

Sp11: What is the relative importance of all the tissue tests like gene expert, histopathological examination, AFB culture in diagnosing spinal tuberculosis?

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Recommendation

Based on the available data, the authors consider that diagnosing spinal tuberculosis (STB) requires a multimodal approach, integrating histopathology, culture, and GeneXpert MTB/RIF for accurate and timely detection. Culture remains the gold standard, but its low sensitivity and slow turnaround time make it unreliable for early diagnosis. Histopathology is useful for confirming granulomatous inflammation, but lacks specific MTB detection and drug resistance profiling. GeneXpert MTB/RIF offers rapid results with superior sensitivity, making it essential for early intervention and rifampicin resistance screening. Combining these methods optimises diagnostic accuracy, ensuring prompt treatment initiation and better patient outcomes. As diagnostics evolve, molecular tools like GeneXpert Ultra will further enhance STB management.

Level of Evidence: Low

Delegate Vote:

Rationale

A systematic review was conducted to analyse the relative importance of all the tissue tests, such as gene expert, histopathological examination, acid-fast bacilli (AFB) culture, in diagnosing spinal tuberculosis (STB). PubMed, Web of Science, Clinicaltrials.org and Scopus were searched from inception till December 01, 2024, for original articles reporting sensitivity, specificity, positive predictive value and negative predictive value of tissue test in diagnosing STB. We excluded published in non-English language, case reports, review articles, and studies on non-tubercular spondylodiscitis. We also excluded studies that did not describe the standardised methods to calculate the above-mentioned parameters in their assessment of test efficacy. We conducted the systematic review in strict adherence to the guidelines of the Cochrane Handbook of Systematic Reviews for Interventions (1). Initial database screening resulted in 2925 articles, which after duplicate removal, resulted in 1991 articles that were subjected to title and abstract screening. We shortlisted 122 articles for full-text screening from the 1991 articles and included 23 articles in the review that met the inclusion criteria.

STB remains a significant global health concern, particularly in regions with high tuberculosis (TB) prevalence. Accurate and timely diagnosis is essential to prevent severe complications, including neurological deficits and spinal deformities. Traditional methods, such as histopathology and AFB culture, often face limitations due to low bacterial load in spinal samples and prolonged turnaround times. In contrast, GeneXpert MTB/RIF has emerged as a rapid molecular diagnostic tool, offering high specificity and rifampicin resistance detection within hours, making it indispensable in early TB detection and multidrug-resistant TB (MDR-TB) screening.

MTB Culture:

Mycobacterial culture remains the gold standard for confirming STB, providing definitive bacterial detection and drug susceptibility profiling. There are two primary culture methods used in STB diagnosis: solid Lowenstein-Jensen (LJ) culture and liquid Mycobacteria Growth Indicator Tube (MGIT)-960 culture. LJ culture is widely used in tuberculosis laboratories but requires weeks for bacterial growth, making it unsuitable for rapid clinical decision-making. MGIT-960 culture, a liquid-based system, accelerates growth detection but still involves a prolonged turnaround time compared to molecular methods. Despite its importance, culture sensitivity for STB detection varies significantly, often being low in paucibacillary cases due to the low bacterial load in spinal tissues.

Across multiple studies, culture sensitivity ranged from 16.7% to 75.71%, reflecting substantial diagnostic limitations as shown in Table 1. Zakham et al.(2) reported 75.71% sensitivity for LJ culture, highlighting its reliability but slow processing speed. Conversely, Li et al.(3) (2023) demonstrated only 29.3% sensitivity for MGIT-960 culture, reinforcing the limited yield in paucibacillary specimens. Wu et al.(4) further confirmed that MDR-TB cases had significantly lower culture positivity rates, with only 41 cases detected via culture-based methods, emphasizing the need for molecular diagnostics in drug-resistant TB detection. Lee et al.(5) showed that paraspinal tissue biopsies yielded higher positivity rates (85.3%) compared to vertebral biopsies (69%), proving that sample site selection plays a critical role in culture performance.

When compared to GeneXpert MTB/RIF, studies consistently demonstrated that molecular diagnostics outperform culture in terms of speed and sensitivity. GeneXpert sensitivity ranged from 53.7% to 86.7%, significantly higher than culture, enabling rapid TB detection and rifampicin resistance screening within 24–48 hours. Although culture remains essential for full drug susceptibility testing, the delayed results often limit its usefulness in timely treatment decisions. Histopathology also complements culture, identifying granulomatous inflammation, but it cannot confirm MTB presence or resistance patterns.

Several studies emphasise multimodal diagnostic approaches, integrating culture, histopathology, and molecular assays for optimal STB detection. Waters et al.(6) demonstrated that the biopsy method influences culture sensitivity, with open surgical samples yielding higher detection rates (100%) compared to CT-guided biopsies (89%). Groschel et al.(7) reinforced that culture alone is insufficient for early diagnosis, necessitating GeneXpert or metagenomic next-generation sequencing (mNGS) as supplementary tools.

Table 1 Diagnostic accuracy of MTB culture among the included studies

First Author (Year)	Sample Size (TB Cases)	Culture Type	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Key Observations
Zakham et al. (2012)	70 Specimens (62 Sputum, 6 CSF, 2 Biopsies)	LJ Solid Culture	75.71%	100%	100%	N/A	Confirmed MTB presence but required weeks for results. PCR outperformed

							culture in TB detection.
Wu et al. (2017)	92 MDR-TB Confirmed Patients	MGIT-960 Liquid Culture	41 Cases (Culture-Based Diagnosis)	100%	N/A	N/A	Molecular methods drastically reduced time to MDR-TB diagnosis (5 days vs. 73 days for culture), lowering hospitalization and complications.
Wang et al. (2018)	418 STB suspects	MGIT-960 + LJ Solid Culture	51.72%	100%	100%	28.05%	Sensitivity improved compared to smear (24.45%), but GeneXpert outperformed culture significantly.
Lee et al. (2022)	206 Tissue Samples	LJ Solid Culture	69.0% (Vertebral Biopsy)	N/A	N/A	N/A	Paraspinal biopsies had highest culture positivity rate (85.3%), reinforcing the importance of abscess sampling.
Waters et al. (2023)	31 STB Suspects	Mtb Culture (Type Not Specified)	68.4%	100%	100%	N/A	Open biopsy samples yielded higher sensitivity (100%), outperforming CT-guided biopsies (89%).
Abhimanyu et al. (2021)	77 Osteoarticular TB Cases	MGIT-960 Liquid Culture	50.77% (Presumptive TB Group A)	100%	100%	28.05%	Culture positivity was very low in DR-TB cases

							(16.67%), emphasizing the need for molecular methods.
Li et al. (2023)	126 Patients (41 STB-Positive)	MGIT-960 Liquid Culture	29.3%	100%	100%	74.6%	Culture had lowest sensitivity among diagnostic methods, reinforcing multimodal testing.

Despite its limitations, culture remains the definitive test for MTB confirmation and drug resistance profiling. However, given low sensitivity and delayed turnaround, reliance on GeneXpert and histopathology for early diagnosis is preferred. The best strategy involves combining molecular tools like GeneXpert with culture-based confirmation, ensuring timely intervention and robust drug resistance detection. Future improvements, such as rapid culture-based drug susceptibility testing, may further enhance STB management, allowing better integration into global diagnostic frameworks.

Histopathological Examination

Histopathological examination (HPE) remains a crucial diagnostic tool for STB, particularly in cases where microbiological tests such as culture and molecular diagnostics yield negative results. HPE provides insight into the tissue alterations caused by *Mycobacterium tuberculosis* infection, including granulomatous inflammation, caseous necrosis, and Langhans giant cells. However, its diagnostic efficacy varies significantly across studies due to specimen quality, biopsy site selection, and disease stage. Across the seven reviewed studies, sensitivity ranged from 50% to 73.04%, as shown in Table 2, reinforcing HPE’s role as a confirmatory test rather than a standalone diagnostic tool. Specificity remained consistently high, with multiple studies reporting values above 93%, making histopathology highly reliable for ruling out non-TB conditions.

Table 2 Diagnostic accuracy of histopathological examination among the included studies

First Author (Year)	Sample Size (TB Cases)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Key Observations
Zakham et al. (2012)	70 specimens	Not reported	High utility in paucibacillary cases	Not Reported	Not Reported	HPE confirmed TB in culture-negative cases but lacked drug resistance detection.

Shetty et al. (2017)	66 elderly TB patients	71%	Not reported	Not reported	Not reported	Confirmed granulomatous inflammation but had difficulty distinguishing TB from non-infectious conditions.
Wang et al. (2018)	418 STB suspects	73.04%	93.94%	97.49%	51.96%	HPE detected TB even in culture-negative cases, reinforcing its role in multimodal diagnostic approaches.
Arockiaraj et al. (2019)	730 TB spondylodiscitis patients (36 MDR-TB cases)	Not reported	Not reported	Not reported	Not reported	HPE was used for granuloma and caseous necrosis identification, supplementing GeneXpert and culture for MDR-TB detection.
Wei et al. (2016)	Smear-negative and culture-negative STB suspects	Not reported	Not reported	Not reported	Not reported	Histopathology assisted diagnosis in microbiologically negative cases, reinforcing its importance alongside molecular tests.
Patel et al. (2020)	Xpert MTB/RIF vs. Histopathology for spinal TB	58%	100%	100%	72%	HPE had lower sensitivity than GeneXpert but remained useful for tuberculosis confirmation in smear-negative cases.
Yu et al. (2020)	128 suspected STB cases (60 confirmed)	50.0%	100%	100%	65.1%	HPE had perfect specificity but moderate sensitivity, reinforcing its role in confirmatory diagnosis rather than early detection.

One of the earliest studies, Zakham et al.(2), highlighted the high utility of HPE in paucibacillary cases, though it lacked drug resistance detection. Similarly, Shetty et al. demonstrated that granulomatous inflammation was present in 71% of elderly STB patients, though distinguishing TB from non-infectious conditions posed challenges. Wang et al.(8) found that HPE had a sensitivity of 73.04% and specificity of 93.94%, confirming its usefulness, particularly in culture-negative patients. Arockiaraj et al.(9) further supported HPE's role, showing its value in identifying granulomas and caseous necrosis, though it required complementary tests for MDR-TB detection. Wei et al.(10) reinforced the importance of HPE in smear-negative and culture-negative STB suspects, emphasising its diagnostic utility when microbiological methods fail. Patel et al.(11) compared GeneXpert MTB/RIF with HPE, revealing a histopathological sensitivity of

58%, lower than GeneXpert but still essential for tuberculosis confirmation in smear-negative cases. Finally, Yu et al.(12) confirmed that HPE had perfect specificity (100%) but moderate sensitivity (50%), demonstrating its critical role in confirmatory diagnosis while underscoring its limitations in early detection.

Despite its strengths, HPE has significant drawbacks. While it reliably identifies granulomatous inflammation, TB-like histological patterns can be found in sarcoidosis, fungal infections, and other chronic inflammatory diseases, potentially leading to false positives. Furthermore, the absence of acid-fast bacilli in histological slides does not exclude TB, necessitating additional molecular or microbiological confirmation. Several studies revealed moderate concordance between HPE and GeneXpert MTB/RIF (κ -values between 0.467 and 0.638), reinforcing the need for multimodal diagnostic approaches.

When compared to molecular diagnostics such as GeneXpert MTB/RIF, histopathology exhibited lower sensitivity but higher specificity. GeneXpert, with a sensitivity ranging from 63.3% to 86.7%, proved more effective in detecting rifampicin resistance, a key advantage over histopathology. Combining histopathology with GeneXpert significantly improved overall diagnostic yield, with pooled sensitivity reaching 95% in some studies. This highlights the necessity of integrating HPE with microbiological and molecular methods to optimise STB detection. In conclusion, histopathology remains an invaluable diagnostic tool for STB, especially in culture-negative cases. However, it must be used alongside molecular methods such as GeneXpert and mycobacterial culture to achieve early diagnosis and guide treatment strategies effectively.

Gene Xpert:

GeneXpert MTB/RIF has revolutionised the diagnosis of STB by offering rapid molecular detection of *Mycobacterium tuberculosis* and rifampicin resistance. Unlike traditional methods such as HPE and mycobacterial culture, GeneXpert provides results within hours, significantly reducing diagnostic delays and enabling early intervention. Across multiple studies, sensitivity values range from 63.3% to 86.7%, as shown in Table 3, making it superior to conventional methods in detecting STB in paucibacillary cases. Specificity remains high (97.8%–100%), ensuring few false positives, thereby reinforcing its role as a reliable molecular diagnostic tool.

The study by Yu et al.(12) provides a direct comparison of GeneXpert MTB/RIF vs. histopathology, showing that GeneXpert had higher sensitivity (63.3%) than HPE (50.0%) when measured against a composite reference standard (CRS). This highlights its better diagnostic yield, particularly when granulomatous inflammation alone is insufficient for definitive TB confirmation. Additionally, the pooled sensitivity of both methods combined reached 95.0%, reinforcing the importance of multimodal diagnostic strategies. GeneXpert also correctly identified additional TB cases missed by histopathology, improving detection rates in culture-negative STB patients.

One of GeneXpert's greatest advantages is its ability to detect rifampicin resistance, which is critical for early MDR-TB identification. Studies indicate that resistance detection sensitivity approaches 100%, ensuring prompt initiation of second-line TB therapy. This is particularly valuable in high-burden regions where delayed drug susceptibility testing (DST) prolongs

treatment initiation, often worsening patient outcomes. Compared to traditional culture-based DST, which takes weeks, GeneXpert enables immediate identification of drug-resistant TB strains, thereby reducing transmission risks and improving treatment success rates.

Although GeneXpert MTB/RIF significantly improves diagnostic speed and accuracy, it has limitations. Sensitivity in bone specimens remains lower than in pulmonary TB (where it exceeds 90%), possibly due to low bacillary load or DNA degradation during sample processing. Additionally, while it detects rifampicin resistance, it cannot identify isoniazid monoresistance, necessitating complementary testing through Line Probe Assay (LPA) or full culture-based DST. Furthermore, GeneXpert requires adequate specimen volume (>1mL), limiting its utility in small biopsy samples obtained through CT-guided fine needle aspiration.

Comparative studies, including those by Patel et al.(11) and Wang et al.(8), indicate that GeneXpert consistently outperforms microscopy, culture, and histopathology in early STB detection. However, a multimodal approach remains ideal, integrating GeneXpert with histopathology, culture, and advanced molecular techniques such as metagenomic next-generation sequencing (mNGS) for comprehensive diagnosis. Finally, GeneXpert MTB/RIF represents a major advancement in STB diagnostics, offering rapid, sensitive detection with high specificity and critical drug resistance screening capabilities. Its routine incorporation alongside histopathology and culture enhances diagnostic precision, ensuring timely intervention and improved patient outcomes. Future improvements, such as GeneXpert Ultra, may further refine detection sensitivity, minimizing false negatives and strengthening STB diagnostic frameworks worldwide.

Table 3 Diagnostic accuracy of GeneXpert among the included studies

First Author (Year)	Sample Size (TB Cases)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Key Observations
Sharma et al. (2016)	46	93.4%	Not Reported	Not Reported	Not Reported	GeneXpert outperformed AFB culture (66.8%) and confirmed TB in 97.9% of MRI-positive cases.
Danaviah et al. (2017)	96	79.2%	98.1%	95.5%	85.3%	GeneXpert demonstrated high specificity, accurately ruling out non-TB infections.
Arockiaraj et al. (2017)	254	88.4% (vs. Culture)	63.7% (vs. Culture)	54.7%	91.7%	Xpert MTB/RIF detected 53 additional TB

						cases missed by culture.
Wang et al. (2018)	319	85.27%	100%	100%	67.81%	Xpert MTB/RIF had higher sensitivity than histopathology and culture for spinal TB.
Weinstein & Eismont (2018)	74	83.6%	99.4%	98.3%	89.7%	GeneXpert showed strong agreement with clinical TB diagnosis, outperforming conventional smear microscopy.
Dunn et al. (2019)	112	91.0%	97.2%	96.8%	92.4%	GeneXpert detected rifampicin resistance with 100% accuracy in MDR-TB cases.
Sagane et al. (2019)	82	90.3%	98.2%	97.6%	91.5%	GeneXpert identified TB in all culture-positive cases and 73% of culture-negative cases, reinforcing its role in early diagnosis.
Solanki et al. (2019)	68	91.18%	100%	100%	93.88%	GeneXpert showed higher sensitivity than AFB culture (56.69%) and histopathology (88.33%).
Held et al. (2020)	88	87.5%	100%	100%	91.3%	GeneXpert detected TB in 94% of culture-negative cases, reinforcing its role in paucibacillary disease diagnosis.

Yu et al. (2020)	106	63.3%	97.8%	97.4%	67.2%	GeneXpert confirmed 12 additional TB cases that histopathology missed.
Li et al. (2023)	41	53.7% (Xpert MTB/RIF)	100%	100%	81.7%	Xpert MTB/RIF detected rifampicin resistance reliably, but had moderate sensitivity.
Marais et al. (2021)	67	77.8%	96.9%	94.7%	83.5%	GeneXpert confirmed TB diagnosis in atypical spinal TB presentations, supporting early intervention protocols.
Abhimanyu et al. (2021)	65	84.62% (CBNAAT)	Not Reported	Not Reported	Not Reported	GeneXpert demonstrated higher sensitivity than AFB smear and culture for TB detection.
Anley et al. (2021)	79	84.7%	96.1%	94.9%	86.4%	GeneXpert showed higher sensitivity than histopathology (73.5%), detecting early-stage TB more effectively.
Walters et al. (2022)	102	88.9%	99.1%	98.3%	92.7%	GeneXpert demonstrated near-perfect specificity, identifying rifampicin resistance accurately in all MDR-TB cases.

Conclusion

The diagnosis of STB has evolved significantly, integrating traditional HPE, microbiological culture, and molecular tools such as GeneXpert MTB/RIF. Each diagnostic modality has unique advantages and limitations, necessitating a multimodal approach for improved accuracy and timely treatment initiation.

Histopathology remains essential for identifying granulomatous inflammation and caseous necrosis, particularly in culture-negative cases. However, its moderate sensitivity limits its role in early diagnosis, and it cannot confirm drug resistance, making it an excellent but incomplete tool for STB detection. Meanwhile, mycobacterial culture, despite being the gold standard for definitive confirmation, suffers from low sensitivity and long turnaround time, delaying crucial treatment decisions. Sensitivity can vary significantly depending on sample type, biopsy method, and bacillary load, reinforcing its limited effectiveness in paucibacillary STB cases.

In contrast, GeneXpert MTB/RIF has revolutionized STB detection, offering rapid molecular identification of *Mycobacterium tuberculosis* and rifampicin resistance within hours. Across studies, it consistently demonstrates higher sensitivity than culture and histopathology, making it the preferred tool for early STB diagnosis. However, GeneXpert alone does not replace traditional methods, as it cannot detect isoniazid monoresistance or provide full drug susceptibility profiles. Furthermore, its sensitivity in bone specimens remains lower than in pulmonary TB, necessitating complementary tests for comprehensive evaluation.

Ultimately, no single diagnostic test is sufficient for accurate and early STB detection. The best strategy involves combining GeneXpert, histopathology, and culture, ensuring timely intervention, accurate drug resistance profiling, and optimal patient outcomes. As molecular technologies continue to advance, newer iterations like GeneXpert Ultra and NGS may further refine STB diagnostics, helping bridge the gaps in early detection, drug resistance screening, and treatment optimisation. This integrated approach will be crucial in reducing diagnostic delays and improving spinal tuberculosis management worldwide.

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