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Genital Tuberculosis in the Molecular Era Conventional Diagnosis, Molecular Testing, Specimen Selection and Emerging Genomic Technologies: A Critical Review

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Review Article

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Abstract

Background: Genital tuberculosis (GTB), particularly female genital tuberculosis (FGTB), is an important form of extrapulmonary tuberculosis and an under-recognized cause of infertility and reproductive morbidity in tuberculosis-endemic settings. Diagnosis is difficult because disease is frequently paucibacillary, may be focal or clinically silent, and often requires invasive sampling. Conventional methods including acid-fast bacilli microscopy, mycobacterial culture, histopathology, imaging and endoscopy provide complementary information but have important sensitivity, turnaround-time and interpretation limitations. Molecular diagnostics have expanded the diagnostic toolbox, but reported performance varies markedly according to specimen type, molecular target, assay architecture and reference standard.

Objective: To critically review conventional and molecular diagnostic approaches for genital tuberculosis, with particular emphasis on infertility-associated disease, specimen selection, molecular target selection, rapid nucleic acid amplification tests (NAATs), drug-resistance detection, emerging sequencing technologies and the methodological problem of imperfect reference standards.

Methods: A structured literature search was undertaken using PubMed/MEDLINE and targeted searches of WHO resources and major journal databases, with literature considered from database inception through 12 August 2026. Search concepts included genital/genitourinary tuberculosis, infertility, diagnosis, PCR, qPCR, GeneXpert, Xpert Ultra, Truenat, LAMP, sequencing and next-generation sequencing. Priority was given to primary diagnostic studies, systematic reviews/meta-analyses, major reviews and WHO guidance. Because this manuscript is a critical narrative review rather than a registered systematic review, no pooled de novo meta-analysis was performed. Diagnostic accuracy estimates were therefore interpreted in relation to specimen, population and reference-standard methodology.

Results: The evidence consistently indicates that no single test reliably excludes GTB across all clinical phenotypes. AFB microscopy and culture are highly specific when positive but often insensitive in paucibacillary disease. Histopathology can provide strong supportive evidence but may be negative when disease is focal or granulomatous inflammation is absent. PCR-based methods generally improve analytical detection, although target selection and reference-standard definitions strongly influence reported performance. Xpert MTB/RIF and Xpert Ultra offer rapid standardized testing and rifampicin-

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resistance information, but genital-TB-specific evidence remains much smaller than the broader extrapulmonary TB evidence base. Menstrual blood is a promising less-invasive specimen, but the evidence is heterogeneous. Targeted next-generation sequencing is increasingly relevant for resistance profiling after TB confirmation, although WHO recommendations currently focus on bacteriologically confirmed pulmonary TB and evidence for direct genital-TB diagnosis is limited. WHO's March 2026 update introduced near-point-of-care NAATs, tongue-swab sampling and sputum pooling for TB diagnosis; these developments are primarily respiratory and should not be extrapolated to genital specimens without validation.

Conclusion: Genital tuberculosis should be diagnosed through an integrated, specimen-aware pathway rather than a single-test paradigm. The major research priorities are standardized genital specimen collection, multicentre diagnostic-accuracy studies using carefully designed composite reference standards, direct comparison of modern rapid NAATs, validation of non-invasive specimens such as menstrual blood, and genital-TB-specific evaluation of targeted sequencing and other emerging molecular technologies.

Keywords: Genital Tuberculosis; Female Genital Tuberculosis; Infertility; Endometrial Tuberculosis; PCR; qPCR; GeneXpert; Xpert Ultra; Truenat; Culture; Histopathology; Molecular Diagnostics; Next-Generation Sequencing

Abbreviations

WHO: World Health Organization; PCR: Polymerase Chain Reaction; FGTB: Female Genital Tuberculosis; EPTB: Extrapulmonary Tuberculosis.

Introduction

Tuberculosis remains a major global infectious disease, and diagnostic practice has shifted rapidly from dependence on microscopy and culture toward rapid molecular testing. The World Health Organization (WHO) consolidated its diagnostic policy framework in 2025, combining guidance for TB infection, TB disease and drug-resistance testing and introducing new diagnostic technology classes. The accompanying operational handbook provides updated performance estimates and implementation guidance for Xpert MTB/RIF Ultra, Truenat MTB Plus, Truenat Rif-Dx, TB-LAMP and targeted next-generation sequencing (tNGS) [1,2].

Extrapulmonary tuberculosis presents additional diagnostic challenges because organism burden is often lower than in respiratory disease, specimens may be difficult to obtain, and disease can be anatomically focal. Accordingly, the performance of a molecular assay in sputum cannot simply be extrapolated to an endometrial biopsy, menstrual blood sample or pelvic specimen. WHO's current guidance recognizes molecular diagnostics as central to TB diagnosis, but the evidence and recommendations remain specimen- and population-specific [1–4].

Genital tuberculosis is particularly important in reproductive medicine. FGTB can damage the fallopian tubes and endometrium and can present with infertility, menstrual disturbance, recurrent pregnancy loss or recurrent implantation failure, sometimes without constitutional symptoms. Older Indian studies illustrate the diagnostic problem: in an infertility cohort, laparoscopy was suggestive of GTB in 59.7% of women, whereas AFB smear, culture, histopathology and PCR were positive in 8.3%, 5.6%, 6.9% and 36.7%, respectively [5]. In another early molecular study, PCR targeting mpt64 detected M. tuberculosis DNA in 14 of 25 infertile women (56%), compared with one AFB-positive sample and two culture-positive samples [6].

These observations do not establish PCR as a gold standard. Rather, they illustrate the central problem of GTB diagnostics: different tests interrogate different biological dimensions of disease. Culture detects viable organisms, histopathology detects host tissue response, endoscopy detects structural phenotype, and PCR detects microbial nucleic acid. A negative result in one dimension does not necessarily exclude disease in another.

The literature has consequently produced apparently conflicting estimates of diagnostic sensitivity. A 2025 systematic review and meta-analysis of pelvic-derived specimens reported pooled sensitivity of approximately 10% for ZN staining, 23% for culture, 54% for PCR and 14% for GeneXpert, with high specificity for the conventional and molecular tests [7]. These estimates should not be interpreted as universal test characteristics because the included studies differed in patient spectrum, specimen type, reference standard and assay methodology.

The present review therefore focuses on the diagnostic pathway rather than on a simple ranking of tests. We examine how clinical probability, specimen selection, pre-analytical

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processing, molecular target, assay design and reference-standard construction interact to determine diagnostic performance.

Pathogenesis and Clinical Spectrum

FGTB is usually considered a secondary manifestation of M. tuberculosis infection following hematogenous or lymphatic dissemination, although direct extension and ascending infection have also been described. The fallopian tubes are commonly affected, with endometrial, ovarian, cervical and vulvovaginal involvement occurring less frequently [8,9].

Tubal disease can produce salpingitis, mucosal destruction, fibrosis, obstruction, hydrosalpinx and pelvic adhesions. Endometrial involvement can result in chronic inflammation, fibrosis and intrauterine adhesions. Importantly, the anatomical damage may persist after viable organisms have become difficult to demonstrate, creating a temporal disconnect between previous infection and current microbiological positivity.

The clinical spectrum ranges from subclinical disease discovered during infertility evaluation to advanced pelvic disease with hydrosalpinx, tubo-ovarian lesions, dense adhesions or intrauterine synechiae. Infertility may be the presenting manifestation, particularly in high-burden settings [8,9]. This spectrum is important when interpreting diagnostic accuracy because sensitivity is likely to differ between minimally symptomatic patients and those with extensive disease.

The biological basis of paucibacillary disease provides a mechanistic explanation for low sensitivity of microscopy and culture. A limited endometrial biopsy samples only a small portion of the reproductive tract, and bacilli may be unevenly distributed. Thus, a negative result can reflect sampling failure rather than true absence of infection. Previous treatment may further reduce viable organisms while residual DNA or histopathological abnormalities remain.

Conventional Diagnostic Approaches

Clinical Assessment

Clinical assessment establishes pre-test probability but cannot confirm GTB. Relevant factors include prior TB, exposure history, residence in a high-burden setting, infertility or tubal-factor infertility, menstrual abnormalities, pelvic symptoms and characteristic reproductive tract abnormalities. The absence of fever, weight loss, cough or other constitutional features does not exclude genital disease [8,9].

Imaging and Hysterosalpingography

Ultrasound and hysterosalpingography are valuable for identifying structural consequences such as hydrosalpinx, tubal obstruction and intrauterine abnormalities. However, these findings are not specific for TB and should not be treated as microbiological confirmation. Endometrial or tubal abnormalities can also arise from non-tuberculous pelvic inflammatory disease, endometriosis, previous surgery and other inflammatory conditions.

Laparoscopy and Hysteroscopy

Laparoscopy can identify tubal beading, adhesions, hydrosalpinx, peritoneal nodules and other characteristic abnormalities while permitting tissue acquisition. Hysteroscopy can demonstrate intrauterine adhesions, synechiae and endometrial abnormalities. A 2025 systematic review of 13 studies involving 2,201 participants found variable sensitivity and specificity for endoscopic findings and concluded that diagnostic utility was greatest when endoscopy was integrated with molecular and histopathological methods [10].

Endoscopy should therefore be conceptualized as phenotypic evidence. It is particularly useful for selecting biopsy sites and defining reproductive anatomy, but endoscopic appearance alone should not be equated with microbiological proof.

Histopathology

Histopathological findings can include epithelioid granulomas, Langhans-type giant cells, caseous necrosis, chronic inflammatory infiltrates and fibrosis. Characteristic granulomatous pathology in an appropriate clinical setting provides strong supportive evidence. However, granulomas may be absent in paucibacillary disease, disease may be focal, and granulomatous inflammation is not entirely specific for TB. Histopathology also reflects host response rather than necessarily demonstrating viable bacilli [8,9].

AFB Microscopy

Ziehl–Neelsen or fluorochrome staining is rapid and highly specific when typical acid-fast bacilli are detected in an appropriate specimen. Its major limitation is poor sensitivity in paucibacillary GTB. The low positivity reported in infertility cohorts supports its use as an adjunct rather than an exclusion test [5,7].

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Mycobacterial Culture

Culture remains important because it demonstrates viable organisms and provides material for phenotypic drug-susceptibility testing. Solid media such as Lowenstein–Jensen and liquid systems such as MGIT are used. However, culture is slow relative to molecular testing and can be negative in paucibacillary disease or after treatment. Consequently, culture is an important reference method but not a perfect gold standard for GTB [3,7].

Conventional and Real-Time PCR

PCR transformed GTB diagnostics by allowing direct detection of M. tuberculosis nucleic acid without requiring viable organisms. Early studies targeting mpt64 demonstrated substantially greater detection than smear and culture in infertility populations [6]. Thangappah, et al. evaluated IS6110 and TRC4 targets in 72 infertile women; PCR positivity was 36.7%, compared with 8.3% by AFB smear, 5.6% by culture and 6.9% by histopathology. The sensitivity of TRC4 against their clinical/laparoscopic criteria exceeded that of IS6110, illustrating the importance of target selection [5].

The choice of molecular target is particularly relevant in regions where IS6110 copy number varies. IS6110 is widely used because it is usually multicopy, but dependence on a single insertion sequence may produce false-negative results in strains with low or absent IS6110 copies. Multi-target approaches using mpt64/MPB64 and IS6110 have therefore been explored for extrapulmonary disease and GTB [5,11].

Nested PCR can increase analytical sensitivity by a second amplification step, but the additional handling increases contamination risk and complicates routine diagnostic workflows. Real-time PCR, especially probe-based assays, offers closed-tube amplification, reduced contamination risk, faster turnaround and straightforward integration of internal controls

DNA-PCR Versus RT-PCR

Rana et al. evaluated DNA-PCR and reverse-transcriptase PCR in 200 women with infertility. The study was designed around an important biological distinction: DNA-PCR may detect present or past microbial DNA, whereas RNA-based detection may provide information more closely related to viable or metabolically active organisms [12]. The concept is important even where RNA assays are not routinely available. A positive DNA result should not automatically be interpreted as proof of viable bacilli, particularly in patients with prior treatment.

Multiplex and Multi-Target PCR

Multi-target PCR has a theoretical advantage in paucibacillary disease because amplification failure at one locus may be compensated by another target. A 2022 study of urogenital TB compared multiplex-nested PCR targeting mpt64/Rv1980c and IS6110 with multiplex PCR and GeneXpert; the nested assay demonstrated higher sensitivity in that study, while GeneXpert retained high specificity [11]. These findings support the principle that analytical sensitivity depends not only on the platform but also on the target architecture and extraction workflow.

Rapid Molecular NAATs

Xpert MTB/RIF

Xpert MTB/RIF provides automated real-time PCR detection of MTB complex DNA with simultaneous detection of mutations associated with rifampicin resistance. Evidence across extrapulmonary TB shows substantial variation in sensitivity by specimen type, while specificity is generally high. A systematic review of Xpert for extrapulmonary TB found sensitivity ranging from 31% to 97% across specimen categories, with high specificity in several specimen types [13].

GTB-specific studies generally show high specificity but variable sensitivity. In a prospective infertility cohort using a composite reference standard, PCR positivity was considerably higher than Xpert positivity and Xpert sensitivity was approximately 36% despite 100% specificity [14]. The implication is clinically important: a positive Xpert result can strongly support MTB infection, but a negative result may not exclude paucibacillary GTB.

Xpert MTB/RIF Ultra

Xpert Ultra was developed to improve analytical sensitivity, particularly for paucibacillary disease. A systematic review of Xpert Ultra and Xpert MTB/RIF in extrapulmonary TB demonstrated that performance varies substantially across specimen types and reference standards [15]. However, direct evidence for endometrial and other genital specimens remains limited compared with cerebrospinal fluid, lymph node, pleural fluid, urine and other extrapulmonary specimens.

Therefore, improved analytical sensitivity of Xpert Ultra should not be assumed to translate into a validated sensitivity for FGTB. Dedicated genital-specimen studies are required.

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Truenat

Truenat represents a low-complexity molecular testing approach that can support decentralized TB diagnosis. WHO's 2025 operational handbook consolidated performance and implementation information for Truenat MTB Plus and Truenat Rif-Dx within the low-complexity automated NAAT framework [2]. As with Xpert Ultra, however, the broader TB evidence base is substantially larger than the genital-TB-specific evidence base.

PAMP ASSAY

PAMP-based assays detect broad pathogen-associated molecular patterns rather than organism-specific targets. The FcMBL-based ELLecSA assay demonstrated rapid PAMP detection from whole blood in systemic infections and suspected sepsis [16]. However, its application to genital TB remains unvalidated because GTB is often localized and paucibacillary. Therefore, GTB-specific studies are needed to determine its diagnostic utility.

TB-LAMP

TB-LAMP uses isothermal amplification and has potential advantages in laboratories where conventional thermocycling infrastructure is limited. In a 300-patient infertility study, PCR demonstrated higher reported sensitivity than conventional tests, while LAMP also outperformed smear, culture and histopathology [17]. Interpretation should nevertheless account for the study's composite reference standard and the lack of broad multicentre validation.

Specimen Selection: The Critical Pre-Analytical Variable

The choice of specimen may be as important as the choice of molecular platform. Endometrial biopsy or aspirate remains the most studied specimen in FGTB because it can provide material simultaneously for histopathology, culture and molecular testing. The 2025 pelvic-specimen meta-analysis concluded that endometrial biopsy/aspirate remains the most suitable specimen on the current evidence base [7].

Other specimens—including menstrual blood, pelvic washings, cervical samples, tubal tissue and urine in selected genitourinary presentations—may be useful in particular clinical contexts. However, their performance is variable and often supported by smaller studies.

Menstrual blood

Menstrual blood is particularly attractive because it can reduce the need for invasive endometrial sampling. A 2019 study of 194 infertile women used nested PCR targeting hsp65/groEL2 and reported similar detection between menstrual blood and endometrial tissue in that cohort [18]. A 2026 systematic review of nine comparative studies found sensitivity ranges of 33.3–91.7% for menstrual-blood testing and 64.8–95.8% for endometrial biopsy, with specificity ranges of 82.9–97.3% and 84.3–97.5%, respectively [19].

These data support menstrual blood as a promising research specimen, but not yet as a universal replacement for endometrial biopsy. Important unresolved issues include standardization of collection timing, menstrual-blood volume, DNA extraction, host-DNA background, contamination, assay target and independent reference standards.

Multiple-site sampling

Because genital TB may be focal, multiple-site sampling may increase diagnostic yield. The early mpt64 PCR study found PCR positivity in endometrial biopsies and aspirates at higher rates than in pouch-of-Douglas fluid, supporting the concept that specimen choice materially influences molecular detection [6]. When clinically feasible, sampling should therefore be directed toward the anatomically involved site rather than relying on a single generic specimen.

Drug-Resistance Detection

The ability to identify drug resistance rapidly is an important advantage of modern molecular diagnostics. Xpert MTB/RIF and related assays provide rapid rifampicin-resistance information, while other molecular platforms and line-probe assays can expand resistance characterization.

WHO's 2025 diagnostic framework incorporated targeted next-generation sequencing as a technology class for resistance testing. The operational handbook describes tNGS as a follow-on approach for broader molecular drug-susceptibility testing after TB confirmation [1,2]. WHO's recommendations are specifically framed around bacteriologically confirmed TB and, for several tNGS applications, pulmonary specimens [20].

A 2026 review of tNGS implementation emphasizes that tNGS can bridge rapid molecular testing and phenotypic DST by interrogating multiple resistance-associated loci and potentially shortening turnaround time [21]. However, there is currently insufficient evidence to recommend tNGS as a routine direct diagnostic test for genital TB itself. Its most plausible near-term role is resistance characterization after microbiological confirmation, with extrapolation to genital specimens requiring validation.

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WGS and Metagenomic Sequencing

Whole-genome sequencing can provide comprehensive strain and resistance information, while metagenomic sequencing could theoretically identify MTB and other pathogens without a predefined target. Genital specimens present special challenges, including low microbial biomass, high host-DNA content, contamination and uncertain clinical thresholds. Consequently, WGS and metagenomics should currently be regarded as emerging or research technologies for GTB rather than replacements for validated NAATs.

The Imperfect Reference Standard Problem

A central methodological challenge in GTB diagnostic research is the absence of a universally accepted gold standard. Culture is highly specific but imperfectly sensitive; histopathology demonstrates tissue response but may miss focal disease; endoscopy provides structural evidence but is not microbiological proof; and PCR detects DNA without necessarily establishing viability.

Several infertility studies have therefore used composite reference standards incorporating combinations of clinical findings, laparoscopy, hysteroscopy, histopathology, culture, AFB microscopy and molecular results [14,22]. Composite reference standards may better reflect the multifactorial diagnosis of FGTB, but incorporation bias becomes a concern if the index molecular test is included in the disease definition.

This problem explains why apparently contradictory diagnostic estimates may both be internally valid. A study using culture as the reference standard may underestimate PCR sensitivity because culture misses paucibacillary disease. A study incorporating PCR into the composite reference standard may overestimate PCR performance. Future diagnostic studies should prespecify independent or carefully constructed composite reference standards and report the contribution of each component.

Spectrum bias

The clinical spectrum of participants can also substantially influence diagnostic accuracy. Patients with advanced tubal disease, pelvic masses or characteristic endoscopic findings may have a greater microbial burden than asymptomatic infertility patients. A test evaluated predominantly in advanced disease may therefore appear more sensitive than when applied to subclinical FGTB. Studies should report disease spectrum and ideally stratify performance by clinical phenotype.

Beyond the Band: How Modern TaqMan PCR Rewrote the Rules of TB Detection

The clinical utility of polymerase chain reaction (PCR) in diagnosing paucibacillary conditions like Female Genital Tuberculosis (FGTB) and Extrapulmonary Tuberculosis (EPTB) is frequently hindered by an outdated clinical perception of high false-positive rates. This skepticism stems from the widespread use of conventional, first-generation gel-based PCR methods over a decade ago. Those early assays suffered from inadequate primer design, subjective interpretation of faint bands on agarose gels, and an open-tube workflow that exposed reaction mixes to post-amplification carry-over contamination, leading to genuine laboratory-induced false positives.

Modern molecular pathology laboratories have eliminated these vulnerabilities by universally transitioning to closed-tube TaqMan probe-based real-time PCR chemistry. This advanced technique introduces a triple-layer sequence specificity requiring a dual-labeled fluorogenic probe to hybridize perfectly between flanking primers, preventing non-specific amplification or primer-dimers from generating a signal. Because the entire amplification and optical detection process occurs within a sealed reaction vessel that is never reopened, the risk of environmental cross-contamination is virtually non-existent, bringing the false-positive rate to near zero.

To maximize diagnostic yield in low bacterial load tissue specimens, an optimized laboratory-developed workflow utilizing semi-nested or nested TaqMan PCR targeting the IS6110 and MPB64 genes provides an exceptional diagnostic standard. The multi-copy IS6110 insertion element ensures ultra-high analytical sensitivity, while the complementary MPB64 secretory protein gene safeguards against rare strains lacking IS6110, ensuring near-perfect specificity. Operating on identical molecular principles to automated systems like GeneXpert, this highly strategic dual-target approach delivers equivalent clinical accuracy and debunking the historical myth of unreliable PCR results for EPTB and FGTB management.

WHO Diagnostic Evolution Through 2026

WHO's 2025 consolidated guideline unified policy guidance on TB infection, TB disease and drug resistance and established new diagnostic technology classes. The companion operational handbook provided updated algorithms and pooled performance estimates for Xpert MTB/RIF Ultra, Truenat MTB Plus, Truenat Rif-Dx and TB-LAMP, and included implementation material for targeted NGS [1,2].

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In March 2026, WHO announced new recommendations for near-point-of-care NAATs, tongue swabs and sputum pooling. The new near-point-of-care class is intended to bring molecular diagnosis closer to peripheral laboratories, primary-care centres and communities, while tongue swabs provide an alternative respiratory specimen for adults and adolescents unable to produce sputum. Sputum pooling is intended as a cost-saving strategy for respiratory TB testing in resource-constrained settings [23,24].

These 2026 innovations are important for the future of decentralized molecular diagnosis, but they should not be misrepresented as genital-TB-specific recommendations. Tongue swabs and sputum pooling are respiratory strategies, and evidence for NPOC-NAATs using endometrial tissue, menstrual blood or other genital specimens remains absent or insufficient. The appropriate conclusion for GTB is therefore that the WHO diagnostic trajectory strongly supports decentralization and rapid molecular testing, while genital-specific validation remains a major research gap [25-30] (Tables 1,2).

Conventional versus Molecular Diagnosis: Comparative Interpretation

Method	Primary signal	Main strength	Main limitation in GTB	Role
Clinical assessment	Clinical probability	Low cost; determines pre-test probability	Non-specific; silent disease common	Essential first step
Ultrasound/ HSG	Anatomical phenotype	Identifies tubal/ endometrial abnormalities	Not microbiologically specific	Adjunct
Laparoscopy/ hysteroscopy	Direct structural phenotype	Visualizes lesions; permits tissue sampling	Invasive; operator dependent	Adjunct/biopsy guidance
	Host tissue response	Characteristic granulomas can be strong evidence	Focal disease; granulomas may be absent	Complementary
AFB smear	Acid-fast bacilli	Rapid and inexpensive	Very low sensitivity in paucibacillary disease	Rule-in when positive
Culture	Viable MTB	Microbiological confirmation; DST	Slow; low sensitivity in paucibacillary disease	Reference/ complementary
Conventional PCR	MTB DNA	Rapid; can detect low-level DNA	Target-dependent; viability not established	Important molecular adjunct
qPCR/ multiplex PCR	MTB DNA	Closed-tube, rapid, multi-target potential	Requires validation and controls	High-value molecular test
Xpert MTB/ RIF	MTB DNA + rifampicin resistance	Standardized and rapid	GTB sensitivity may be insufficient to exclude disease	Rapid rule-in/ resistance screen
Xpert Ultra	Higher analytical sensitivity + rifampicin resistance	Improved low-burden detection in some extrapulmonary specimens	Limited genital-specific evidence	Promising; requires GTB validation
PAMP-based assay	Broad microbial patterns	Rapid, broad-spectrum detection in whole blood	Not MTB-specific; diagnostic performance in GTB has not been established	Investigational; requires GTB-specific validation [16]
Truenat	MTB DNA ± reflex resistance	Decentralizable; rapid	Limited genital-specific evidence	Promising decentralized option
TB-LAMP	MTB DNA	Isothermal; lower complexity	Limited multicentre GTB evidence	Potential resource-limited option
tNGS	Resistance-associated variants	Broad resistance profiling	Usually follow-on; limited genital evidence	Emerging resistance test
WGS/ metagenomics	Genome/pathogen sequences	Comprehensive information	Cost, host DNA, bioinformatics, low biomass	Research/emerging

Table 1: Current Diagnostic Methods and its Advantages and limitations.

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Evidence Summary by Specimen

Specimen	Evidence level	Potential advantage	Major limitation	Current interpretation
Endometrial biopsy/ aspirate	Highest for FGTB	Allows HPE + culture + molecular testing	Invasive; sampling error	Best-supported specimen
Menstrual blood	Emerging but expanding	Non-invasive; repeatable	Variable sensitivity; collection/processing not standardized	Promising alternative; needs validation
Peritoneal/ POD fluid	Limited	May sample pelvic disease	Often low yield; invasive collection	Selective use
Tubal/ovarian tissue	Limited	Direct sampling of affected site	Requires surgery	Useful when clinically indicated
Cervical/ vaginal samples	Limited	Accessible	May not represent upper-genital disease	Not a universal replacement for endometrial sampling
Urine	Useful mainly in genitourinary disease	Non-invasive	May not reflect isolated genital disease	Context-dependent

Table 2: Comparative Evaluation of Clinical Specimens for the Diagnosis of Female Genital Tuberculosis (FGTB): Evidence, Advantages, Limitations, and Diagnostic Utility.

Diagnostic Algorithm Proposed for Infertility-Associated FGTB

A practical diagnostic pathway should begin with clinical probability and anatomical assessment, followed by collection of the most informative specimen and parallel testing rather than sequential dependence on a single negative test.

Step 1: Identify clinical and epidemiological risk and the reproductive phenotype.

Step 2: Perform appropriate anatomical assessment when clinically indicated, including ultrasound, HSG, hysteroscopy or laparoscopy.

Step 3: Obtain an anatomically relevant specimen, preferably endometrial tissue/aspirate when endometrial disease is suspected; consider additional specimens according to disease location.

Step 4: Perform parallel histopathology, mycobacterial culture and molecular testing rather than relying on a single modality.

Step 5: If MTB is detected, perform resistance testing according to the applicable national and WHO algorithm.

Step 6: If molecular testing is negative but clinical suspicion remains high, evaluate specimen adequacy and consider repeat/alternative sampling, culture, histopathology and an alternative molecular target or platform.

Step 7: Interpret discordant findings through multidisciplinary clinical, microbiological, pathological and reproductive-medicine correlation.

Research Gaps and Future Directions

- Standardization collection, transport, homogenization and nucleic-acid extraction from genital specimens.
- Conduct multicentre prospective diagnostic-accuracy studies in well-characterized infertility populations.
- Develop composite reference standards that minimize incorporation bias and explicitly report each component.
- Directly compare Xpert Ultra, Truenat, qPCR and culture on the same genital specimens.
- Evaluate multi-target molecular assays in geographically diverse MTB populations.
- Validate menstrual blood using standardized collection and extraction procedures against clinically independent reference standards.
- Evaluate the potential role of PAMP-based assays for detecting GTB, particularly in paucibacillary disease.
- Determine the clinical significance of low-level PCR positivity in culture-negative patients.
- Define how previous anti-TB therapy affects DNA, RNA and culture positivity.
- Generate genital-TB-specific evidence for rapid molecular drug-resistance testing.
- Evaluate whether tNGS can be applied directly to paucibacillary genital specimens.
- Investigate metagenomic sequencing while addressing host-DNA background and contamination.
- Assess cost-effectiveness and feasibility of decentralized molecular testing in high-burden settings.

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- Develop digital pathology/AI tools as adjuncts rather than replacements for microbiological diagnosis

Strengths and Limitations of the Evidence Base

The principal strength of the current evidence is the convergence of multiple diagnostic domains clinical, endoscopic, histopathological, microbiological and molecular across several decades of research. The literature also includes relatively large infertility cohorts and recent systematic reviews that permit identification of recurring methodological problems.

However, the evidence base is limited by small single-centre studies, heterogeneous patient populations, inconsistent specimen processing, varying molecular targets, differences in reference standards and incorporation of index tests into composite disease definitions. Many contemporary rapid molecular technologies have not been directly evaluated in genital specimens. Accordingly, numerical diagnostic estimates should be regarded as study-specific rather than universal assay characteristics [31-34].

Conclusion

Genital tuberculosis remains diagnostically challenging because the biological and anatomical characteristics of disease undermine the sensitivity of individual tests. Conventional methods retain important complementary roles: culture provides evidence of viable organisms and enables susceptibility testing; histopathology identifies tissue response; and endoscopy identifies structural phenotype and facilitates targeted sampling. However, microscopy and culture may be insufficiently sensitive in paucibacillary disease.

Molecular diagnostics have substantially improved the speed and analytical detection of M. tuberculosis. PCR and qPCR can detect microbial DNA when conventional tests are negative, while multi-target strategies may reduce dependence on a single genomic locus. Xpert-based assays and Truenat offer standardized rapid testing and, in selected workflows, resistance information. Yet a negative molecular result cannot reliably exclude GTB when disease probability is high, specimen quality is uncertain or bacillary burden is very low.

The most defensible diagnostic model is therefore multimodal and specimen aware. The key scientific challenge is no longer simply to identify the most sensitive assay, but to determine which specimen should be tested, which molecular target is most robust, how viability should be interpreted, and which reference standard best represents clinically meaningful GTB.

The 2025 WHO diagnostic framework and its operational handbook demonstrate a global shift toward rapid molecular diagnosis, decentralization and broader molecular resistance testing. WHO's March 2026 recommendations for near-point-of-care NAATs, tongue swabs and sputum pooling further accelerate this trajectory [1,2,23]. However, these 2026 developments are principally respiratory and should not be directly extrapolated to genital specimens. The major opportunity for the next generation of GTB research is to adapt the principles of rapid, decentralized molecular diagnosis to validated genital specimens, including potentially less-invasive specimens such as menstrual blood.

Ultimately, an integrated strategy combining clinical probability, appropriate specimen selection, histopathology, culture, molecular NAATs and resistance testing is more scientifically defensible than a single-test paradigm. Future multicentre studies should establish standardized, clinically independent reference frameworks and directly compare modern molecular platforms in infertility-associated genital TB Tables 3-7 and Figures 1-4.

Study	Population/specimen	Method(s)	Key result	Interpretive point
Vijaya Bhanu, et al., 2005 [6]	25 infertile women; 61 genital/pelvic samples	mpt64 PCR, smear, culture, laparoscopy	PCR detected MTB DNA in 14/25 (56%); smear 1/61; culture-positive 2/61	Early evidence of increased molecular detection; PCR positivity requires clinical correlation
Thangappah, et al., 2011 [5]	72 infertile women; endometrial samples; PCR subset 49	IS6110/TRC4 PCR, AFB, culture, HPE, laparoscopy	PCR 36.7%; AFB 8.3%; culture 5.6%; HPE 6.9%; TRC4 outperformed IS6110 in their clinical comparison	Target selection and imperfect reference standard matter
Rana et, al., 2011 [12]	200 infertility patients	DNA-PCR and RT-PCR with conventional methods	Designed to distinguish DNA detection from viable-organism-associated RNA	Highlights viability problem

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Sethi et, al., 2016 [17]	300 infertile women; endometrial biopsy	ZN, LJ, BACTEC, HPE, PCR, LAMP	Reported sensitivity: ZN 2.94%; culture 10.29%; HPE 8.82%; PCR 95.59%; LAMP 66.18%	Promising molecular performance; composite reference standard requires caution
Chaubey, et al., 2019 [18]	194 infertile women; menstrual blood and endometrial tissue	Nested PCR; hsp65/ groEL2	126/194 PCR-positive; menstrual blood and endometrial tissue showed similar detection in this cohort	Supports non-invasive specimen research
Sharma, et al., 2020 [14]	Infertility cohort; CRS-defined FGTB	PCR, Xpert, culture, HPE, AFB, endoscopy	PCR positivity substantially exceeded Xpert and culture; Xpert specificity 100%, sensitivity ~36%	Illustrates reference-standard and sensitivity problem
Kamra, et al., 2022 [11]	Urogenital TB; urine/ endometrial/menstrual specimens	mpt64 + IS6110 multiplex-nested PCR, multiplex PCR, GeneXpert	Nested PCR more sensitive in study; Xpert highly specific	Assay architecture influences analytical detection
Nayak, et al., 2026 [19]	9 comparative studies	Menstrual blood vs endometrial biopsy	MB sensitivity 33.3–91.7%; EB 64.8–95.8%; specificity broadly comparable	Most current synthesis of non-invasive specimen evidence

Table 3: Selected Diagnostic Studies Informing the Molecular Evidence Base.

Characteristic	Conventional methods	PCR/qPCR	Rapid NAATs	Sequencing
Turnaround	Hours to weeks	Hours	~hours	Hours to days; variable
Viable organism	Culture: yes	No	No	No, unless culture-derived
Paucibacillary performance	Often limited	Potentially high	Variable by platform/ specimen	Potentially high but DNA input dependent
Drug resistance	Culture-based DST possible	Limited unless resistance targets included	Rifampicin on selected platforms	Broad resistance profiling
Standardization	Variable	Variable	High for cartridge systems	Variable; requires bioinformatics
Infrastructure	Microbiology laboratory	Molecular laboratory	Low/moderate depending platform	Advanced molecular/ bioinformatics
GTB-specific evidence	Moderate	Moderate/high	Limited/moderate	Limited
Main role	Viability/reference/DST + tissue diagnosis	Sensitive molecular detection	Rapid detection/ resistance	Resistance profiling/ research

Table 4: Practical Comparison of Diagnostic Modalities.

Target	Rationale	Potential limitation	Review interpretation
IS6110	Commonly multicopy MTB insertion sequence	Copy-number variation; low/ absent copies in some strains	Useful but should not always be the sole target
mpt64/ Rv1980c	MTB-specific protein-coding target used in GTB PCR studies	Single-target dependence; assay-specific performance	Useful target; often incorporated into multi-target strategies
MPB64	Protein-coding MTB target used in EPTB multiplex assays	Performance depends on assay design	Useful complementary target

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TRC4	Alternative repetitive target evaluated in Indian GTB studies	Less standardized than IS6110	Important evidence for target-selection discussion
hsp65/groEL2	Used in menstrual blood/ endometrial nested PCR	Nested workflow and contamination considerations	Relevant to non-invasive specimen studies
Multi-target combinations	Redundancy can improve analytical robustness	More complex assay validation	Promising strategy for paucibacillary GTB

Table 5: Common Molecular Targets and Their Rationale.

Year	WHO Development	Relevance to GTB	Important Caveat
2024	Third-edition rapid diagnostic guidance; tNGS class incorporated	Establishes modern molecular/ resistance framework	tNGS evidence mainly pulmonary/confirmed TB
2025	Consolidated Module 3: diagnosis	Unifies TB infection, disease and resistance diagnostics; new diagnostic classes	Recommendations remain specimen/population specific
2025	Operational handbook Module 3	Updates Xpert Ultra, Truenat and TB-LAMP implementation/performance	Genital-specific evidence remains limited
2026	NPOC-NAATs recommended	Supports future decentralization of molecular testing	Primarily respiratory evidence
2026	Tongue swabs recommended in selected adults/adolescents unable to produce sputum	Demonstrates expansion of alternative specimen strategies	Not a genital specimen recommendation
2026	Sputum pooling recommended in constrained settings	Shows cost-efficient molecular testing strategy	Respiratory only; no direct GTB application

Table 6: WHO Diagnostic Evolution Relevant to the Review.

Research Question	Why it Matters	Suggested Design
Is Xpert Ultra sufficiently sensitive for endometrial tissue/aspirate?	Current genital-specific evidence is limited	Prospective multicentre paired-specimen study
Can menstrual blood provide equivalent clinical information to endometrial biopsy?	Could reduce invasive sampling	Prospective paired-sample diagnostic study with independent CRS
Which molecular target is most robust across geographic MTB diversity?	Single-target assays may vary by strain	Multicentre analytical + clinical comparison
Can tNGS detect resistance directly from genital specimens?	Could enable broader resistance profiling	Analytical validation followed by prospective clinical study
How should PCR-positive/culture-negative cases be classified?	Core clinical interpretation problem	Longitudinal outcome study with treatment response and repeat testing
What is the optimal diagnostic algorithm for infertility clinics?	No universally validated pathway	Prospective diagnostic pathway and cost-effectiveness study
Can PAMP based assays detect genital TB when applied to appropriate genital specimens or blood?	GTB is often localized and paucibacillary	Comparative diagnostic study of PAMP and MTB-specific assays

Table 7: Priority Research Questions.

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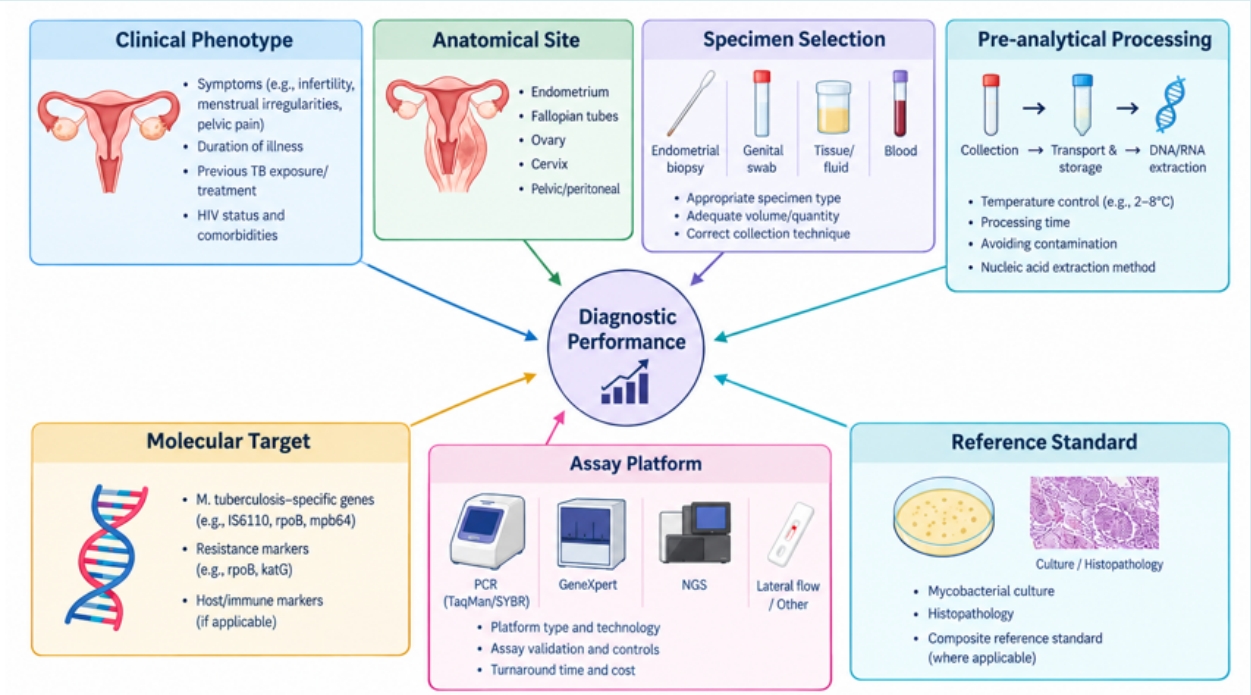


Figure 1: Proposed Conceptual Model of Genital Tuberculosis Diagnosis. Clinical Phenotype, Anatomical Site, Specimen Selection, Pre-Analytical Processing, Molecular Target, Assay Platform and Reference Standard Interact to Determine Observed Diagnostic Performance.

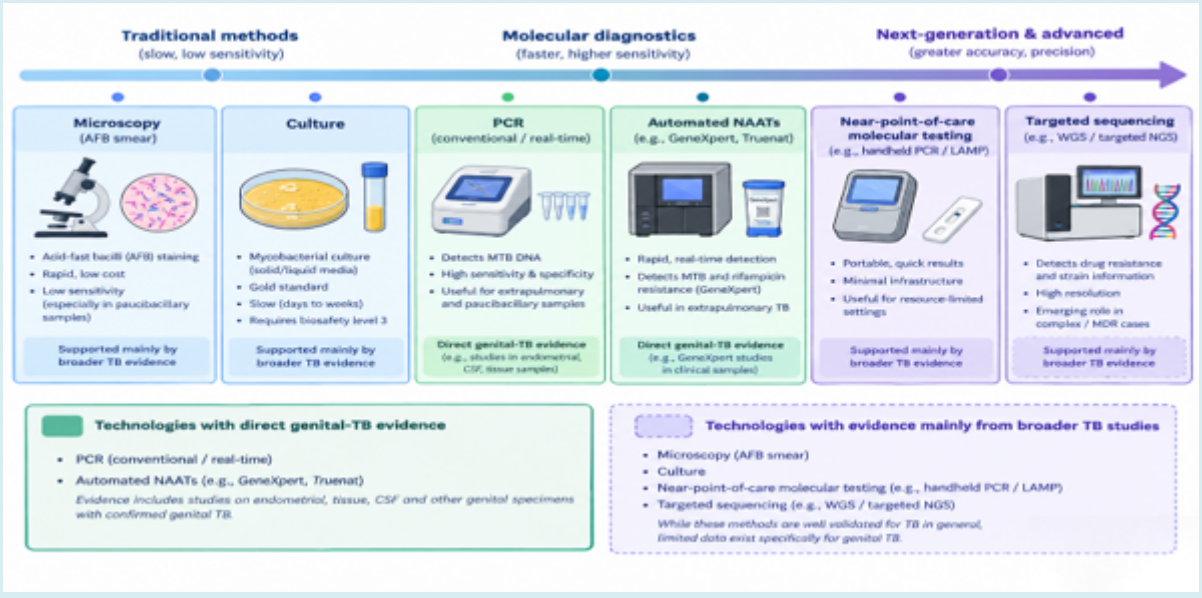


Figure 2: Evolution Of Tuberculosis Diagnostics from Microscopy and Culture To PCR, Automated Naats, Near-Point-Of-Care Molecular Testing and Targeted Sequencing. The Figure Should Distinguish Technologies with Direct Genital-TB Evidence from Those Currently Supported Mainly by Broader TB Evidence.

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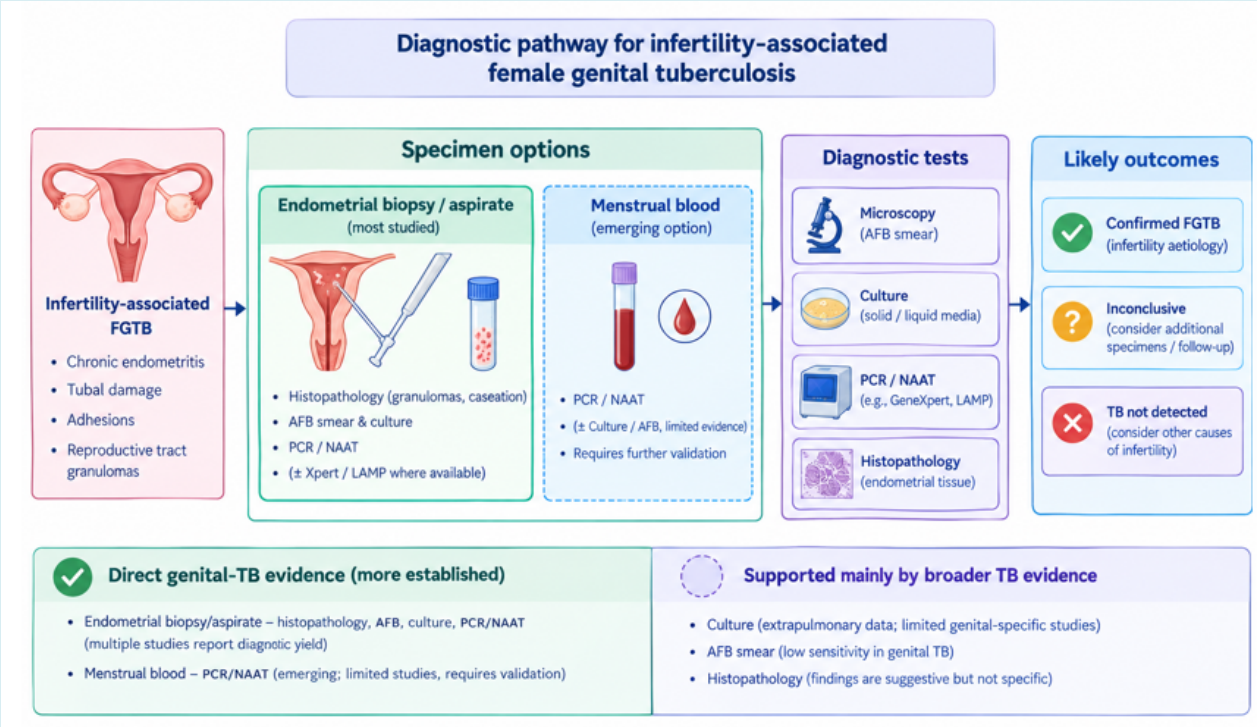


Figure 3: Specimen-Specific Diagnostic Pathway for Infertility-Associated Female Genital Tuberculosis. Endometrial Biopsy/Aspirate Remains the Most Studied Specimen; Menstrual Blood Is Shown as An Emerging Less-Invasive Option Requiring Further Validation.

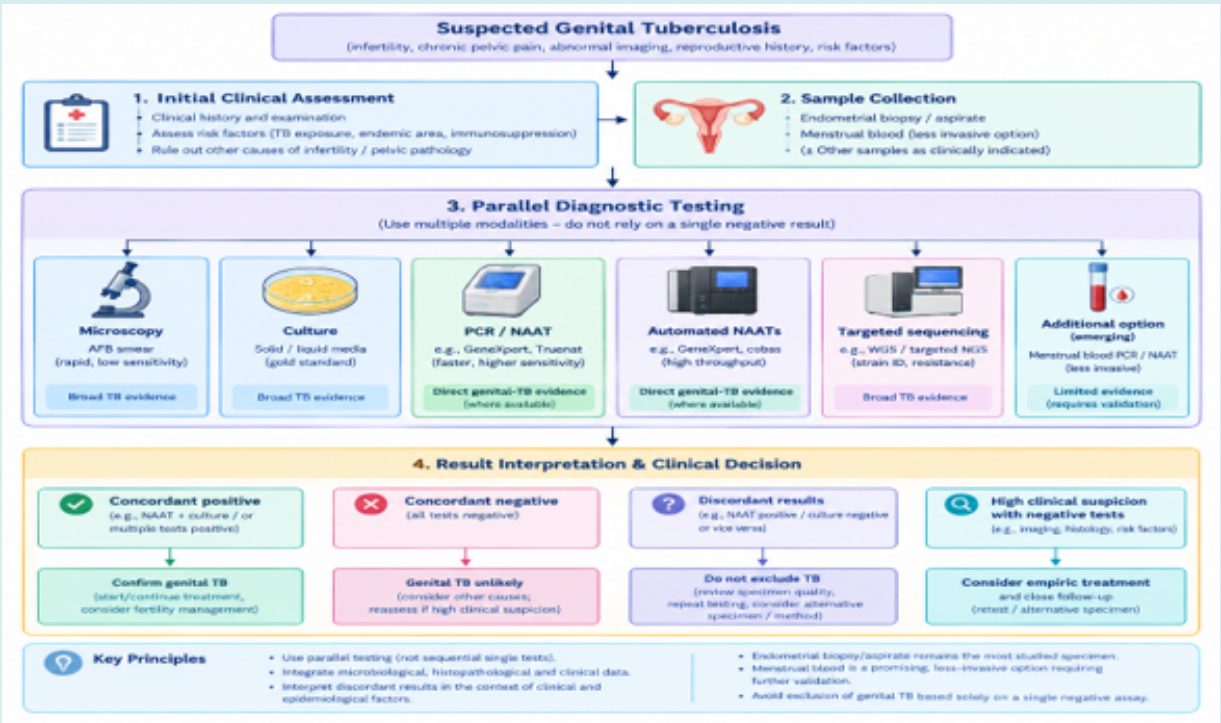


Figure 4: Proposed Integrated Diagnostic Algorithm for Suspected Genital Tuberculosis. The Algorithm Emphasizes Parallel Testing and Clinical Interpretation of Discordant Results Rather Than Exclusion Based on a Single Negative Assay.

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Declarations

Ethics approval

Not applicable. This manuscript is a literature-based review and does not involve collection of new human or animal data.

AI-assisted Writing and Image Generation Disclosure

AI tools were used as research-assistance and language-support tools during literature organization, manuscript drafting, and the generation of illustrative figures and scientific images for the manuscript. The authors retain full responsibility for the accuracy, interpretation, and final content of the manuscript.

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