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Review Article

Diagnosing Extrapulmonary Tuberculosis: Current Tools and a Specimen -Centred Multimodal Approach

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ABSTRACT

Extrapulmonary tuberculosis (EPTB) remains diagnostically difficult because disease manifestations are heterogeneous, affected sites may be inaccessible and most clinical specimens are paucibacillary. No single investigation performs consistently across all anatomical forms. Rapid molecular assays can provide early detection of *Mycobacterium tuberculosis* complex and rifampicin resistance, but their sensitivity varies markedly by specimen type and a negative result cannot exclude disease. Culture remains essential for recovery of viable bacilli and phenotypic drug-susceptibility testing, whereas cytology and histopathology demonstrate tissue response and help identify alternative diagnoses. Biochemical, antigen and immune-based tests are useful only in defined settings, and imaging is most valuable for localisation, complication assessment and image-guided sampling. This Short Communication proposes a pragmatic specimen-centred approach: establish clinical probability and urgency, identify the highest-yield sampling target, divide representative material before formalin fixation and perform molecular testing, microscopy, culture and pathology in parallel. Results should then be integrated with pre-test probability, specimen quality and disease severity. This multimodal strategy reduces diagnostic delay, preserves opportunities for resistance testing and avoids over-reliance on any single negative assay.

KEYWORDS

• Extrapulmonary Tuberculosis • Diagnosis • Xpert MTB/RIF Ultra • Mycobacterial Culture • Histopathology • Drug-Susceptibility Testing • Specimen-Centred Pathway

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INTRODUCTION

Tuberculosis remains a leading cause of death from a single infectious agent, and extrapulmonary disease contributes substantially to its clinical burden.[1-4] EPTB may involve lymph nodes, pleura, meninges, bones and joints, the abdomen, genitourinary tract, pericardium, skin or multiple organs. Pulmonary and extrapulmonary disease may coexist, particularly in children and people living with human immunodeficiency virus (HIV).

Diagnosis is difficult because clinical and radiological appearances overlap with malignancy, autoimmune disease, pyogenic infection, deep fungal infection and non-tuberculous mycobacterial disease. Lesions may be hazardous to sample, while fluids and tissue often contain bacillary concentrations below the detection threshold of microscopy, culture or molecular assays.[5-8] Culture, although conventionally used as a microbiological reference standard, is itself insensitive in paucibacillary disease. Apparent disagreement between tests may therefore reflect limitations in the specimen or reference standard rather than failure of the index test.

This Short Communication is based on a focused narrative review of PubMed/MEDLINE, the Cochrane Library and World Health Organization (WHO) publications available through 30 June 2026. Current WHO guidance, systematic reviews, meta-analyses and major prospective diagnostic-accuracy studies were prioritised. The purpose is not to identify a universally superior test, but to present a clinically usable framework for selecting specimens and combining complementary modalities.

A SPECIMEN-CENTRED DIAGNOSTIC STRATEGY

The diagnostic process should begin by defining the likely anatomical site, host risk factors and urgency. Tuberculous meningitis, pericarditis, disseminated disease and spinal cord compromise require accelerated investigation because irreversible complications may occur before bacteriological confirmation. Ultrasound, computed tomography or magnetic resonance imaging should be used when necessary to identify the safest and highest-yield target. Concurrent pulmonary assessment is appropriate because a respiratory specimen may provide easier bacteriological confirmation and resistance information.

Specimen quality frequently determines the performance of every subsequent test. Aspirate, pus, cerebrospinal fluid (CSF) or tissue is preferable to a superficial swab. Adequate volume should be collected and transported promptly. Tissue must be divided before fixation: a fresh sterile portion should be reserved for molecular testing and culture, while a separate portion is placed in formalin for histopathology. Non-sterile material requires appropriate decontamination, whereas concentration of body fluids may improve yield. These pre-analytical principles are emphasised in WHO and national guidance and remain especially important when material is limited.[2,3,6-8]

Testing should proceed in parallel rather than as a slow sequential cascade. Representative material should be allocated simultaneously for a WHO-recommended rapid molecular test, acid-fast bacillus microscopy, mycobacterial culture with drug-susceptibility testing (DST), and cytology or histopathology when tissue is available. Site- and host-specific adjuncts may be added, but they should not consume material needed for bacteriological confirmation.

CORE DIAGNOSTIC MODALITIES

Rapid molecular testing and resistance detection

Xpert MTB/RIF and Xpert MTB/RIF Ultra automate nucleic acid amplification and detect *M. tuberculosis* complex (MTBC) together with rifampicin resistance. The 2025 Cochrane update and earlier meta-analysis show a consistent pattern across EPTB specimens: specificity is generally high, making a positive result clinically valuable, but sensitivity is heterogeneous and insufficient for exclusion.[9,10] Ultra improves analytical sensitivity and may return a “trace detected” result in very paucibacillary specimens. Such results require clinical context, particularly after previous treatment, but are treated as bacteriological confirmation in WHO guidance for people evaluated for EPTB.[2,3]

Anatomical site strongly modifies performance. In lymph-node aspirate, pus and many tissue specimens, molecular testing is

often productive. In pleural effusion, however, bacilli are usually concentrated in pleural tissue rather than free fluid. Xpert MTB/RIF therefore has excellent specificity but low sensitivity in pleural fluid, and Ultra improves sensitivity without becoming a reliable rule-out test.[11,12] A negative result in a patient with persistent high probability should prompt pleural biopsy or another targeted investigation rather than repeated low-yield fluid testing.

CSF requires urgent, volume-conscious processing. Prospective studies show that Ultra can increase microbiological confirmation of tuberculous meningitis, particularly in adults with HIV, but performance varies by population, specimen volume and reference definition.[13-15] Neither Xpert nor Ultra has adequate negative predictive value to exclude tuberculous meningitis. When the clinical syndrome and CSF profile are strongly compatible, treatment should not be delayed solely because the initial molecular result is negative.

Rifampicin resistance detected by a rapid molecular assay should trigger prompt resistance work-up and clinical review. Line-probe assays can identify additional resistance-associated mutations when sufficient bacillary DNA is present. Targeted next-generation sequencing offers broader genotypic resistance prediction and has shown high overall accuracy in systematic review, but direct application to paucibacillary EPTB material remains constrained by low DNA concentration and inhibitors.[16] Culture-based phenotypic DST is still needed when sequencing fails, genotype and phenotype are discordant, or susceptibility beyond validated mutation targets must be established.

Microscopy, culture and tissue pathology

Ziehl-Neelsen staining and fluorochrome microscopy provide same-day evidence of acid-fast bacilli at low cost. Their principal limitation is very low sensitivity in many extrapulmonary fluids and tissue homogenates. A positive smear does not reliably distinguish MTBC from non-tuberculous mycobacteria and supplies no resistance information. A negative smear should never be used to rule out EPTB.

Mycobacterial culture detects viable organisms, supports species identification and enables phenotypic DST. Automated liquid systems generally recover organisms earlier than solid media, but results still require days to weeks, and contamination or false-negative culture may occur. Culture should be requested whenever sufficient material is available, especially when drug resistance is possible, the molecular resistance result is indeterminate or susceptibility to drugs beyond rifampicin is required.

Cytology and histopathology are particularly useful in lymph-node, pleural, abdominal, cutaneous and osteoarticular disease. Caseous necrosis, epithelioid granulomas and Langhans-type giant cells support TB but are not pathognomonic; fungal infection, sarcoidosis, foreign-body reactions and non-tuberculous mycobacteria may produce similar patterns. Immunosuppression may also result in poorly formed or absent granulomas. Pathology should therefore be interpreted with microbiology, and all tissue should not be placed in formalin before a fresh portion is secured.

Adjunctive biomarkers and immune-based tests

Pleural-fluid adenosine deaminase (ADA) is the best-established biochemical adjunct. A high ADA concentration in a lymphocyte-predominant exudate can increase the probability of tuberculous pleuritis in a high-burden setting, while a low value may reduce it. Meta-analysis demonstrates good overall accuracy but important heterogeneity; empyema, lymphoma, rheumatoid pleuritis and other infections may also elevate ADA.[17] Unstimulated pleural-fluid interferon-gamma may have greater diagnostic accuracy, but cost, availability and non-standardised thresholds restrict routine use.[18] Neither test provides drug-resistance information or replaces tissue sampling when malignancy remains possible.

The tuberculin skin test and commercial interferon-gamma release assays demonstrate immunological sensitisation rather than active disease. They cannot reliably distinguish latent infection from EPTB and should not be used as stand-alone confirmatory tests.[19] Negative results may occur in disseminated TB, advanced HIV infection, malnutrition or immunosuppression.

Urine lipoarabinomannan is a rapid rule-in adjunct for selected people living with HIV, particularly those with advanced

immunosuppression or serious illness. Sensitivity increases as CD4 count declines, but a negative result does not exclude TB.[20] A multicountry randomised trial showed that lipoarabinomannan-guided treatment initiation reduced mortality among hospitalised HIV-positive adults with suspected TB, supporting its value as a patient-outcome intervention rather than merely a laboratory assay.[21] It does not identify the anatomical site or drug resistance and should be used concurrently with molecular testing and specimen-based investigation.

Blood host signatures, cytokine combinations, antibodies, metabolites, cell-free mycobacterial DNA and next-generation antigen assays remain under evaluation. A systematic review found extensive heterogeneity and limited prospective validation against robust reference standards[22] These technologies may ultimately help when the disease site is inaccessible, but they are not yet replacements for representative specimen testing.

Imaging and image-guided sampling

Imaging is indispensable for localising disease, defining extent, detecting complications and guiding aspiration or biopsy. It is rarely definitive because necrotic lymph nodes, rim-enhancing collections, serosal thickening, marrow lesions and granulomatous masses can resemble malignancy or other infections.[23,24] Ultrasound is useful for superficial nodes, effusions, ascites and abdominal lesions; computed tomography delineates thoracic, abdominal and deep-organ disease; and magnetic resonance imaging is preferred for brain, meninges, spine, marrow and soft-tissue extension. Point-of-care ultrasound may accelerate recognition and sampling in resource-limited or HIV-care settings, although protocols and diagnostic thresholds remain heterogeneous.[25]

SITE-DIRECTED CLINICAL APPLICATION

The value of each modality depends on matching the test to the anatomical compartment. Table 1 summarises a pragmatic approach.

Table 1: Site-directed specimen selection and parallel diagnostic strategy

Clinical context	Preferred specimen or target	Tests performed in parallel	Key interpretive caution
Peripheral lymph-node disease	Fine-needle aspirate; core or excision biopsy if nondiagnostic	Molecular test, smear, culture/DST and cytology or histology	Preserve fresh material before formalin; exclude lymphoma and fungal disease
Pleural disease	Pleural fluid plus pleural biopsy when feasible	Molecular test and culture on fluid/tissue; histology on biopsy	Negative fluid molecular testing does not exclude disease; ADA is an adjunct
Tuberculous meningitis	Adequate-volume CSF processed urgently	Ultra or another rapid molecular test, microscopy and culture	A negative rapid test cannot safely rule out disease or justify treatment delay
Abdominal or pericardial disease	Image-guided fluid, node, peritoneal or pericardial tissue	Molecular testing, culture/DST and histopathology	Biochemical markers modify probability but do not confirm resistance
Osteoarticular or spinal disease	Deep aspirate, synovial sample or bone/soft-tissue biopsy	Molecular test, culture/DST and histopathology	Superficial swabs have poor value; imaging defines complications and biopsy route
HIV-associated or disseminated disease	Sample the safest involved site; obtain respiratory specimens when possible	Molecular testing and culture; urine lipoarabinomannan in eligible patients	Negative urine testing does not exclude TB; actively search for multiple sites

INTEGRATED INTERPRETATION AND CLINICAL ACTION

A positive molecular test or culture provides bacteriological confirmation and should lead to treatment initiation or refinement, resistance evaluation and notification according to national policy. Histopathology may strongly support the diagnosis and reveal alternative disease, but granulomatous inflammation alone should not be equated automatically with TB. Concordance among microbiology, pathology, imaging and clinical findings raises confidence; discordance should trigger structured reassessment.

When initial tests are negative, clinicians should reconsider pre-test probability,

specimen type and volume, transport and processing, prior treatment and competing diagnoses. If probability remains moderate or high, repeat sampling or tissue biopsy is generally more informative than serially ordering low-yield blood tests. In immediately life-threatening EPTB, appropriate specimens should be collected before treatment whenever safely possible, but therapy should not be delayed while awaiting culture or because a single rapid test is negative.

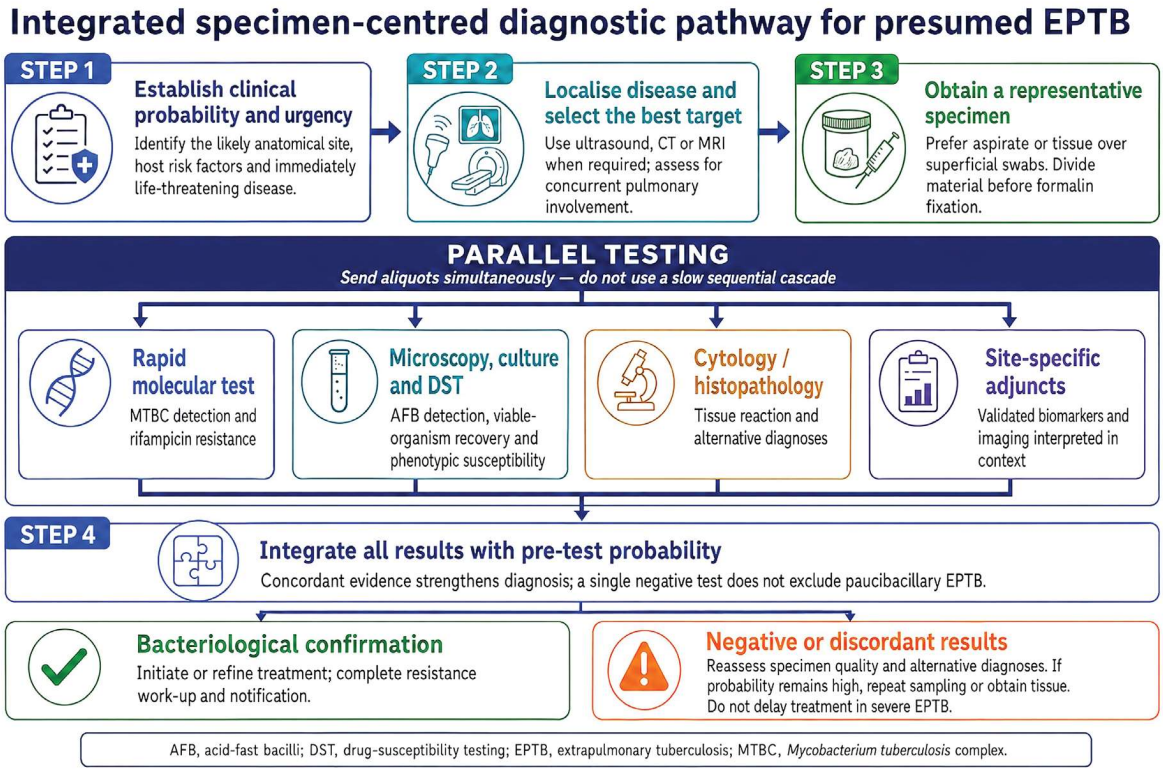

Figure 1: Integrated specimen-centred diagnostic pathway for presumed extrapulmonary tuberculosis. Testing should proceed in parallel rather than as a slow sequential cascade. DST, drug-susceptibility testing; EPTB, extrapulmonary tuberculosis; MTBC, Mycobacterium tuberculosis complex.

The proposed pathway also supports diagnostic stewardship. It prevents exhaustion of limited material by low-value testing, reduces delays created by sequential requests and preserves opportunities for DST. Its principal limitation is dependence on access to safe sampling, pathology, culture and molecular platforms. Where resources are constrained, the same hierarchy remains useful: prioritise the best specimen, a rapid high-specificity test and culture, then add pathology or validated site-specific adjuncts according to availability.

LIMITATIONS AND RESEARCH PRIORITIES

EPTB diagnostic studies frequently use small, site-specific cohorts and heterogeneous reference standards. Culture-only standards underestimate true disease, while composite standards may incorporate clinical response or the index test itself. Accuracy estimates may therefore not transfer directly across populations, specimen-processing protocols or prevalence settings. Evidence for direct sequencing and emerging non-sputum biomarkers is also less mature in EPTB than in pulmonary TB.

This communication is a focused narrative synthesis rather than a systematic review; study selection was guided by methodological quality and clinical relevance, and no formal risk-of-bias assessment or meta-analysis was performed. Future studies should use prespecified case definitions, report specimen volume and processing in detail, and evaluate patient-important outcomes such as time to treatment, invasive procedures avoided, disability and mortality.

CONCLUSION

Accurate EPTB diagnosis depends less on selecting a single "best" assay than on obtaining the best available specimen and using complementary tests simultaneously. Rapid molecular testing provides early MTBC and rifampicin-resistance detection, culture preserves viable-organism and phenotypic DST information, and pathology identifies tissue response and alternative diagnoses. Imaging and validated biomarkers should guide sampling or modify probability rather than substitute for bacteriological investigation. Negative results must be interpreted against specimen quality, anatomical site, pre-test probability and disease severity. A specimen-centred, parallel-testing pathway offers the most practical means of improving confirmation while limiting diagnostic delay.

Abbreviations

ADA: adenosine deaminase;
CSF: cerebrospinal fluid;
DST: drug-susceptibility testing;
EPTB: extrapulmonary tuberculosis;
HIV: human immunodeficiency virus;
MTBC: Mycobacterium tuberculosis complex;
WHO: World Health Organization.

Declarations

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