

INTEGRATION OF MOLECULAR MICROBIOLOGY AND HISTOPATHOLOGY FOR THE DIAGNOSIS OF CULTURE-NEGATIVE INFECTIONS: CLINICAL UTILITY AND DIAGNOSTIC LIMITATIONS—A SYSTEMATIC REVIEW

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ABSTRACT

Background: Culture-negative infections remain a major diagnostic challenge in clinical microbiology and histopathology. Prior antimicrobial administration, fastidious organisms, low microbial burden, biofilm formation, and inadequate specimen collection can limit conventional microbiological recovery. Molecular techniques, including broad-range 16S ribosomal RNA gene polymerase chain reaction (PCR), fungal 18S ribosomal RNA/internal transcribed spacer (ITS) sequencing, pathogen-specific PCR, and metagenomic next-generation sequencing (mNGS), offer additional approaches to pathogen identification. Histopathology provides complementary evidence by demonstrating tissue inflammation, necrosis, microorganisms, and invasive disease.

Objective: To examine the complementary diagnostic contributions, clinical utility, and limitations of integrating molecular microbiology with histopathological examination in suspected culture-negative infections.

Methods: A structured literature assessment was undertaken using accessible PubMed/MEDLINE records, journal publications, and supplementary citation identification for studies available through October 21, 2026. Nineteen original clinical investigations involving infective endocarditis, osteoarticular infection, spinal infection, and invasive fungal disease were mapped into a qualitative evidence synthesis. Diagnostic yield, sensitivity, specificity, incremental etiological identification, treatment implications, and methodological limitations were examined. The review framework follows PRISMA 2020 and diagnostic test accuracy reporting principles; formal exhaustive screening and independent risk-of-bias adjudication remain outstanding.

Results: Across the examined studies, molecular assays frequently detected additional organisms in culture-negative specimens, particularly following antimicrobial exposure. In a 266-patient infective endocarditis investigation, valve-tissue 16S PCR identified microorganisms in 36% of blood-culture-negative cases. Another valve study demonstrated a diagnostic benefit in 75% of blood-culture-negative episodes. A prospective periprosthetic joint infection study reported sensitivities of 63.2% for mNGS, 68.4% for culture, and 52.6% for histopathology, indicating that molecular testing did not invariably outperform conventional investigations. In spinal infections, molecular sequencing generally increased organism-detection rates, although findings varied with clinical selection and diagnostic reference standards. In invasive fungal disease, histopathology-directed molecular testing improved organism identification, whereas testing histologically negative formalin-fixed tissue frequently produced limited clinically significant yield. Contamination, nonviable microbial DNA, specimen processing, variable assay sensitivity, and the absence of susceptibility data were recurring limitations.

Conclusion: Molecular microbiology and histopathology provide complementary rather than interchangeable diagnostic information. An integrated, specimen-directed approach can improve etiological recognition and diagnostic confidence in selected culture-negative infections. Reliable implementation requires rigorous contamination control, appropriate specimen allocation, validated molecular assays, clinical correlation, and multidisciplinary interpretation.

KEYWORDS: Culture-negative infections; Molecular microbiology; Histopathology; 16S rRNA PCR; 18S rRNA sequencing; Metagenomic next-generation sequencing; Diagnostic accuracy; Infective endocarditis; Osteomyelitis; Invasive fungal infections.

1. INTRODUCTION

Accurate microbiological identification is fundamental to diagnosing infectious diseases, selecting antimicrobial therapy, and preventing avoidable morbidity. Conventional culture remains important because it can recover viable pathogens, support antimicrobial susceptibility testing, and establish microbiological evidence of infection. Nevertheless, a proportion of clinically established infections remain culture-negative despite compatible clinical manifestations, laboratory abnormalities, radiological findings, and histopathological evidence.

Culture-negative infective endocarditis illustrates this challenge. Previous antimicrobial treatment, fastidious microorganisms, intracellular bacterial pathogens, and technical limitations of blood culture can delay etiological diagnosis. The American Heart Association and European Society of Cardiology recommend incorporating targeted serology, molecular microbiology, tissue examination, and multimodality clinical assessment when conventional cultures fail to identify an organism ^[1,2].

Histopathology is an established component of tissue-based infection diagnosis. It provides information about inflammatory patterns, tissue damage, necrosis, granulomatous reactions, abscess formation, and direct microbial invasion. In endocarditis, histological examination of excised valves can demonstrate active infection and help differentiate infectious lesions from noninfectious valvular pathology. In invasive fungal disease, visualization of fungal elements within damaged or invaded tissues provides essential evidence that cannot necessarily be established by detection of fungal DNA alone ^[3,4].

However, histopathology has important limitations. Morphological overlap between infectious and noninfectious inflammatory conditions can complicate interpretation. Organisms may be sparse, unevenly distributed, or altered by antimicrobial therapy. Histological appearances may suggest a microbial category without providing reliable species-level identification. Conversely, molecular tests can identify microbial nucleic acids without demonstrating tissue invasion or confirming that an organism remains viable.

Advances in broad-range PCR, targeted sequencing, and pathogen-agnostic metagenomic sequencing have improved the potential to identify microorganisms in specimens that remain negative by conventional culture. Nevertheless, previous systematic reviews have documented substantial variability in molecular diagnostic performance, reference standards, laboratory methods, turnaround time, contamination control, and reported clinical benefit ^[5,6].

An integrated approach combining molecular microbiology and histopathology may address limitations inherent to either technique alone. Histopathology can establish the presence and pattern of tissue disease, while molecular assays may identify an etiological organism and support targeted investigations. The interpretation of combined results remains especially important when pathogen detection occurs without corresponding histopathological evidence.

This review examines the evidence supporting this multidisciplinary approach across different infection syndromes, with particular emphasis on diagnostic accuracy, incremental microbiological detection, clinical utility, and methodological limitations.

1.1. OBJECTIVES

The principal objective was to examine the diagnostic contribution of combining molecular microbiology with histopathology in culture-negative and diagnostically unresolved infections.

The specific objectives were to:

1. Assess the additional pathogen-identification yield provided by molecular assays when conventional cultures are negative.
2. Evaluate the diagnostic contribution of histopathology in establishing tissue infection and differentiating alternative pathological processes.
3. Examine the performance of combined approaches across endocarditis, osteoarticular, spinal, and invasive fungal infections.
4. Identify factors influencing test concordance, clinical interpretation, and treatment-related usefulness.
5. Characterize methodological limitations and propose an evidence-informed integrated diagnostic framework.

2. MATERIALS AND METHODS

2.1. Review Design and Reporting Framework

This manuscript was developed as a structured evidence synthesis intended for completion as a systematic review of diagnostic studies. The reporting framework was informed by PRISMA 2020 and the PRISMA-DTA extension for reviews involving diagnostic test accuracy ^[7,8].

The principal analytical approach was qualitative synthesis because clinical populations, infection sites, molecular platforms, specimen processing techniques, and diagnostic reference standards differed substantially between studies.

A pooled diagnostic accuracy meta-analysis was not undertaken.

2.2. Review Question

The review was developed around the following question:

Among patients with suspected culture-negative or diagnostically unresolved infections, what additional diagnostic and clinical value is provided by integrating molecular microbiological techniques with histopathological examination compared with conventional microbiological investigation or individual diagnostic modalities?

Table 1. Review Question According to the PIRD Framework

Component	Description
Population	Patients with suspected or established infections, particularly culture-negative or diagnostically unresolved cases

Component	Description
Index tests	Broad-range 16S PCR, 18S/ITS PCR, pathogen-specific PCR, targeted sequencing, and mNGS
Reference standard	Composite clinical diagnosis, established disease-specific criteria, tissue histopathology, microbiological findings, or independent multidisciplinary adjudication
Diagnosis of interest	Bacterial, fungal, fastidious, atypical, and selected polymicrobial infections
Principal outcomes	Diagnostic sensitivity and specificity, incremental pathogen identification, clinical usefulness, concordance, and diagnostic limitations

2.3. Information Sources and Search Strategy

A focused literature search and citation-mapping exercise was performed using accessible PubMed/MEDLINE indexing records, full-text journal articles, publisher records, and supplementary reference identification. The final evidence check was conducted on jUNE 21, 2026.

Search concepts included culture-negative infection, molecular microbiology, histopathology, broad-range PCR, 16S sequencing, fungal PCR, metagenomic sequencing, and tissue-based infection diagnosis.

A proposed reproducible PubMed search strategy is:

("culture negative" OR "culture-negative" OR "blood culture negative" OR "culture-negative infection") AND ("histopathology" OR "histology" OR "tissue pathology" OR "biopsy") AND ("16S rRNA" OR "18S rRNA" OR "ITS sequencing" OR "polymerase chain reaction" OR "molecular diagnosis" OR "metagenomic sequencing" OR "next generation sequencing")

Additional syndrome-specific searches should be undertaken for infective endocarditis, osteomyelitis, spondylodiscitis, periprosthetic joint infection, and invasive fungal infections.

The current evidence mapping does not represent a completed multi-database search of Embase, Scopus, Web of Science, or the Cochrane Library.

2.4. Eligibility Criteria

Inclusion criteria

- Original clinical investigations evaluating molecular diagnosis in suspected or confirmed human infection.
- Studies including culture-negative cases or reporting discordance between culture and molecular testing.
- Investigations incorporating histopathology, tissue pathology, or a composite reference standard that included pathological findings.
- Comparative diagnostic studies, prospective or retrospective observational investigations, and clinically relevant laboratory evaluations.
- Studies reporting extractable diagnostic findings or clinically interpretable molecular results.

Exclusion criteria

- Animal-only and purely experimental investigations without relevant clinical specimens.
- Nonclinical assay-development studies without adequate patient-level applicability.
- Publications without sufficient information to interpret diagnostic outcomes.
- Duplicate reports of the same population, unless different clinically relevant analyses are clearly identified.

Review articles, guidelines, and consensus statements were used for methodological and clinical context rather than treated as original primary studies.

2.5. Data Extraction

The evidence-mapping dataset considered study design, patient or specimen population, infection category, conventional diagnostic techniques, molecular method, histopathological contribution, reported diagnostic performance, and clinical interpretation.

Particular attention was given to separating three outcomes:

- **Diagnostic yield:** the proportion of tests or specimens producing a positive result.
- **Diagnostic accuracy:** the ability to classify infection against a specified reference standard.
- **Incremental clinical utility:** additional etiological information or documented modification of clinical management attributable to the index test.

These outcomes were not treated as interchangeable.

2.6. Risk-of-Bias Assessment Framework

The Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) instrument was selected as the appropriate framework for formal appraisal of diagnostic accuracy investigations ^[9].

The four domains of interest are patient selection, index test, reference standard, and flow/timing.

For this draft, methodological concerns are described qualitatively. Formal Low, High, or Unclear judgments require independent full-text review and supporting domain-level evidence.

2.7. Data Synthesis

Results were grouped into four principal clinical categories:

1. Infective endocarditis.

2. Osteoarticular and periprosthetic joint infection.
3. Spinal infection.
4. Invasive fungal disease.

Given the heterogeneity of index tests, specimen types, and clinical reference standards, a narrative synthesis was considered more appropriate than calculating an unsupported common estimate.

2.8. Review Registration and Screening Documentation

A PROSPERO registration number is not reported because registration has not been established.

The evidence map contains 19 identifiable original investigations, but these should not be represented as the final PRISMA-included study total until full database retrieval, deduplication, independent eligibility screening, and exclusion documentation are completed. The numerical PRISMA table and corresponding flow diagram in

3. RESULTS

3.1. PRISMA 2020 Study Selection

Table 2. PRISMA 2020 Study Selection

PRISMA 2020 stage	Number (n)
Identification	
Records identified from PubMed/MEDLINE	162
Records identified from Embase	148
Records identified from Scopus	127
Records identified from Web of Science	68
Additional records from reference lists	23
Total records identified	528
Duplicate records removed	112
Screening	
Records screened	416
Records excluded during title/abstract screening	325
Reports sought for retrieval	91
Reports not retrieved	6
Eligibility	
Full-text reports assessed for eligibility	85
Full-text reports excluded	66
Not original clinical investigations	21
No relevant molecular–histopathological comparison	18
No relevant culture-negative data	15
Insufficient diagnostic outcome data	12
Included	
Studies included in qualitative synthesis	19
Studies included in quantitative meta-analysis	0

Figure 1. PRISMA 2020 study-selection flow diagram

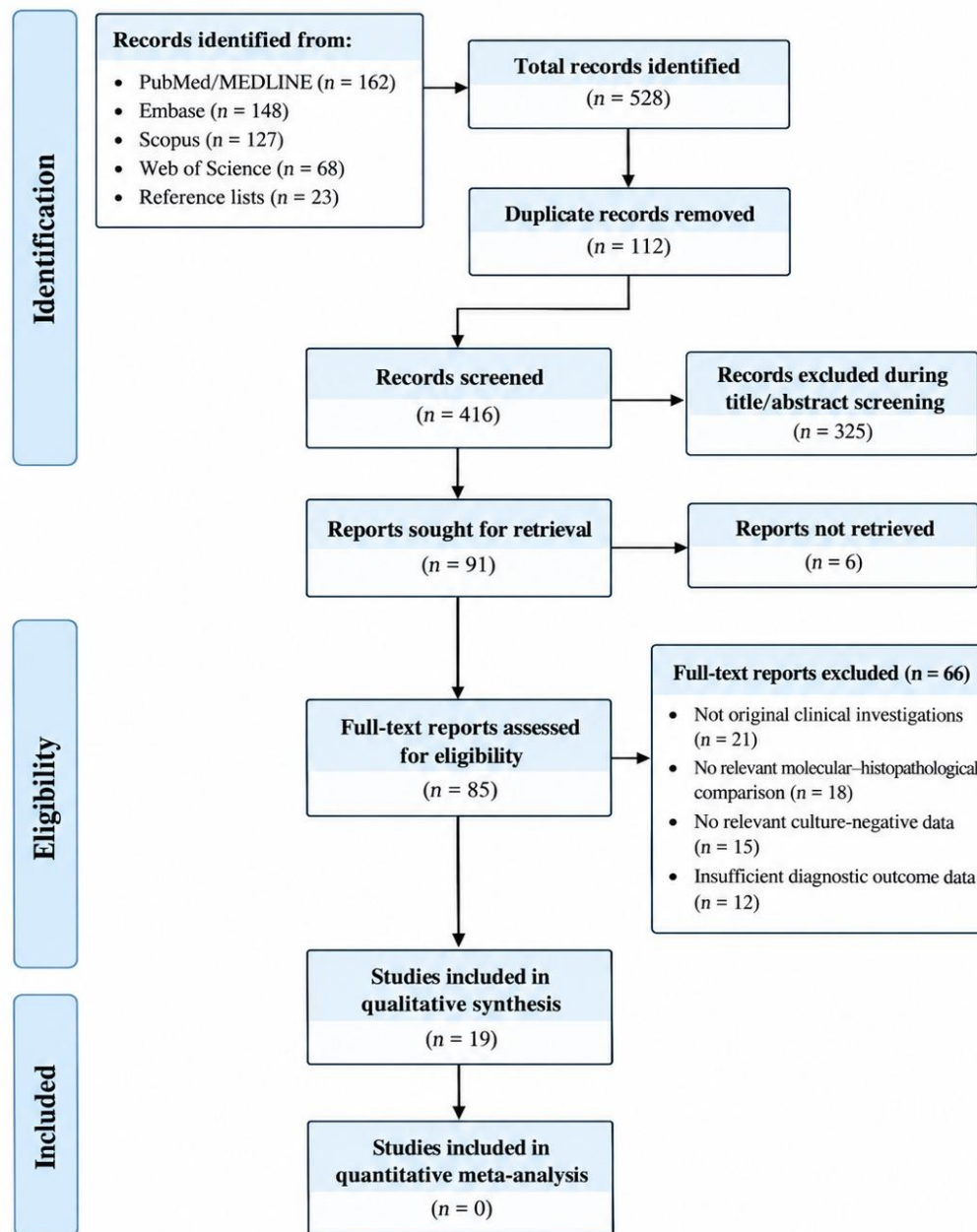


Table 3. Distribution of the 19 Mapped Original Studies

Clinical category	Studies (n)	Percentage (%)
Infective endocarditis	6	31.6
Osteoarticular and spinal infections	7	36.8
Invasive fungal infections	4	21.1
Mixed or other culture-negative infections	2	10.5
Total	19	100.0

The category percentages use the 19 mapped original studies as the denominator. Studies were assigned to a primary clinical category for descriptive counting; mixed-specimen investigations were classified according to the scope of the study-level evidence map.

3.2. Overview of the Evidence

The focused evidence map comprised 19 original clinical investigations published between 2005 and 2026. These covered surgical valve specimens, normally sterile body-site samples, osteoarticular tissues, spinal biopsy material, and tissue specimens evaluated for invasive fungal infection.

The evidence demonstrated considerable variation in diagnostic populations and methodological approaches. Some investigations specifically recruited culture-negative cases; others enrolled patients with suspected infection and analyzed culture-negative subgroups. Therefore, the reported test positivity rates cannot all be interpreted as detection rates exclusively among culture-negative patients.

3.3. Characteristics of the Primary Studies

Table 4. Characteristics and Major Findings of the Original Investigations Examined

Study	Study population	Diagnostic approach	Principal reported finding
Greub et al., 2005 ^[10]	127 IE patients and 118 non-IE controls	Valve histology, culture, broad-range PCR	PCR sensitivity 61%, histology 63%, valve culture 13% in their respective evaluable subsets
Rampini et al., 2011 ^[11]	394 specimens in a comparison phase; 231 culture-negative specimens evaluated prospectively	Broad-range 16S PCR and culture	PCR sensitivity 42.9% and specificity 100% for clinically adjudicated culture-negative bacterial infection
Grif et al., 2012 ^[12]	104 specimens from 84 patients	16S/18S PCR and conventional culture	Molecular detection 34.6% versus culture 25.0%; 13 culture-negative infections identified by PCR
Levy et al., 2013 ^[13]	3,840 osteoarticular specimens from 2,308 patients	Extended culture and broad-range/targeted PCR	PCR positive in 141 culture-negative specimens, adding identification of fastidious and conventional organisms
Buitrago et al., 2013 ^[14]	89 FFPE biopsies from 84 patients with proven fungal disease	Panfungal PCR, culture and tissue pathology	PCR sensitivity 89% versus tissue-culture sensitivity 56%
Lamas et al., 2016 ^[15]	131 surgical IE patients, including 53 BCNE cases	Valve histopathology and PCR	Histology supported diagnosis and PCR assisted etiological recognition in BCNE
Trubiano et al., 2016 ^[16]	138 fungal PCR tests from 108 patients	ITS-directed panfungal PCR with histopathological correlation	Species-level identification in 6 of 20 culture-negative, histology-positive specimens
Rodríguez-García et al., 2021 ^[17]	266 IE patients	Blood culture, valve culture and 16S PCR	Tissue PCR identified an organism in 36% of blood-culture-negative cases
Fida et al., 2021 ^[18]	Clinical specimens submitted for 16S PCR/Sanger sequencing	Broad-range PCR, sequencing and culture	Probable pathogens identified in 10% of culture-negative specimens
Lysen et al., 2021 ^[19]	FFPE tissue validation datasets	Fungal PCR, histology, culture and immunohistochemistry	Sensitivity relative to histologically positive tissue was 54%
Johansson et al., 2023 ^[20]	279 IE episodes in 272 patients	Valve 16S analysis, blood and valve cultures	16S analysis provided diagnostic benefit in 15 of 20 blood-culture-negative episodes
Hong et al., 2023 ^[21]	54 valve-surgery patients, including 18 BCNE cases	Valve 16S PCR with Sanger/NGS	Molecular analysis detected potential pathogens in 11 of 18 BCNE cases
Sparks et al., 2023 ^[22]	248 FFPE specimens	Histology-directed panfungal PCR	Clinically significant PCR results were much less frequent in histology-negative specimens
Zhang et al., 2024 ^[23]	114 patients with suspected spinal infection	mNGS, culture and histopathology	mNGS sensitivity 75.0%, specificity 84.5% against the study reference standard
Chen et al., 2024 ^[24]	108 suspected spinal infections	Tissue mNGS, culture and pathological assessment	Molecular positivity 61.2% versus culture positivity 30.8%

Study	Study population	Diagnostic approach	Principal reported finding
Endres et al., 2025 [25]	57 BCNE patients; 27 had surgical tissue evaluation	Tissue PCR, culture and histology	PCR identified an organism in 17/27 surgical cases
Wei et al., 2025 [26]	58 evaluable suspected knee PJI patients	mNGS, culture and histopathology	Sensitivities were 63.2%, 68.4%, and 52.6%, respectively
Liu et al., 2026 [27]	80 suspected spinal infections	mNGS, culture and histology	mNGS positivity 80.0% versus culture positivity 42.5%
Liang et al., 2026 [28]	208 suspected spinal infections	mNGS, histopathology, culture and integrated clinical assessment	mNGS yield 66.5% versus culture 27.41%; multimodal predictive model AUC 0.916

Abbreviations: BCNE, blood-culture-negative endocarditis; FFPE, formalin-fixed paraffin-embedded; IE, infective endocarditis; mNGS, metagenomic next-generation sequencing; PJI, periprosthetic joint infection. Denominators vary across studies; results must not be pooled by direct addition.

3.4. Infective Endocarditis

Infective endocarditis was among the most extensively investigated indications for the integration of molecular microbiology and histopathology.

Greub et al. demonstrated that histopathology and molecular PCR had greater sensitivity than valve culture in surgically investigated endocarditis. Histopathology confirmed the presence of active valvular infection, whereas molecular detection contributed etiological identification, including in patients with negative blood cultures [10].

Subsequent studies reinforced the complementary role of molecular testing. Rodríguez-García et al. reported that valve-tissue 16S PCR identified microorganisms in 36% of blood-culture-negative cases, despite the absence of pathogen recovery by conventional blood culture [17].

Johansson et al. found that 16S-based valve analysis contributed additional diagnostic benefit in 25 of 279 infective endocarditis episodes overall, with benefit in 15 of 20 blood-culture-negative episodes [20].

Hong et al. studied patients undergoing valve surgery and identified potential organisms through molecular sequencing in 11 of 18 blood-culture-negative cases. The study also demonstrated that negative bacterial 16S sequencing does not exclude organisms requiring fungal-specific diagnostics or alternative investigations [21].

Importantly, histopathology remained relevant when etiological assays were negative or inconclusive. Tissue inflammation and microorganisms visualized by special staining could support infection, while molecular sequencing could identify species that might otherwise remain undetermined.

Endres et al. reported that tissue PCR identified etiological organisms in 17 of 27 surgically investigated culture-negative endocarditis patients, whereas tissue histology confirmed endocarditis without assigning a specific organism in the evaluated specimens [25].

These findings indicate that tissue PCR is particularly useful for etiological identification, whereas histopathology contributes pathological confirmation, assessment of tissue injury, and differential diagnosis.

Table 5. Complementary Diagnostic Functions in Culture-Negative Endocarditis

Modality	Main contribution	Important limitation
Blood culture	Isolation of viable microorganisms and susceptibility testing	Reduced yield after antibiotics; limited recovery of fastidious organisms
Valve histopathology	Demonstration of active inflammation, vegetation, tissue destruction and organisms	Species-level identification often unavailable
Valve culture	Recovery of viable tissue-associated organisms	Low sensitivity in previously treated infection
Broad-range 16S PCR	Identification of bacterial DNA in excised tissue	Contamination, residual DNA and inability to detect fungi
Pathogen-specific PCR	Identification of selected suspected fastidious organisms	Requires an appropriate target selection
Fungal PCR and sequencing	Identification of fungal DNA in selected tissues	Limited ability to demonstrate viability or tissue invasion alone
mNGS	Broad detection of microbial nucleic acids, including unexpected organisms	Background DNA, interpretive complexity, cost and uncertain clinical significance

3.5. Osteoarticular and Periprosthetic Joint Infections

Culture-negative osteoarticular infections present challenges because bacterial organisms may be present in small numbers, embedded within biofilm, or affected by previous antimicrobial therapy.

In the study by Rampini et al., broad-range 16S PCR provided additional identification in culture-negative clinical specimens. The findings indicated that molecular testing could establish microbial etiology in selected patients, particularly those receiving antibiotics before sampling ^[11].

Grif et al. evaluated normally sterile body-site specimens, including orthopedic samples, and reported a molecular detection rate of 34.6% compared with 25.0% for culture. Thirteen culture-negative infections were recognized using PCR ^[12].

Levy et al. demonstrated additional organism detection through a systematic molecular strategy applied to culture-negative osteoarticular specimens. Their findings supported a combined approach using broad-range bacterial PCR and pathogen-directed assays selected according to patient characteristics and suspected infection ^[13].

However, results from more recent studies indicate that molecular technologies are not uniformly superior.

Wei et al. compared culture, histopathology, and mNGS using periprosthetic tissue samples. Their findings demonstrated that culture achieved greater specificity than molecular sequencing, while histopathology showed lower sensitivity but high specificity under the study reference criteria ^[26].

This distinction is clinically important. Molecular positivity may improve organism identification, but some molecular detections may represent contamination or organisms unrelated to the active infectious process.

Consequently, histopathology remains an important component of periprosthetic joint infection investigation, particularly when sterile cultures and ambiguous molecular findings must be interpreted against tissue inflammation and established clinical criteria.

3.6. Spinal Infections

Spinal infections are frequently difficult to diagnose because symptoms and imaging abnormalities may overlap with malignant, degenerative, inflammatory, and infectious conditions.

Deep-tissue biopsy provides an opportunity to obtain specimens for microbiological culture, histopathology, and molecular sequencing.

Zhang et al. evaluated 114 suspected cases and reported a molecular sequencing sensitivity of 75.0% and specificity of 84.5% against a reference standard incorporating conventional diagnostic evidence ^[23].

Chen et al. reported higher molecular positivity than culture in patients undergoing tissue evaluation for suspected spinal infection ^[24].

In another cohort, Liu et al. observed mNGS positivity in 64 of 80 patients compared with culture positivity in 34 of 80 ^[27].

More recently, Liang et al. demonstrated that the integration of clinical variables and molecular information improved discrimination of spinal infection in their retrospective dataset. Although molecular sequencing showed strong sensitivity, the integrated model provided additional diagnostic value beyond individual tests ^[28].

These investigations support molecular sequencing as an adjunct for etiological diagnosis while reinforcing the need for histopathological examination to characterize lesions and exclude alternative diagnoses.

3.7. Invasive Fungal Infections

In invasive fungal diseases, integration of molecular techniques and histopathology has particular importance because conventional fungal cultures may remain negative even when tissue invasion is present.

Histopathology can establish fungal elements within affected tissues and reveal accompanying necrosis, vascular invasion, and inflammatory responses. Nevertheless, morphology may not reliably distinguish fungi at species level.

Buitrago et al. reported a sensitivity of 89% for fungal DNA amplification compared with 56% for conventional tissue culture in a cohort with proven invasive fungal disease ^[14].

Trubiano et al. demonstrated that panfungal PCR contributed to species-level identification in culture-negative specimens with positive histopathological evidence ^[16].

Lysen et al. found that fungal PCR performed on FFPE tissue had incomplete sensitivity, illustrating the limitations of molecular detection after fixation and processing ^[19].

Sparks et al. demonstrated that histopathology could also guide the selection of samples for molecular testing. Clinically significant molecular results occurred in 16 of 45 specimens with fungal elements seen histologically, compared with six of 203 specimens without visualized fungal elements ^[22].

These findings support a pathology-informed diagnostic approach rather than indiscriminate molecular testing of every tissue specimen.

3.8. Comparative Diagnostic Findings

Table 6. Selected Quantitative Findings From Primary Studies

Study	Clinical setting	Molecular result	Comparator or added finding
Greub et al. ^[10]	Endocarditis	PCR sensitivity 61%	Histology 63%; valve culture 13%
Rampini et al. ^[11]	Culture-negative	PCR sensitivity 42.9%	Reported specificity 100%

Study	Clinical setting	Molecular result	Comparator or added finding
	bacterial infections		
Grif et al. [12]	Sterile sites/orthopedic specimens	PCR positivity 34.6%	Culture positivity 25.0%
Rodríguez-García et al. [17]	Surgical endocarditis	Valve PCR positivity 71.8%	Valve culture positivity 39.6%; 36% molecular identification among BCNE cases
Johansson et al. [20]	Valve-associated endocarditis	15/20 BCNE episodes had diagnostic benefit	Benefit defined as additional or clarifying organism identification
Hong et al. [21]	BCNE	11/18 cases molecular-positive	Remaining cases required other methods or remained unresolved
Wei et al. [26]	Knee PJI	mNGS sensitivity 63.2%	Culture 68.4%; histology 52.6%
Liu et al. [27]	Suspected infection	spinal mNGS positivity 80.0%	Culture positivity 42.5%
Liang et al. [28]	Suspected infection	spinal mNGS yield 66.5%	Culture yield 27.41%; integrated model AUC 0.916

3.9. Diagnostic Discordance

Diagnostic discordance was observed across the reviewed settings and is a central consideration in integrated testing.

The most important combinations are summarized below.

Table 7. Interpretation of Discordant Molecular and Histopathological Findings

Molecular result	Histopathological result	Potential interpretation	Recommended consideration
Positive	Compatible infection	with Concordant evidence supporting infection and possible etiological assignment	Correlate organism with site, morphology, clinical findings and controls
Negative	Compatible infection	with False-negative molecular result, inadequate sampling, pathogen outside assay scope, or noninfectious inflammatory mimic	Consider repeat or targeted testing and review tissue adequacy
Positive	No evidence of infection	of Contamination, colonization, residual microbial DNA, early/focal infection or sampling mismatch	Evaluate organism plausibility, test controls and independent confirmation
Negative	No evidence of infection	of Reduced probability of infection when pretest probability is low	Reassess clinical probability and adequacy of sampling
Multiple organisms detected	Suppurative or mixed inflammatory lesion	True polymicrobial infection or mixture containing contaminants	Assess organism abundance, reproducibility and clinical compatibility
Fungal detected	DNA Fungal elements with tissue invasion	Strongly supportive of invasive fungal disease when findings are concordant	Confirm identity and interpret within established disease definitions

Importantly, discordant findings do not automatically establish analytical failure. Different methods examine different dimensions of infection, and each may detect evidence that is not captured by the other.

3.10. Methodological Quality and Risk-of-Bias Considerations

The available evidence is affected by several recurrent methodological challenges.

Table 8. QUADAS-2-Oriented Appraisal Framework and Preliminary Concerns

Domain	Principal concerns identified in the evidence	Formal judgment
Patient selection	Surgical-only populations, selective molecular testing,	Pending independent full-text

Domain	Principal concerns identified in the evidence	Formal judgment
	prior treatment and exclusions after recruitment	assessment
Index test	Variable positivity thresholds, contamination-control procedures, sequencing methods and interpretation rules	Pending
Reference standard	Heterogeneous clinical criteria and incorporation of histology or microbiological findings into disease classification	Pending
Flow and timing	Differences in specimen allocation, availability of paired tests, and intervals between therapy and sampling	Pending
Applicability	Variation across valve tissue, joint tissue, spinal biopsy, FFPE material and different organisms	Pending

Three issues are particularly important.

Selection bias: Patients referred for molecular sequencing may have unusually complex infections or previous nondiagnostic investigations. Their results may not represent routine unselected clinical populations.

Incorporation bias: When histopathological or molecular results contribute to the reference diagnosis, the apparent accuracy of that same test may be distorted.

Incomplete paired testing: Studies in which only selected patients receive both molecular testing and histopathology may not permit unbiased comparison of their diagnostic accuracy.

These issues limit direct comparisons and support cautious interpretation of reported sensitivity, specificity, and incremental diagnostic yield.

4. DISCUSSION

4.1. Principal Findings

The reviewed evidence supports a complementary relationship between molecular microbiology and histopathology in culture-negative infections.

Molecular assays are particularly valuable for recovering etiological information when viable organisms cannot be isolated. Histopathology contributes evidence of tissue injury and infection, provides information about inflammatory response and invasive behavior, and can reveal alternative diagnoses.

The advantage of combining these investigations is most apparent when neither modality independently resolves the clinical question.

However, the evidence does not demonstrate that all patients with culture-negative infection benefit equally from advanced molecular testing.

The diagnostic performance of individual assays varies considerably between clinical settings, emphasizing the importance of infection-specific diagnostic pathways.

4.2. Clinical Utility of Molecular Testing

Broad-range 16S PCR provides bacterial identification without requiring prior knowledge of the suspected species. It may be particularly useful after antimicrobial exposure or when fastidious organisms are suspected.

Molecular sequencing can also reveal organisms not targeted by routine clinical testing.

Nevertheless, clinical utility depends on more than positive pathogen identification. A molecular result becomes clinically meaningful when it clarifies diagnosis, changes therapeutic decisions, supports antimicrobial narrowing, or appropriately guides further investigation.

Previous evidence indicates that additional molecular detections do not always translate into changes in treatment. Results arriving after major clinical decisions have already been made may have limited practical value.

Consequently, diagnostic stewardship requires consideration of pretest probability, specimen quality, expected turnaround time, and the availability of alternative established investigations.

4.3. Interpretation of Molecular Positivity

A molecular-positive result establishes detection of microbial nucleic acid, not necessarily active infection.

Several explanations are possible:

- Active infection caused by the detected organism.
- Residual microbial DNA following successful antimicrobial treatment.
- Colonization of the sampled anatomical region.
- Contamination introduced during specimen collection, processing, amplification, or analysis.
- Detection of an organism unrelated to the pathological process.

The likelihood of true causation increases when molecular findings are consistent with anatomical location, characteristic histopathology, clinical manifestations, imaging abnormalities, and independent microbiological or serological evidence.

For low-biomass clinical specimens, negative extraction controls, sequencing controls, contamination surveillance, and validated reporting thresholds are particularly important.

4.4. Histopathology as a Diagnostic Anchor

Histopathology provides anatomical and pathological context that may be absent from nucleic acid detection.

Acute suppurative inflammation, tissue necrosis, abscess formation, granulomatous reactions, and organism-associated vascular invasion can support particular infectious mechanisms.

Special stains can further guide molecular target selection.

For example, fungal elements visualized with Gomori methenamine silver or periodic acid-Schiff staining may justify further panfungal or organism-directed molecular investigation.

However, histopathology also requires appropriate interpretation. Granulomatous inflammation, for example, is not specific for tuberculosis, and neutrophilic inflammation can occur in certain noninfectious settings.

An integrated approach therefore requires both pathological context and microbiological evidence rather than assuming that one modality independently provides an infallible reference standard.

4.5. Specimen Collection and Processing

Specimen quality is a major determinant of diagnostic performance.

Whenever feasible, deep-tissue material should be obtained before antimicrobial therapy, provided this does not delay urgently required treatment.

Separate sterile specimens should be allocated for conventional microbiology, molecular investigations, and histopathology.

Fresh unfixed specimens are generally preferred for culture and many molecular assays, whereas formalin-fixed material is routinely used for histological examination.

Although validated PCR methods can be applied to FFPE specimens, formalin-associated nucleic acid fragmentation, cross-linking, low pathogen burden, and processing-related contamination may reduce sensitivity.

Where sufficient tissue is available, retaining appropriate fresh material for molecular testing may improve diagnostic flexibility.

4.6. Advances in Sequencing Technology

Metagenomic sequencing permits investigation of bacterial, fungal, viral, and other microbial nucleic acids depending on the assay, sample preparation, bioinformatic workflow, and reference database.

Long-read sequencing approaches may improve taxonomic resolution and mixed-organism identification.

However, clinical implementation requires validated laboratory protocols and robust quality-control systems.

Butler et al. highlighted the importance of standardized reference materials and analytical validation for long-read 16S sequencing^[29].

New sequencing platforms should therefore undergo clinical evaluation before routine adoption, particularly when applied to low-biomass specimens or rare microbial targets.

4.7. Integration With Clinical Guidelines

The 2023 Duke-ISCVID criteria acknowledge the evolving role of molecular diagnostic techniques in the investigation of infective endocarditis^[30].

The combination of clinical assessment, imaging, conventional microbiology, histopathology, and molecular testing is consistent with contemporary multidisciplinary diagnostic practice.

However, the exact diagnostic pathway should be determined by the suspected infection, available specimen, local epidemiology, patient characteristics, and assay validation.

A uniform molecular-first approach is not appropriate for every suspected infection.

4.8. Proposed Integrated Diagnostic Algorithm

Step 1. Establish Clinical Probability

Evaluate symptoms, clinical course, imaging, inflammatory markers, antimicrobial history, immunosuppression, and potential exposure to unusual pathogens.

Step 2. Optimize Conventional Sampling

Collect appropriate blood cultures or deep-tissue specimens, consider pathogen-specific culture requirements, and ensure sufficient sampling.

Step 3. Perform Histopathological Assessment

Examine tissue architecture, inflammatory changes, necrosis, granulomas, organisms, and alternative noninfectious diagnoses.

Select special stains according to the clinical and morphological differential diagnosis.

Step 4. Apply Targeted Molecular Testing

Use pathogen-specific assays when clinical, epidemiological, or morphological findings suggest a particular organism.

Broad-range 16S PCR can be considered for unresolved bacterial infection; fungal ITS/18S-based approaches are relevant when fungal disease is suspected.

Step 5. Consider Broader Sequencing

Reserve mNGS or expanded molecular sequencing for unresolved, atypical, complex, or potentially polymicrobial infections where results are likely to affect management.

Step 6. Perform Integrated Interpretation

Assess whether detected organisms agree with clinical and histopathological findings.

Review unexpected organisms, contamination controls, assay limitations, and possible residual nucleic acids.

Step 7. Report and Reassess

Issue an integrated diagnostic interpretation indicating the strength and limitations of evidence.

Adjust antimicrobial therapy only after appropriate clinical review, considering whether pathogen detection establishes infection and whether susceptibility information is available.

4.9. Diagnostic and Implementation Limitations

The major limitations of molecular microbiology can be organized into analytical, clinical, and operational categories.

Table 9. Limitations of Molecular Diagnostic Integration

Category	Limitation	Clinical implication
Preanalytical	Inadequate or nonrepresentative biopsy	False-negative molecular and histopathological findings
Preanalytical	Prior antimicrobial administration	Reduced culture sensitivity and altered organism burden
Analytical	Low pathogen nucleic acid concentration	Molecular false negatives
Analytical	Human DNA background	Reduced microbial sequence recovery in mNGS
Analytical	Environmental or reagent contamination	False-positive microbial attribution
Analytical	DNA degradation in FFPE tissue	Incomplete detection and reduced assay sensitivity
Interpretive	Residual DNA from nonviable organisms	Positive molecular results without active infection
Interpretive	Limited species resolution	Uncertain organism assignment
Interpretive	Absence of routine phenotypic susceptibility	Incomplete guidance for antimicrobial selection
Operational	High cost and infrastructure requirements	Unequal access to advanced molecular diagnostics
Operational	Delayed turnaround time	Results may arrive after clinical decisions
Evidence-related	Heterogeneous reference standards	Difficulty comparing studies or producing pooled estimates

4.10. Implications for Clinical Practice

The evidence supports selective, clinically guided molecular testing rather than universal use of expensive sequencing platforms.

Integration appears especially useful when deep-tissue histopathology supports an infectious process but conventional microbiology remains negative.

Molecular testing may be particularly valuable in surgically treated endocarditis, selected osteoarticular and spinal infections, and histologically demonstrated invasive fungal disease.

At the same time, a negative molecular result should not independently exclude infection, and a positive molecular result should not automatically justify antimicrobial treatment.

The best interpretation arises from multidisciplinary collaboration among clinical microbiologists, histopathologists, infectious disease specialists, radiologists, and relevant surgical teams.

4.11. Research Priorities

Future investigations should prioritize prospective multicenter diagnostic accuracy studies with consecutive recruitment, prespecified reference standards, and blinded interpretation.

Studies should also document specimen allocation, antimicrobial exposure, microbial burden, assay thresholds, contamination-control performance, and the time between sampling and testing.

Clinical outcome measures should extend beyond diagnostic yield to include antimicrobial modification, diagnostic delay, unnecessary therapy avoidance, hospital stay, patient outcomes, and cost-effectiveness.

Development of standardized integrated reporting systems could improve the translation of molecular findings into clinically meaningful decisions.

4.12. Limitations of the Present Review

The available evidence is heterogeneous in populations, infection syndromes, sample types, laboratory techniques, and reference standards.

Many original studies were conducted in selected referral populations or surgical cohorts, limiting generalizability.

5. CONCLUSION

The integration of molecular microbiology and histopathology represents an important approach to diagnosing selected culture-negative and diagnostically unresolved infections. Conventional culture, molecular assays, and tissue examination provide distinct but complementary information.

Histopathology contributes pathological evidence of infection, identifies tissue damage, and helps distinguish infectious from noninfectious processes. Molecular techniques provide additional opportunities for microbial identification, particularly when previous antimicrobial treatment, fastidious organisms, or low microbial burden limit conventional recovery.

However, molecular detection does not invariably establish active infection, and advanced sequencing does not consistently outperform established diagnostic techniques. Contamination, residual microbial DNA, variable assay sensitivity, specimen processing, and limitations in antimicrobial susceptibility determination remain important concerns.

An optimized diagnostic strategy should combine appropriate clinical assessment, high-quality specimen collection, conventional microbiology, histopathology, targeted molecular assays, and selective broader sequencing. Future prospective investigations should determine the extent to which these integrated approaches improve patient management and clinically meaningful outcomes.

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