25–55% and specificities of 75-95%. The newer FujiLAM assay, currently under evaluation, shows higher sensitivity (40–85%) and specificity (85-95%). Stool Xpert Ultra achieves 35–85% sensitivity and 85–100% specificity, offering particular value in children unable to expectorate sputum. These findings highlight best-use scenarios such as urine LAM in advanced HIV, stool testing in children, and oral swabs in elderly or sputum-scarce populations. Future progress in biomarker discovery, simplified processing, and portable molecular platforms will be critical for scaling up non-sputum diagnostics and closing diagnostic gaps in the vulnerable groups.

Keywords: tuberculosis, non-sputum-based diagnostic tests, saliva, urine, stool

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Introduction

Tuberculosis (TB) has regained its status as the leading cause of death worldwide from a single infectious agent, surpassing the coronavirus disease, 2019 (COVID-19), with an estimated 1.25 million deaths reported in 2023. The disease occurs predominantly in low- and middle-income countries, particularly in rural areas where access to healthcare is limited. In 2023, an estimated 10.8 million people developed TB, yet only 8.2 million were notified and received care, leaving 2.6 million either undiagnosed or unreported. The diagnosis and treatment of TB are further complicated by the emergence of multidrug- or rifampicin-resistant TB (MDR/RR-TB). An estimated 400,000 people developed MDR/RR-TB, yet only 175,923 were diagnosed and treated, representing just 44% of those affected[1]. This persistent diagnosis–treatment gap underscores the urgent need for improved diagnostic strategies.

TB is caused by *Mycobacterium tuberculosis* (Mtb) and is transmitted via airborne infectious aerosols through close contact with an index patient. TB primarily affects the lungs, resulting in pulmonary TB (PTB). Currently, diagnosis relies mainly on sputum-based methods such as culture, smear microscopy, and nucleic acid amplification tests (NAATs), but each has important drawbacks. Culture, while considered the gold standard and highly sensitive, is slow and resource-intensive. Smear microscopy is rapid and cheap but lacks sensitivity and specificity. NAATs such as Xpert®, Truenat®, and FluoroType® provide accuracy but need costly equipment and laboratory infrastructure, while TB-LAMP is point-of-care but cannot detect drug resistance (Table 1)[2,3]. Consequently, these approaches fall short in many high-burden, resource-limited settings. These limitations highlight the need for alternative, non-sputum-based diagnostics.

Sputum collection itself poses significant barriers. The act of coughing generates infectious aerosols, increasing biosafety risks for healthcare workers and close contacts. Furthermore, many patients, particularly infants, young children, the elderly, and immunocompromised individuals, face difficulty in expectorating sputum. This challenge is compounded in paucibacillary disease, where the bacterial load is low and detection rates decline[4-6].

Invasive respiratory-related specimens such as induced sputum (IS), gastric aspiration (GA), bronchoalveolar lavage (BAL), and nasopharyngeal aspirates (NPA) can improve diagnostic yield. However, these procedures require facility-based collection, trained staff, and specialised equipment, making them impractical for large-scale use. For example, in children, GA and IS have higher diagnostic sensitivity than expectorated sputum, yet they remain resource-intensive and uncomfortable for patients[7]. Thus, while useful, these approaches cannot close the diagnostic gap in most routine care settings.

Taken together, these limitations highlight the need for non-invasive, non-sputum-based diagnostic alternatives. Such approaches have the potential to expand access, reduce biosafety risks, and improve case detection, particularly in rural and resource-limited environments. This review will critically examine the feasibility and performance of alternative specimens, including saliva, oral swabs, urine, and stool, for PTB diagnosis. For each specimen, we summarise key biomarkers, diagnostic performance, operational considerations, and any remaining challenges to inform clinicians and researchers on the future direction of TB diagnostics.

Saliva

Saliva is generally easier to collect than sputum and carries a lower risk of infection transmission, making it more suitable for point-of-care testing in various healthcare settings. Its feasibility is supported by recent global experience, such as the World Health Organization (WHO) endorsement of saliva for COVID-19 testing as a

Table 1 Comparison of commonly used sputum-based diagnostic methods for pulmonary TB

Diagnostic method	Turnaround time	Sensitivity (bacilli/mL)	Advantages	Limitations
Culture				
Solid culture (e.g., Löwenstein–Jensen)	Colony growth up to 3 weeks; DST adds 2–6 weeks	~10^1–10^2	Gold standard; allows full drug susceptibility testing	Very slow; needs biosafety facilities; resource-intensive
Liquid culture (e.g., MGIT)	7–14 days for detection; DST adds 1–2 weeks	~10^1–10^2	Gold standard; faster and higher sensitivity than solid culture	Still lab-dependent; needs biosafety facilities; costly; contamination risk
Smear microscopy				
Ziehl–Neelsen (ZN); Auramine O fluorescence (AO)	Same day (hours)	~5×10^3 – 1×10^4	Simple, rapid, inexpensive; AO higher sensitivity than ZN	Low sensitivity and specificity; cannot detect drug resistance
Nucleic acid amplification tests (NAATs)				
Xpert MTB/RIF; Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA)	~2 hours	~131 (Xpert) ~16 (Ultra)	Automated; simultaneous detection of TB and rifampicin resistance; Xpert Ultra higher sensitivity than Xpert; WHO-recommended	Cartridge-based; requires infrastructure and cost barriers
Truenat MTB / MTB Plus / MTB-RIF Dx (Molbio Diagnostics, Goa, India)	~1 hour (MTB/Plus); additional ~1 hour for MTB-RIF Dx	~100 (MTB) ~30 (Plus)	Portable, battery-operated; chip-based real-time PCR; suitable for near-point-of-care; detects rifampicin resistance (RIF Dx); WHO-recommended	Requires separate MTB/MTB Plus test before RIF resistance detection (MTB-RIF Dx)
TB-LAMP (Loop-mediated Isothermal Amplification) (Eiken Chemical, Tokyo, Japan)	~1 hour	~100	Low-cost; minimal equipment; suitable for point-of-care; WHO-recommended	Does not detect drug resistance
FluoroType MTBDR (Bruker/Hain Lifescience, Nehren, Germany)	~3 hours	~16	Simultaneous detection of TB and rifampicin and isoniazid resistance; WHO-recommended	Lab-based; requires PCR platform and trained staff

AFB=Acid-fast bacilli, AO=Auramine O fluorescence microscopy, DST=Drug susceptibility testing, LAMP=Loop-mediated isothermal amplification, MGIT=Mycobacteria Growth Indicator Tube, MTB=*Mycobacterium tuberculosis*, NAAT=Nucleic acid amplification test, PCR=Polymerase chain reaction, RIF Dx=Rifampicin resistance detection, TB=Tuberculosis, ZN=Ziehl–Neelsen microscopy

more acceptable and safer alternative to nasopharyngeal swabs[8]. For TB, saliva offers significant potential as a diagnostic specimen, with studies exploring host biomarker signatures, antibody responses, and direct Mtb detection.

In infectious diseases, host biomarkers provide valuable information about immune response, disease severity, and treatment outcomes, and thus have potential diagnostic utility. A major limitation of saliva, however, is the lower concentration of informative analytes compared with serum[9]. Despite this, several salivary biomarkers have shown promise in TB diagnosis. Multiplex cytokine analyses of saliva from TB patients demonstrated that combinations of host biomarkers can differentiate TB from non-TB cases with high sensitivity and specificity. Jacobs et al. (2016) reported that a 7-marker panel (CRP, ferritin, serum amyloid P, MCP-1, α2-macroglobulin, fibrinogen, and tissue plasminogen activator) achieved 78.1% sensitivity and 83.3% specificity[10]. The same group later proposed a 5-marker panel (IL-1β, IL-23, ECM-1, HCC1, and fibrinogen) with 88.9% sensitivity and 89.7% specificity regardless of HIV status, and an 8-marker panel [ECM1, myoglobulin, HCC1, IL-21, ENA-78, TPA, IL-12(p40), and IL-13] with 100% sensitivity and 95.0% specificity in HIV-negative patients[11]. Importantly, cytokine changes during treatment were also observed, suggesting potential use in monitoring therapy[10,11]. Other studies have identified alternative biomarker panels, such as a 4-marker panel (fractalkine, IP-10, IL-1α, and VEGF)[12], and elevated levels of fractalkine, IL-17, IL-6, IL-9, MIP-1β, CRP, VEGF, and IL-5 in TB patients[13]. In addition, proteomic analysis using mass spectrometry identified a 5-protein marker panel (P01011, Q8NCW5, P28072, A0A2Q2TTZ9, Q99574) with 100% sensitivity and 90.9% specificity[14]. However, reproducibility across cohorts remains limited, and these findings require validation in larger, multicentre studies before clinical implementation. Cost and technical complexity of multiplex assays may also limit use in peripheral settings.

Saliva contains immunoglobulins generated during adaptive immune responses. Secretory IgA (SIgA), the dominant antibody in mucosal secretions, has been evaluated for TB diagnosis. Saliva-based assays have measured SIgA responses against Mtb antigens such as the 38-kDa protein[15–17] and purified protein derivative (PPD)[16,17]. The 38-kDa antigen, actively secreted on the bacterial surface, elicits both B- and T-cell responses[18,19]. In contrast, PPD is a heterogeneous antigen mixture also present in other mycobacteria, resulting in low specificity[20]. Comparative studies show stronger reactivity with the 38-kDa antigen than with PPD, in both TST-positive and TST-negative TB patients (28.0% vs 16.6% and 14.2% vs 0%, respectively). Moreover, studies of household contacts with latent TB infection (LTBI) revealed elevated IgA responses to additional antigens, such as lipoarabinomannan (LAM), PstS1, cell membrane fraction (CMF), and culture filtrate proteins (CFP), suggesting roles for SIgA in both TB pathogenesis and transmission dynamics[21].

Direct detection of Mtb in saliva remains challenging because bacterial load is substantially lower than in sputum (approximately 0.1–1.0% of sputum bacillary concentration). Semiquantitative analysis using Xpert Ultra showed higher mycobacterial loads in sputum, with 78.0% of samples graded as high/medium, compared to only 14.0% of saliva samples reaching medium grade. Nevertheless, in sputum culture-confirmed TB patients, saliva tested by Xpert Ultra achieved 90.0% sensitivity[22]. To enhance detection, innovative enrichment techniques have been developed. One example is the lipobiotin-functionalized magnetic bead (LMB) assay, which exploits the affinity of lipobiotin for mycobacterial surface lipids. The method increased mycobacterial DNA concentration by 4.2–8.7-fold in saliva spiked with Mtb, improving clinical detection rates from 47.1% to 70.6%[23].

Overall, saliva demonstrates potential as a non-invasive diagnostic specimen, but significant limitations remain. The lower bacillary concentration and variability in analyte levels reduce sensitivity and reproducibility, particularly in routine settings. Biomarker panels, though showing potential, remain largely exploratory, and cost-effective assays that can reliably distinguish active from latent TB are still lacking. Further validation is required before saliva-based testing can be integrated into clinical practice.

Oral swab

Oral swab analysis (OSA) is a potential non-sputum approach for TB diagnosis. Mtb DNA can be detected in the oral cavity and demonstrates diagnostic performance comparable to sputum in patients with high bacillary loads. For example, Xpert-OSA achieved sensitivities of 90.0% and 79.0% in patients with high and medium sputum bacillary loads, although sensitivity dropped to 38.0% in those with low or very low loads[24]. In children, Xpert-OSA was less sensitive than sputum testing (43.0%) but still detected 24.0% of sputum-negative, clinically diagnosed TB cases, suggesting potential to identify otherwise missed infections[25].

Diagnostic yield depends on several factors, including swab site, swab type, number of swabs, timing of collection, and patient characteristics. Tongue and buccal swabs generally yield higher bacterial loads than gum swabs[26,27]. Swab type may also influence biomass collection, with FLOQSwabs (Copan, USA) and PurFlock Ultra (Puritan, USA) often performing better than other brands[27,29], though some studies found no significant differences[27]. Variability across studies appears largely attributable to patient factors, and adjusted analyses suggest overall similar performance between swab types[29,30]. The number of swabs collected is also critical, as single OSA tests are less sensitive than sputum, but using at least two to three swabs substantially improves detection rates[25,26,31]. However, this requirement may be dependent on the swab type, with higher-capacity swabs showing improved performance[28]. Timing matters as well. Early-morning swabs consistently demonstrate higher sensitivity than spot or evening samples, and combining multiple daily swabs can increase sensitivity to 82.6% with a specificity of 94.5%[31,32]. For the best results, samples should be processed promptly, ideally within 30 days, as prolonged storage reduces positivity rates[33].

From a feasibility perspective, oral swabs are easy to collect, painless, and more acceptable to patients than sputum induction or invasive procedures. They can be obtained in community settings by minimally trained staff, offering clear advantages for large-scale screening. However, diagnostic accuracy remains variable, particularly in individuals with a low bacillary burden, and standardisation of collection protocols is still required.

Urine

Urine-based diagnostics for TB focus mainly on lipoarabinomannan (LAM), a glycolipid of the *Mtb* cell wall. The Alere Determine™ TB LAM Ag test (AlereLAM; Abbott, Palatine, IL) is a lateral flow assay using polyclonal antibodies. In 2015, the World Health Organization (WHO) recommended AlereLAM for diagnosing TB in HIV-infected patients with CD4 counts ≤100 cells/μL[34], and in 2019 extended its use to both inpatient and outpatient settings[35]. Operationally, the test is inexpensive, requires minimal infrastructure, and provides results within 25 minutes, which makes it particularly useful in low-resource, high-burden settings. However, sensitivity is limited, particularly among HIV-negative individuals and those with higher CD4 counts. A meta-analysis reported higher pooled sensitivity among adults with CD4 ≤100 cells/μL (56.0%) compared to those with CD4 >100 cells/μL (26.0%)[36]. In children, AlereLAM (73.2%) outperformed smear microscopy (2.8%), Xpert (20.0%), and culture (17.8%)[37]. It has shown particular utility in HIV-positive and malnourished children, with sensitivity and specificity among HIV-positive children <15 years were 46.6% and 76.5%, and among HIV-negative children were 32.3% and 79.1%[38]. Among malnourished children <5 years, 37.8% were AlereLAM-positive while Xpert was negative for all samples[39], highlighting its role as an important complementary diagnostic tool in vulnerable paediatric populations.

To improve sensitivity, Fujifilm SILVAMP TB LAM assay (FujiLAM; Fujifilm, Tokyo, Japan) was developed. This lateral flow test uses high-affinity monoclonal antibodies directed against LAM epitopes and incorporates a silver-amplification step to enhance band intensity. Although more expensive than AlereLAM, FujiLAM is more cost-effective because of its substantially higher sensitivity. LAM is relatively stable in urine, and FujiLAM results have shown strong agreement between fresh samples (≤4 hours) and biobanked samples stored for one month (93.4%)[40]. In adults with HIV, FujiLAM demonstrated markedly superior sensitivity compared to AlereLAM (70.4% vs 42.3%), while maintaining similar specificity (90.8% vs 95.0%). Performance was strongly associated with immune status: sensitivity was highest among patients with CD4 ≤100 cells/µL (84.2% vs 57.3%) and decreased in those with CD4 101–200 (60.6% vs 26.4%) and >200 cells/µL (44.0% vs 12.2%)[41]. These findings are supported by Bjerrum et al. (2020)[42] and Broger et al. (2020)[43], who confirmed higher sensitivity in both inpatient and outpatient cohorts of HIV-positive patients with advanced immunosuppression. In children under 15 years, FujiLAM also performed better than AlereLAM (52.3% vs 45.9%). Subgroup analyses showed that in HIV-positive children, FujiLAM achieved 57.9% sensitivity and 87.7% specificity, compared with 51.0% and 89.5% in HIV-negative children[38]. Nicol et al. (2021) reported comparable overall sensitivity between FujiLAM (41.7%) and AlereLAM (50.0%), but FujiLAM showed advantages in HIV-positive children (60.0% vs 36.0%) and malnourished children (61.9% vs 66.7%), along with higher specificity (91.7% vs 65.8%)[44]. Despite these gains, both assays demonstrated reduced detection in HIV-negative patients. To address this, Paris et al. (2017) developed a hydrogel nanocage assay incorporating a copper-complex dye that enriched urinary LAM, achieving >95% sensitivity and >80% specificity in HIV-negative adults, particularly those with a high bacillary burden, weight loss, or cough[45].

Beyond LAM, several Mtb antigens have been evaluated in urine. The 38-kDa antigen showed good diagnostic accuracy in children, with an area under the curve (AUC) of 84.3%, sensitivity of 83.0%, and specificity of 71.4%, which were higher than corresponding serum values (63.5%, 53.2%, and 57.1%)[46]. To address the challenge of low antigen concentrations in urine, novel approaches such as the MagPlas ELISA have been developed. This assay uses Fe_3O_4–Au nanozyme reagents for magnetic enrichment and catalytic signal amplification, enabling reliable detection of secretory antigens such as CFP-10 and Ag85[47]. In addition to antigen-based assays, antibodies against Mtb antigens have also been detected in urine, with modest sensitivities including anti-CFP (55.0%), anti-MPT32 (64.0%), and anti-GlcB (53.0%) antibodies[48].

Urine also contains *Mtb* cell-free DNA (cfDNA). These short transrenal fragments are filtered into urine, but their detection is challenging due to low concentrations and fragment sizes of only 25–150 bp. Novel DNA probe–magnetic bead assays have demonstrated the ability to reliably detect as few as five copies of cfDNA in 10 mL of urine[49]. In clinical studies, this approach achieved 83.7% sensitivity and 100% specificity, independent of HIV or CD4 status, and was able to identify cases missed by smear, Xpert, or AlereLAM[50].

Overall, urine is an attractive, non-invasive sample for TB diagnosis with strong applicability in high-burden, resource-limited settings. AlereLAM is already WHO-endorsed for people with advanced HIV and hospitalised patients, while FujiLAM shows improved performance but awaits formal policy approval. Novel assays detecting antigens or cfDNA are still in the research pipeline. Although urine-based testing is rapid, low-cost, and operationally feasible, its clinical use outside HIV-positive and paediatric populations remains limited by sensitivity.

Stool

Stool has gained attention as a non-invasive alternative specimen for TB diagnosis, particularly in children unable to produce sputum. Gastric aspirates (GA), long used in such cases, show limited sensitivity with conventional methods: among children ≤5 years and >5 years, GA culture achieved 28.9% and 21.9%, and AFB smear only 13.3% and 6.3%, respectively. In contrast, molecular methods have improved yield: Xpert Ultra reached sensitivities of 57.8% and 50.0%, while stool performed even better with 66.7% and 57.3% in the same age groups, compared to

46.7% and 26.0% with Xpert[51]. Similarly, in children <15 years, stool showed higher positivity than induced sputum across culture, Xpert, and Xpert Ultra (0.4%, 2.5%, 13.4% vs 2.0%, 2.7%, 6.3%)[52]. Other respiratory-related samples (RRS), such as nasopharyngeal aspirates and bronchial lavage, showed sensitivities of 50.0% for smear, 65.0% for Xpert, and 77.5% for Xpert Ultra. Xpert Ultra on stool had a comparable sensitivity of 70.0%[53].

Since culture remains an imperfect gold standard in children, stool-based tests often provide incremental yields. Stool Xpert detected an additional 8.0% of TB cases among children with negative sputum Xpert and culture[54]. In children with radiological evidence of severe disease, stool Xpert offered rapid confirmation[55]. Testing two stool samples with Xpert increased sensitivity to 54.5% compared with 41.7% for a single sample, and combining stool with AlereLAM raised sensitivity to 73.3%[56].

Stool testing has demonstrated particular utility in HIV-infected children. A systematic review reported higher pooled sensitivity in HIV-positive children (79.0%) compared with HIV-negative (60.0%)[57]. In HIV-infected children <15 years, sensitivities were 72.4% with induced sputum, 69.0% with NPA, 62.1% with stool, and 36.0% with the string test. Combining NPA with stool increased sensitivity to 75.9%, outperforming sputum-based methods[58]. In HIV-infected children ≤12 years, Xpert-stool had higher sensitivity (63.0%) than Xpert-sputum/GA (60.0%) and AlereLAM (43.0%), rising to 80.0% in severely immunosuppressed children compared with 60.0% for AlereLAM[59].

Stool culture is not recommended because of high contamination (41.5%), low sensitivity (24%), and performing worse than Xpert MTB/RIF (33.3%)[60]. To improve diagnostic yield, a range of stool processing methods has been evaluated. Complex protocols, such as combining chemical buffers (AL buffer, polyvinylpyrrolidone, Sample Reagent) with bead beating and filtration[61], or sucrose flotation with emulsification and centrifugation[62], can improve sensitivity but remain impractical outside well-equipped laboratories. By contrast, simplified methods appear more feasible for field use. For instance, a single centrifugation step raised sensitivity to 68% compared with 35% without centrifugation[63]. Similarly, replacing centrifugation with a 30-minute sedimentation step produced comparable results[64]. Other approaches, such as vortexing in PBS followed by sedimentation, improved concordance with respiratory samples and detected additional cases, possibly due to extrapulmonary involvement[65]. In addition, direct mixing with Sample Reagent, followed by two cycles of 30-second shaking and 10-minute incubation at room temperature, achieved 77.8% positivity in nasogastric aspiration–positive samples and also identified additional negatives[66]. Importantly, diagnostic outcomes also depend on laboratory capacity. For example, in Tanzania, stool Xpert reached 84.0% sensitivity and 93.4% specificity at the Central Reference Laboratory, but performance dropped to 63.0% and 76.7% in peripheral facilities[67].

Overall, stool is a valuable non-invasive specimen for TB diagnosis, particularly in children who cannot produce sputum. Evidence from a recent systematic review and meta-analysis further reinforces these findings. Across 35 studies, the pooled sensitivity of stool Xpert was 60.0% and improved to 73.0% with Xpert Ultra when using bacteriological confirmation as the reference standard. Stool-based Xpert Ultra increased bacteriological confirmation by 38.6% overall, underscoring its incremental value in paediatric TB diagnosis. Importantly, simplified, centrifuge-free stool processing methods improved sensitivity (77.0% vs 61.0% with centrifugation-based protocols)[68]. However, because stool testing still depends on Xpert instruments and lacks standardised protocols, it is not yet suitable for true field implementation, although it remains a strong candidate for future expansion in high-burden settings.

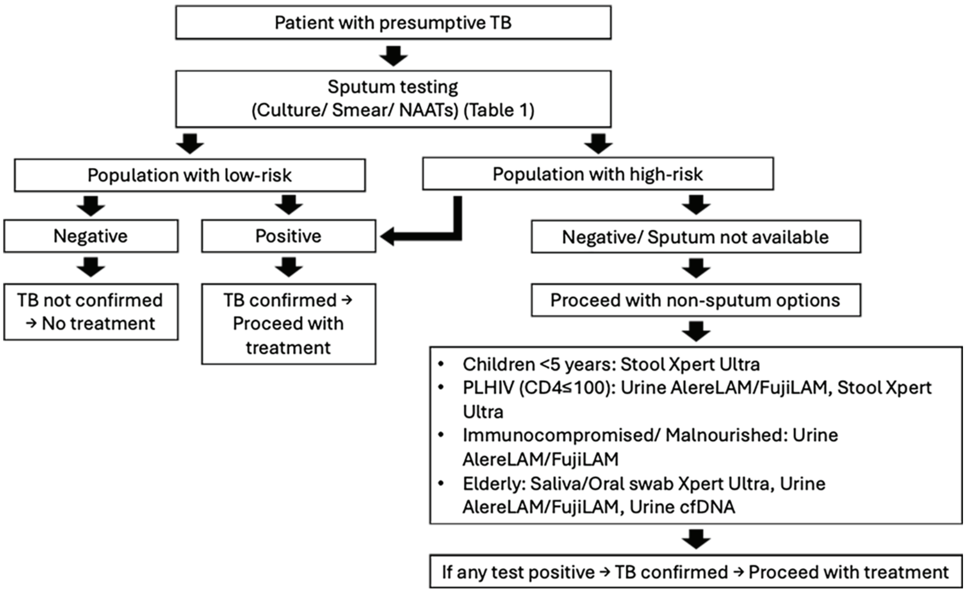
Future prospects of non-sputum-based diagnostic tests

In summary, non-sputum specimens, including saliva, oral swabs, urine, and stool, hold strong potential to close the diagnostic gap in populations where sputum testing has poor sensitivity or is not feasible, such as children, the elderly, severely immunocompromised patients, and people living with HIV. Each specimen type offers distinct advantages and limitations. For example, saliva and oral swabs are simple to collect and well accepted by patients, but their diagnostic yield is constrained by low bacillary loads and variable biomarker concentrations, limiting their role as stand-alone tools. The AlereLAM urine assay is particularly valuable in HIV-positive individuals with CD4 counts ≤100 cells/μL, where WHO has issued policy recommendations supporting its use; FujiLAM demonstrates superior accuracy but remains under evaluation. Urine cfDNA assays also show encouraging performance but are technically demanding. Stool testing with Xpert Ultra achieves sensitivity comparable to respiratory specimens and is especially useful in young children unable to expectorate sputum (Table 2, Supplementary Table S1). The latest WHO guidelines recommend Urine LAM for adults and adolescents living with HIV, molecular testing of respiratory specimens and stool in children, and concurrent use of molecular tests on respiratory samples, stool, and Urine LAM on urine in children living with HIV[1]. As illustrated in Figure 1, these assays can be integrated into TB diagnostic pathways to provide complementary value in high-risk groups. Importantly, non-sputum pathways are not intended to replace sputum testing, but to complement it, particularly when sputum results are negative or specimens cannot be obtained.

Table 2 Summary of diagnostic performance of non-sputum specimens for TB detection

Specimen	Best assay (s)	Best population	Sensitivity	Specificity	Notes
Saliva	• Multiplex cytokines • Xpert Ultra	Adults	75–100%	80–90%	**Advantages:** Non-invasive, easy to self-collect, high patient acceptability. **Disadvantages:** Biomarker performance is variable, requires multi-marker panels, and low bacillary load reduces NAAT yield.
Oral swab	• Xpert Ultra • qPCR	Adults	30–90%	80–100%	**Advantages:** Non-invasive, simple to collect, good patient acceptability. **Disadvantages:** Sensitivity depends on swab type, site, number, and timing of collection.
Urine	• AlereLAM • FujiLAM • cfDNA	Adults and children; PLHIV (CD4 ≤100/μL); malnourished	• AlereLAM: 25–55% • FujiLAM: 40–85% • cfDNA: 75-90%	• AlereLAM: 75–95% • FujiLAM: 85–95% • cfDNA: ~100%	**Advantages:** Non-invasive, suitable for point-of-care testing, especially valuable in PLHIV and children. **Disadvantages:** AlereLAM limited to advanced HIV; FujiLAM still under evaluation; cfDNA requires advanced technologies for detection.
Stool	• Xpert Ultra	Children	35–85%	85–100%	**Advantages:** Key option for children unable to produce sputum. **Disadvantages:** Processing methods vary and not yet standardised for routine use.

cfDNA=Cell-free DNA, LAM=Lipoarabinomannan, NAAT=Nucleic acid amplification test, PLHIV=People living with HIV, qPCR=Quantitative polymerase chain reaction



cfDNA=Cell-free DNA, NAATs=Nucleic acid amplification tests, PLHIV=people living with HIV, TB=Tuberculosis

Figure 1 Non-sputum-based diagnostic pathways into TB care for high-risk populations

When assessed against the WHO's updated Target Product Profiles (TPPs), which set minimal sensitivity at ≥80% and optimal at ≥95%, with specificity ≥98%[69], none of the current non-sputum approaches consistently achieve optimal benchmarks across all patient groups. Urine LAM assays partially meet TPP criteria in people with advanced HIV, while stool Xpert Ultra approaches targets for pediatric TB. Saliva and oral swabs align with the TPP vision of simple, self-collectable point-of-care tests, but they remain in the earlier stages of development and validation.

Future progress in non-sputum TB diagnostics will require scientific innovation and practical implementation strategies. On the scientific side, two priorities stand out: First, expanding biomarker discovery and validation, including multiplex cytokine and antibody panels, Mtb antigens, and cfDNA to improve diagnostic accuracy across specimen types; and second, advancing technological platforms, such as bacterial enrichment, nanotechnology-based capture, microfluidics, isothermal amplification, and portable NAATs, which may enhance sensitivity and specificity while reducing dependence on central laboratories.

Implementation challenges remain equally important. Non-sputum samples are easier to collect and generally more acceptable to patients, but achieving a good yield often requires multiple collections and careful timing, such as early-morning sampling. At the same time, molecular platforms like Xpert remain costly, infrastructure-dependent, and reliant on trained staff, which limits scalability in resource-limited settings. Performance also varies by health system level, with accuracy often higher in central laboratories than in peripheral clinics. To move toward widespread use, new assays must meet WHO TPP expectations, not only for diagnostic performance but also for affordability, simplicity, biosafety, and usability by minimally trained health workers[69]. Addressing these barriers will be essential for translating non-sputum diagnostics into routine frontline and community-based care.

Conclusion

Non-sputum-based specimens can complement sputum testing in cases where bacillary load is low or sputum results are inconclusive, and in some contexts, such as children, severely immunocompromised patients, or settings where sputum collection is impractical, they may even serve as substitutes. Looking forward, progress in biomarker validation, simplified processing methods, and integration with advanced molecular technologies, combined with implementation research, will be vital to transition these approaches from experimental tools to routine practice. According to the WHO TPP, future assays must achieve high sensitivity (≥80–95%), specificity (≥98%), rapid turnaround (<1 hour), and low cost (ideally ≤US$4 per test), while remaining simple enough for use by minimally trained health workers in peripheral or community settings. With further standardisation, simplified protocols, and large-scale validation, non-sputum-based diagnostics have the potential to transform TB control by improving early case detection, reducing diagnostic delays, and supporting global elimination efforts, particularly in high-burden and resource-limited settings.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Supplementary Table 1 Sensitivity and specificity of TB diagnosis using non-invasive, non-sputum-based specimens

Specimen	Assay	Population	Target genes/ Biomarkers	Sensitivity & specificity	References
Saliva	Multiplex cytokines	Adults	CRP, ferritin, serum amyloid P, MCP-1, alpha-2-macroglobulin, fibrinogen, and tissue plasminogen activator	78.1% & 83.3%	10
Saliva	Multiplex cytokines	Adults	IL-1β, IL-23, ECM-1, HCC1 and fibrinogen	88.9% & 89.7%	11
Saliva	Multiplex cytokines	Adults	Fractalkine + IP-10 + IL-1α + VEGF	74.1% & 91.2%	12
Saliva	Multiplex cytokines	Adults	Fractalkine, IL-6, IL-9, MIP-1β, CRP, and IL-5	Higher in TB than non-TB	13
	Mass spectrometry		P01011, Q8NCW5, P28072, A0A2Q2TTZ9, and Q99574	100% & 90.9%	14
Saliva	Antibody	Children <15 years	Secretory IgA against 38 kDa antigens	36.1% & 91.6%	15
Saliva	Antibody	Adults	Secretory IgA against 38 kDa antigens	80% & 36.6%	16
Saliva	Antibody	Children <15 years	SIgA PPD and 38kDa	Sensitivity: 38 kDa: TST+ (28.0%), TST- (16.6%) PPD: TST+ (14.2%), TST- (0%)	17
Saliva	Xpert Ultra	Adults	*rpoB*	Sensitivity: 90.0%	22
Oral swab	Xpert	Adults	rpoB	Sensitivity: High – 90.0%, Medium – 79.0%, Low – 38.0%, Very low – 38.0% Specificity: 100%	24
Oral swab	PCR	Children <15 years	IS6110	43.0% & 93.0%	25
Oral Swab	qPCR	Adults	IS6110	1-swab: 71.2-78.0% & 94.4-97.2% 2-swab: 83.1% & 91.5%	26
Oral swab	Xpert Ultra	Children <15 years	rpoB	33.0% & 100%	27
Oral swab	qPCR	Adults	IS6110	Sensitivity: 1-swab (88.0%), 2-swab (94.4%) Specificity: 79.2%	28
Oral swab	qPCR	Adults	IS6110	51.0% & 96.7%	29
Oral swab	qPCR	Children <15 years	IS6110	21.0% & 99.0%	30
Oral Swab	TB-LAMP	Adults	IS6110	Morning (M): 50% & 96.4% Spot (S): 37% & 98.2% Night (N): 32.6% & 98.2% M+S: 67.4% & 94.5% N+S: 60.9% & 96.4% M+N: 69.6% & 96.4% M+S+N: 82.6% & 94.5%	31
Oral Swab	qPCR	Adults	IS6110	<30 days: 61.0% & 78.0% >30 days: 29.0% & 81.0%	33
Urine	AlereLAM	Adults HIV	Lipoarabinomannan	CD4 count of ≤100 cells/μL: 56.0% & 90.0% CD4 count of >100 cells/μL: 26.0% & 92.0%	36

Supplementary Table 1 (continued)

Specimen	Assay	Population	Target genes/ Biomarkers	Sensitivity & specificity	References
Urine	AlereLAM	Malnourished children <5	Lipoarabinomannan	Sensitivity: 37.8%	39
Urine	FujiLAM vs AlreLAM	Adults	Lipoarabinomannan	70.4% & 90.8% vs 42.3% & 95.0% ≤100 cells/µL: 84.2% & 85.0% vs 57.3% & 94.1% 101-202 cells/µL: 60.6% & 89.6% vs 26.4% & 92.8% >200 cells/µL: 44.0% & 97.0% vs 12.2% & 97.2%	41
Urine	FujiLAM vs AlreLAM	Adults	Lipoarabinomannan	74.2% & 89.3% vs 53.0% & 95.6% ≤100 cells/µL: 84.4% & 69.8% vs 65.6% & 87.9% 101-202 cells/µL: 70.6% & 100.0% vs 47.1% & 98.6% >200 cells/µL: 53.3% & 98.4% vs 26.7% & 99.5%	42
Urine	FijiLAM vs AlereLAM	Adult people living with HIV (PLHIV)	Lipoarabinomannan	70.7% & 90.9% vs 34.9% & 95.3% ≤100 cells/µL: 87.1% & 80.5% vs 56.0% & 93.6% 101-200 cells/µL: 62.7% & 95.0% vs 25.3% & 96.7% >200 cells/µL: 43.9% & 97.0% vs 10.9% & 97.6%	43
Urine	FijiLAM vs AlereLAM	Children <15	Lipoarabinomannan	52.32% & 89.37% vs 45.9% & 80.42% HIV-positive: 57.89% & 87.66% vs 46.59% & 76.45% HIV-negative: 50.95% & 89.47% vs 32.33% & 79.07%	38
Urine	FijiLAM vs AlereLAM	Children <15	Lipoarabinomannan	41.7% & 97.4% vs 50.0% & 74.4% Living with HIV: 60.0% & 93.3% vs 36.0% & 46.7% Living without HIV: 33.9% & 91.4% vs 55.9% & 68.6% Malnourished: 61.9% & 95.7% vs 66.7% & 69.6% Not malnourished: 31.3% & 90.4% vs 45.8% & 62.7%	44
Urine	ELISA	Children <14 years old	38kDa antigen	83.0% & 71.43%	46
Urine	MagPlas ELISA	Adults	CFP-10, Ag85	CFP-10: 85% & 96.7% Ag85: 47% & 92.6%	47
Urine	ELISA	Adults	anti-CFP, anti-MPT-32, anti-GlcB	Sensitivity: anti-CFP (55.0%), anti-MPT-32 (64.0%), anti-GlcB (53.0%)	48

Supplementary Table 1 (continued)

Specimen	Assay	Population	Target genes/ Biomarkers	Sensitivity & specificity	References
Urine	Hybridization probes immobilized on magnetic beads	Adults	Transrenal urine cell-free DNA (cfDNA)	83.7% & 100% HIV-positive: 88.2% & 100% HIV-negative: 73.3% & NA CD4 ≤200 cells/mm3: 90.9% & 100% CD4 >200 cells/mm3: 83.3% & 100% Culture-positive: 88.2% & NA Culture-negative: 75.0% & 100% AFB-positive: 100% & NA AFB-negative: 76.0% & 100% AlereLAM-positive: 100% & NA AlereLAM-negative: 76.5% & 100%	50
Stool	Xpert vs Xpert Ultra	Children <15	rpoB	≤5 years old: 46.7% & 100% vs 66.7% & 87.5% >5 years old: 26.0% & 100% vs 57.3% & 100%	51
Stool	Xpert vs Xpert Ultra	Children <15	rpoB	37.9% & 100% vs 58.6% & 89.7%	52
Stool	Xpert Ultra	Children <15	rpoB	70.0% & 83.7%	53
Stool	Xpert	Children <7 years	rpoB	One sample: 41.7% & 99.0% Two samples: 54.5% & 99.0%	56
Stool	Xpert	HIV-infected Children <12 years	rpoB	63.0% & 99.0%	59
Stool	Xpert	Children < 13	rpoB	33.3% & 98.0%	60
Stool	Xpert	Adults and Children <15	rpoB	All: 84.0% & 93.4% Children <15: 66.7% & 99.1%	67

AFB=Acid-fast bacilli, CD4=Cluster of differentiation 4, cfDNA=Cell-free DNA, CFP=Culture filtrate protein, CRP=C-reactive protein, ECM-1=Extracellular matrix protein 1, ENA-78=Epithelial-derived neutrophil-activating peptide 78, ELISA=Enzyme-linked immunosorbent assay, GA=Gastric aspirate, GlcB=Glucose-6-phosphate isomerase, HCC1=Hemofiltrate CC chemokine 1, HIV=Human immunodeficiency virus, IL=Interleukin, IP-10=Interferon gamma-induced protein 10, LAM=Lipoarabinomannan, MCP-1=Monocyte chemoattractant protein-1, MIP-1β=Macrophage inflammatory protein-1 beta, kDa=kilodalton, NAAT=Nucleic acid amplification test, NPA=Nasopharyngeal aspirate, PCR=Polymerase chain reaction, PPD=Purified protein derivative, qPCR=Quantitative polymerase chain reaction, *rpoB*=RNA polymerase β-subunit gene, SIgA=Secretory immunoglobulin A, TST=Tuberculin skin test, VEGF=Vascular endothelial growth factor