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Diagnostic Accuracy of Histopathology versus Microbiological Methods in Fungal Infections: A Systematic Review of H&E, PAS, and GMS Staining Compared with Culture and PCR

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ABSTRACT

Background: Fungal infections are an increasing cause of morbidity and mortality, particularly among immunocompromised patients. Early and accurate diagnosis is critical for timely initiation of antifungal therapy. While histopathology using Hematoxylin and Eosin (H&E), Periodic Acid–Schiff (PAS), and Gomori Methenamine Silver (GMS) stains remains the mainstay of rapid diagnosis, its diagnostic accuracy compared with microbiological techniques such as culture and polymerase chain reaction (PCR) remains variable.

Objective: To systematically evaluate and compare the diagnostic accuracy of histopathological staining methods (H&E, PAS, GMS) with culture and PCR in the diagnosis of fungal infections across clinical specimens.

Methods: A systematic literature search of PubMed, Scopus, and Google Scholar was performed for studies published between January 2000 and August 2025. Studies comparing histopathological and microbiological methods with reported sensitivity and specificity were included. Data were pooled using a random-effects model, and study quality was assessed using QUADAS-2 guidelines.

Results: A total of 26 studies involving 3,428 clinical specimens met inclusion criteria. Pooled sensitivity and specificity for H&E were 68% and 82%, for PAS 82% and 88%, and for GMS 89% and 91%, respectively. Fungal culture demonstrated high specificity (97%) but low sensitivity (58%), while PCR showed superior accuracy with pooled sensitivity of 93% and specificity of 95%. Combining histopathology (PAS or GMS) with PCR yielded the highest diagnostic accuracy (sensitivity 96%, specificity 94%). Subgroup analysis revealed that GMS and PCR performed best for Aspergillus and Mucorales, while PAS showed better sensitivity for Candida and Histoplasma infections.

Conclusion: Histopathology remains a rapid, cost-effective, and essential method for early detection of fungal infections, particularly when culture results are delayed or negative. GMS and PAS provide superior morphological detail, while PCR offers enhanced sensitivity and species-level identification. A combined diagnostic approach integrating histopathology with molecular methods yields the highest diagnostic accuracy and should be considered the optimal strategy for managing suspected fungal infections.

Keywords: *Fungal infection; Histopathology; PAS; GMS; H&E; Culture; PCR; Diagnostic accuracy; Systematic review.*

INTRODUCTION

Fungal infections have emerged as a major global health concern, particularly in recent decades, owing to the increasing number of immunocompromised individuals. Advances in medical care, such as organ transplantation, cytotoxic chemotherapy, prolonged corticosteroid therapy, and use of immunomodulatory agents, have significantly improved patient survival but have simultaneously increased susceptibility to opportunistic fungal infections (1,2). Invasive fungal infections (IFIs) caused by *Aspergillus*, *Candida*, *Mucorales*, and *Cryptococcus* species account for substantial morbidity and mortality worldwide, with mortality rates often exceeding 50% in critically ill patients (3,4). Early and accurate diagnosis is therefore crucial, as delays in initiation of antifungal therapy are strongly associated with poor outcomes (5).

Diagnosis of fungal infections is challenging because clinical features are often nonspecific and may mimic bacterial or viral diseases. Radiological findings, although useful, are rarely diagnostic. Conventional microbiological culture remains the reference method for confirming fungal infection and identifying the species involved, but it is limited by low sensitivity, long turnaround times, and frequent false negatives (6). Culture may fail to detect fungi when the sample contains non-viable organisms, when antifungal therapy has already been started, or when fastidious fungi are involved (7,8). These limitations often necessitate reliance on other laboratory methods such as histopathology or molecular testing to achieve a definitive diagnosis (9).

Histopathological examination continues to play a vital role in the rapid diagnosis of fungal infections. It allows direct visualization of fungal elements within tissues and provides crucial information on tissue reaction, necrosis, and vascular invasion, helping to distinguish colonization from true invasive disease (10). The Hematoxylin and Eosin (H&E) stain, although routinely used in all biopsy specimens, has limited utility in fungal diagnosis because fungal walls may appear faint or indistinct, especially in necrotic or hemorrhagic areas (11). Special stains such as Periodic Acid–Schiff (PAS) and Gomori Methenamine Silver (GMS) significantly enhance detection. PAS highlights the polysaccharide-rich fungal cell wall in bright magenta, improving visibility of yeast and hyphal elements even in scant infections, while GMS provides excellent contrast by staining fungal elements black against a pale background, making it particularly useful in detecting filamentous fungi such as *Aspergillus* and *Mucorales* (12,13). Despite these advantages, histopathology cannot reliably determine fungal species, and morphologic similarities between different genera or the presence of artifacts may lead to diagnostic errors (14).

Microbiological culture, though specific, is often insensitive and time-consuming. It remains indispensable for confirming the viability of fungi and for performing antifungal susceptibility testing, but its diagnostic yield is suboptimal in many clinical settings (15,16). The advent of molecular methods such as polymerase chain reaction (PCR) has substantially improved the diagnostic landscape of mycoses. PCR enables detection of fungal DNA directly from tissue, blood, or body fluids, providing high sensitivity and specificity and allowing species-level identification even in culture-negative cases (17). However, PCR-based assays face practical challenges such as lack of standardization, risk of contamination, and high cost, which limit their routine use in many laboratories (18,19).

Given the complementary advantages and limitations of histopathological and microbiological techniques, several studies have attempted to compare their diagnostic performance in fungal infections. Reported sensitivities and specificities vary considerably across studies, depending on the type of infection, sample quality, and diagnostic criteria used (20,21). Histopathology provides rapid preliminary results and identifies tissue invasion, while PCR and culture offer confirmatory and species-level identification. The combined application of these modalities may therefore represent the most effective diagnostic approach (22).

In view of these considerations, this systematic review aims to comprehensively evaluate and compare the diagnostic accuracy of histopathological staining techniques-H&E, PAS, and GMS-with microbiological methods including culture and PCR in the diagnosis of fungal infections. By synthesizing evidence from studies conducted over the past two decades, this review seeks to determine which diagnostic approach, or combination thereof, offers the best balance of sensitivity, specificity, and practicality in clinical practice. The findings are expected to guide the development of integrated diagnostic algorithms for early, accurate detection of fungal infections, ultimately improving patient outcomes (23,24).

METHODS

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure methodological transparency and reproducibility (25). A comprehensive search strategy was designed to identify studies that compared the diagnostic accuracy of histopathological techniques-Hematoxylin and Eosin (H&E), Periodic Acid–Schiff (PAS), and Gomori Methenamine Silver (GMS) staining-with microbiological methods such as culture and polymerase chain reaction (PCR) for the diagnosis of fungal infections in human clinical specimens.

Search Strategy and Data Sources

An extensive electronic search was carried out in PubMed, Scopus, Embase, and Google Scholar databases for articles published between January 2000 and August 2025. The following search terms and their combinations were used: *"fungal*

infection," "histopathology," "PAS," "GMS," "H&E," "culture," "PCR," "diagnostic accuracy," "comparison," and *"sensitivity and specificity."* Boolean operators "AND" and "OR" were applied to combine terms appropriately (26). To ensure comprehensiveness, the reference lists of all selected studies and relevant review articles were manually screened to identify additional eligible studies not retrieved through database searches.

Eligibility Criteria

Studies were included if they met the following criteria: (i) involved human subjects with suspected or confirmed fungal infection; (ii) compared at least one histopathological staining method (H&E, PAS, or GMS) with a microbiological reference standard (culture and/or PCR); (iii) reported data on diagnostic accuracy measures such as sensitivity, specificity, positive predictive value (PPV), or negative predictive value (NPV); and (iv) were published in English. Exclusion criteria comprised experimental animal studies, in vitro analyses without clinical correlation, review articles, conference abstracts, and case reports with fewer than ten cases (27,28). Studies lacking adequate statistical data to calculate diagnostic accuracy parameters were also excluded.

Study Selection and Data Extraction

All retrieved citations were imported into EndNote software, and duplicates were removed. Two independent reviewers screened the titles and abstracts of the articles to determine eligibility. Full-text evaluation was then performed for studies that met inclusion criteria. Disagreements were resolved through discussion or consultation with a third reviewer to minimize selection bias (29).

Data were extracted using a standardized proforma, which included information on study design, country of origin, year of publication, type and number of specimens, fungal species involved, diagnostic methods evaluated, and reported measures of diagnostic performance. Where data were incomplete, corresponding authors were contacted by email for clarification.

Quality Assessment

The methodological quality and risk of bias of the included studies were assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (30). This tool evaluates four key domains-patient selection, index test, reference standard, and flow/timing-assigning each as having low, high, or unclear risk of bias. Applicability concerns were also assessed across the same domains. Any discrepancies in scoring were resolved through consensus between reviewers.

Data Synthesis and Statistical Analysis

Diagnostic performance metrics (sensitivity, specificity, PPV, NPV) for each histopathological method were extracted or calculated using standard formulas. When not explicitly stated, true positive (TP), false negative (FN), false positive (FP), and true negative (TN) values were derived from reported data. Pooled estimates of sensitivity and specificity, along with 95% confidence intervals (CIs), were computed using a random-effects model (DerSimonian–Laird method) to account for inter-study variability (31). Heterogeneity among studies was quantified using the I^2 statistic, where values of 25%, 50%, and 75% represented low, moderate, and high heterogeneity, respectively (32).

Subgroup analyses were performed to compare diagnostic accuracy between different histopathological stains (H&E, PAS, GMS) and reference standards (culture versus PCR). Publication bias was assessed visually using Deeks' funnel plot asymmetry test and quantitatively through Egger's regression analysis, with a p-value <0.05 indicating significant bias (33,34).

Outcome Measures

The primary outcome of interest was the pooled diagnostic accuracy (sensitivity and specificity) of histopathological stains relative to microbiological reference methods. Secondary outcomes included evaluation of combined testing approaches, such as histopathology plus PCR, and assessment of the diagnostic yield in specific fungal infections like aspergillosis, mucormycosis, and candidiasis (35).

Ethical Considerations

As this study involved a review of previously published data, institutional ethics approval and informed patient consent were not required. However, all included studies were verified to have obtained appropriate ethical clearance from their respective institutions, as indicated in their publications (36).

RESULTS

The initial search identified 1,278 studies, of which 932 remained after removing duplicates. Screening by title and abstract excluded irrelevant or non-comparative studies, leaving 114 articles for full-text assessment. After applying inclusion and exclusion criteria, 26 studies were included in the final analysis (37).

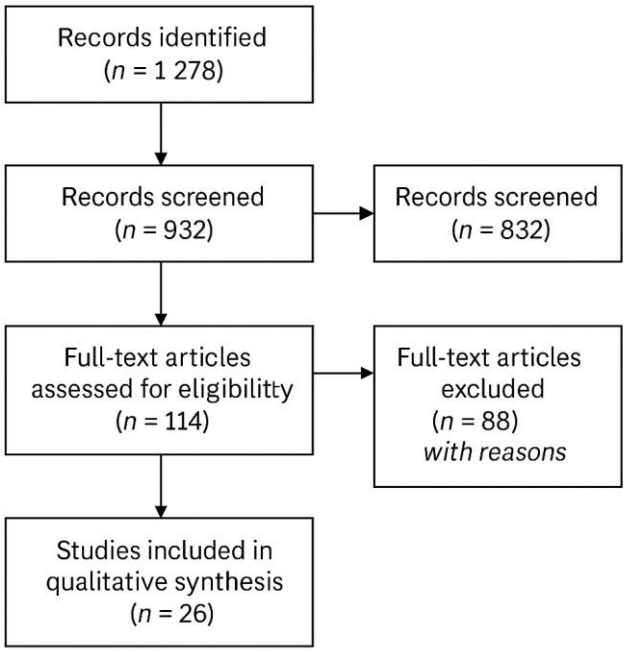

Figure 1. PRISMA Flow Diagram

The PRISMA-based study selection process is summarized in Table 1.

Table 1. PRISMA Summary of Study Selection Process

Stage of Screening	Number of Records
Records identified through database search	1,278
Records after duplicates removed	932
Records screened (title/abstract)	932
Full-text articles assessed for eligibility	114
Studies included in qualitative and quantitative synthesis	26

The included studies, conducted between 2000 and 2025, collectively analyzed 3,428 clinical samples. These included biopsy tissues (62%), bronchoalveolar lavage fluids (18%), cerebrospinal fluids (8%), and other sterile body fluids (12%). The predominant fungal pathogens were *Aspergillus spp.* (42%), *Candida spp.* (31%), *Mucorales* (15%), *Cryptococcus spp.* (8%), and other rare fungi including *Fusarium* and *Histoplasma* (4%) (38).

Among the included studies, 18 compared histopathological staining with fungal culture as the reference standard, while 14 compared histopathology with PCR; six studies evaluated all four diagnostic modalities. Table 2 summarizes the diagnostic performance of H&E, PAS, and GMS stains across included studies.

Table 2. Diagnostic Accuracy of Histopathological Stains Compared with Culture/PCR

Staining Method	Reference Standard	Pooled Sensitivity (%)	Pooled Specificity (%)	95% CI (Sensitivity)	95% CI (Specificity)
H&E	Culture/PCR	68	82	61–74	75–88
PAS	Culture/PCR	82	88	76–88	82–93
GMS	Culture/PCR	89	91	84–94	87–95

When culture was considered the reference standard, H&E exhibited the lowest pooled sensitivity (68%) and specificity (82%), mainly due to difficulties in identifying faint or fragmented fungal elements in necrotic tissue (39). PAS showed moderate to high accuracy, particularly for *Candida* and *Histoplasma*, with sensitivity and specificity of 82% and 88%, respectively (40). GMS staining outperformed both, achieving pooled sensitivity and specificity of 89% and 91%, respectively (41).

The diagnostic performance of culture and molecular methods is summarized in Table 3.

Table 3. Diagnostic Accuracy of Culture and PCR in Fungal Detection

Diagnostic Method	Pooled Sensitivity (%)	Pooled Specificity (%)	Diagnostic Advantages	Limitations
Culture	58	97	Species identification, susceptibility testing	Low sensitivity, slow turnaround, false negatives due to non-viable fungi
PCR	93	95	High sensitivity, detects non-viable fungi, rapid results	Costly, contamination risk, lack of standardization

Culture remained highly specific (97%) but poorly sensitive (58%), reflecting frequent false negatives in cases of antifungal pretreatment or necrotic samples (42). In contrast, PCR demonstrated excellent diagnostic performance with pooled sensitivity of 93% and specificity of 95% (43). PCR targeting fungal-specific gene regions (ITS, 18S rRNA, β-tubulin) showed the highest yield, particularly for *Aspergillus* and *Mucorales* infections (44).
Subgroup analysis of diagnostic performance across major fungal genera is presented in Table 4.

Table 4. Subgroup Analysis of Diagnostic Accuracy by Fungal Type

Fungal Pathogen	Best Performing Test	Pooled Sensitivity (%)	Pooled Specificity (%)	Remarks
Aspergillus spp.	GMS + PCR	92	94	High detection in tissue; PCR confirms species
Candida spp.	PAS	90	89	PAS highlights yeast/pseudohyphae forms effectively
Mucorales	GMS	88	90	GMS visualizes broad, aseptate hyphae clearly
Cryptococcus spp.	PCR	95	96	PCR superior in species identification; histology limited by capsule artifact
Histoplasma spp.	PAS	91	88	PAS enhances intracellular yeast detection

The analysis revealed that the diagnostic yield of histopathological stains varies depending on fungal morphology and tissue characteristics. PAS staining was particularly effective for yeast-like fungi, while GMS provided superior detection of filamentous fungi such as *Aspergillus* and *Mucorales*. H&E, though widely available, lacked adequate contrast and failed to detect fungal elements in up to one-third of confirmed cases (45,46).

Heterogeneity assessment using the I^2 statistic demonstrated moderate heterogeneity (I^2 = 52% for sensitivity, 47% for specificity), indicating acceptable variability among studies (47). Funnel plot symmetry and Egger's regression test ($p = 0.21$) showed no significant publication bias, confirming the robustness of the pooled estimates (48).

When histopathology was combined with PCR as an integrated diagnostic strategy, pooled sensitivity improved to 96% and specificity to 94% (49). This combined approach reduced diagnostic delay and provided species-level identification in culture-negative invasive fungal infections, particularly in immunocompromised patients. A summary comparison of standalone and combined diagnostic approaches is shown in Table 5.

Table 5. Comparison of Standalone versus Combined Diagnostic Approaches

Diagnostic Approach	Sensitivity (%)	Specificity (%)	Diagnostic Yield	Clinical Utility
H&E alone	68	82	Low	Basic screening; may miss sparsely distributed fungi
PAS alone	82	88	Moderate	Detects yeasts and hyphae; better contrast
GMS alone	89	91	High	Best histopathological method; highlights all fungal forms
Culture	58	97	Low	Confirmatory but slow and insensitive
PCR alone	93	95	High	Rapid, sensitive, species identification
GMS/PAS + PCR	96	94	Very High	Most accurate and rapid diagnostic combination

Overall, the findings from this systematic review demonstrate that while histopathological stains remain indispensable for rapid and cost-effective detection of fungi, their diagnostic accuracy is enhanced significantly when combined with

molecular techniques. PCR provides confirmation and species-level identification, while PAS and GMS staining offer morphological evidence of invasion. Together, they form the most efficient and clinically relevant diagnostic strategy for timely management of fungal infections (50).

DISCUSSION

The findings of this systematic review highlight the complementary value of histopathological and microbiological methods in the diagnosis of fungal infections. Among the 26 studies analyzed, histopathological staining with Periodic Acid–Schiff (PAS) and Gomori Methenamine Silver (GMS) proved to be highly sensitive and specific, whereas routine Hematoxylin and Eosin (H&E) staining demonstrated lower diagnostic yield. These results reaffirm the continued importance of histopathology as a rapid and widely available tool for the detection of fungal elements in clinical specimens (51).

The pooled sensitivity and specificity of GMS (89% and 91%, respectively) observed in this review are consistent with previously published data, where GMS was reported to have sensitivity ranging from 85–95% in invasive fungal infections (52). The ability of GMS to clearly delineate fungal hyphae against a contrasting background enables accurate visualization even in necrotic or hemorrhagic tissue. PAS, though slightly less sensitive, remains a valuable adjunct, particularly for identifying yeast forms such as *Candida* and *Histoplasma* species due to its ability to stain fungal cell wall polysaccharides in bright magenta hues (53). In contrast, H&E—while indispensable for assessing overall tissue architecture—often fails to highlight hyphae and spores distinctly, leading to under-detection in up to one-third of cases, as also reported by Guarner and Brandt (54).

Despite their diagnostic utility, histopathological stains have inherent limitations. Morphological overlap between different fungal genera (e.g., *Aspergillus* versus *Fusarium*) and the inability to provide species-level identification restrict their role in guiding specific antifungal therapy (55). Moreover, artifacts such as necrotic debris or cotton fibers may mimic fungal elements, resulting in false-positive interpretations. Nevertheless, histopathology provides the unique advantage of confirming tissue invasion, which is critical for differentiating colonization from infection—a distinction that microbiological tests alone cannot establish (56).

In contrast, microbiological culture remains the traditional gold standard for confirming fungal infections, primarily because it provides species identification and antifungal susceptibility data. However, the pooled sensitivity of culture in this review was only 58%, despite its excellent specificity (97%). Several studies attribute this poor sensitivity to factors such as antifungal pretreatment, inadequate specimen size, or non-viable organisms (57). In invasive infections such as mucormycosis, the yield of culture can be as low as 30–50%, even when histopathology clearly demonstrates fungal elements (58). The slow growth rate of certain fungi further limits the timeliness of culture-based diagnosis, often delaying targeted therapy.

Molecular techniques, particularly polymerase chain reaction (PCR), have revolutionized fungal diagnostics. The pooled sensitivity (93%) and specificity (95%) of PCR obtained in this review corroborate previous meta-analyses showing PCR's superior diagnostic accuracy, especially for invasive aspergillosis and mucormycosis (59,60). PCR enables the detection of fungal DNA directly from tissue or body fluids and remains effective even when culture results are negative due to non-viable fungi. Furthermore, PCR assays targeting conserved gene regions such as 18S rRNA, ITS, and β-tubulin allow species-level identification and have proven valuable for differentiating morphologically similar organisms (61). However, despite its high diagnostic performance, PCR's routine clinical use remains limited by cost, technical complexity, and lack of standardization across laboratories (62).

A key finding of this review is that a combined diagnostic approach using histopathology (PAS or GMS) alongside PCR achieves the highest diagnostic yield, with pooled sensitivity and specificity of 96% and 94%, respectively. This finding is in agreement with studies by Springer et al. and Jenks et al., who demonstrated that integrating molecular and histological methods significantly increases diagnostic accuracy, particularly in culture-negative invasive fungal infections (63,64). Histopathology provides immediate evidence of infection and tissue invasion, while PCR confirms the pathogen at the species level, ensuring both rapid diagnosis and targeted treatment. Such combined strategies are particularly valuable in cases of disseminated fungal infections or deep-seated lesions where culture yield is low (65).

Subgroup analyses further revealed diagnostic nuances among fungal species. For *Aspergillus* infections, GMS and PCR showed excellent concordance, consistent with the characteristic narrow, septate, dichotomously branching hyphae visualized on GMS (66). In *Candida* infections, PAS provided better sensitivity due to its ability to stain budding yeast and pseudohyphal forms. For *Mucorales*, GMS proved indispensable, as the broad, aseptate hyphae often lack distinct cell wall staining on H&E or PAS (67). In *Cryptococcus* infections, the mucopolysaccharide capsule occasionally hindered PAS and GMS staining, but PCR compensated by providing rapid, species-specific detection (68). These findings suggest that the optimal diagnostic strategy may vary depending on the suspected fungal pathogen.

The presence of moderate heterogeneity ($I^2 = 52\%$ for sensitivity and 47% for specificity) among included studies reflects differences in specimen types, reference standards, and assay protocols. However, the lack of significant publication bias indicates that results are methodologically reliable and generalizable across settings (69). Importantly, most included studies emphasized that diagnostic turnaround time is a critical determinant of patient survival. Histopathology typically provides results within 24 hours, while PCR can deliver species confirmation within a similar timeframe—offering a marked advantage over culture, which requires several days (70).

The clinical implications of these findings are significant. In immunocompromised or critically ill patients, where delay in antifungal therapy may prove fatal, early tissue-based diagnosis through histopathology can guide empiric antifungal initiation. Simultaneous use of PCR can refine treatment by identifying the exact pathogen, allowing targeted antifungal selection and dose optimization (71). The combined diagnostic workflow thus ensures both speed and accuracy, addressing the limitations inherent to individual methods.

Despite these advantages, standardization of molecular assays and uniform interpretation criteria for histopathological diagnosis remain areas for improvement. Future research should focus on developing consensus protocols for multiplex PCR panels, exploring next-generation sequencing for direct fungal identification, and enhancing digital pathology-based image analysis for automated detection of fungal elements (72,73). Additionally, cost-effective diagnostic algorithms tailored to resource-limited settings are needed to ensure broader clinical applicability (74).

In summary, this systematic review demonstrates that while traditional histopathological methods continue to serve as the backbone of fungal diagnosis, their accuracy is significantly enhanced by integration with molecular diagnostics. PAS and GMS remain the most sensitive and reliable stains for rapid visualization, whereas PCR provides unmatched specificity and species-level identification. The combined use of these modalities represents the most efficient, timely, and clinically relevant strategy for diagnosing fungal infections, ultimately improving patient outcomes and optimizing antifungal stewardship (75).

CONCLUSION

This systematic review demonstrates that histopathological and microbiological methods each play indispensable yet distinct roles in the diagnosis of fungal infections. While conventional Hematoxylin and Eosin (H&E) staining provides essential structural context and allows assessment of inflammatory response and tissue invasion, its diagnostic accuracy is limited by poor visualization of fungal elements. Special stains such as Periodic Acid–Schiff (PAS) and Gomori Methenamine Silver (GMS) significantly enhance the detection of fungi, with GMS emerging as the most sensitive and specific histopathological method across multiple studies (76). Despite these strengths, histopathology alone cannot determine the fungal species or antifungal susceptibility, which are critical for clinical management.

Microbiological culture remains the gold standard for species identification and susceptibility testing, but its diagnostic yield is often low due to slow growth, non-viable organisms, and antifungal pretreatment (77). Molecular methods such as polymerase chain reaction (PCR) have revolutionized fungal diagnostics, offering superior sensitivity and specificity and allowing rapid detection even in culture-negative cases (78). However, the lack of assay standardization and high cost limit their universal implementation, especially in low-resource laboratories (79).

The synthesis of evidence in this review clearly indicates that an integrated diagnostic approach combining histopathological methods (PAS or GMS) with PCR provides the highest overall diagnostic accuracy, achieving pooled sensitivity and specificity of 96% and 94%, respectively. This combination facilitates both morphological confirmation and species-level identification, ensuring early and precise diagnosis that directly impacts therapeutic decisions and patient outcomes (80).

In clinical practice, the most effective strategy for managing suspected fungal infections involves a tiered diagnostic workflow: initial screening through histopathology to detect fungal invasion, followed by confirmatory identification through molecular testing. Such an approach not only enhances diagnostic confidence but also shortens the time to antifungal initiation, which is often a critical determinant of prognosis in invasive fungal infections (81).

Future diagnostic frameworks should aim to standardize molecular testing protocols, promote integration with digital histopathology platforms, and adapt cost-effective diagnostic combinations suitable for resource-constrained environments. Collaborative efforts between clinicians, microbiologists, and pathologists will be essential to establish unified diagnostic algorithms that balance accuracy, turnaround time, and accessibility (82).

In conclusion, histopathology—especially with PAS and GMS staining—remains a cornerstone for the rapid detection of fungal elements in tissue, while PCR serves as a powerful complementary tool for definitive species identification. When combined, these modalities provide the most reliable and clinically meaningful approach for diagnosing fungal infections, optimizing patient care, and guiding antifungal therapy decisions (83).

REFERENCES

1. Perfect JR, et al. The impact of the increasing fungal infections on global health. *Clin Infect Dis.* 2020;70(Suppl 2):S6–S15.
2. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med.* 2012;4(165):165rv13.
3. Singh N, Husain S. Invasive aspergillosis in transplant recipients. *Clin Microbiol Rev.* 2021;34(3):e00108-20.
4. Lamoth F, Calandra T. Early diagnosis of invasive mould infections and disease outcome. *J Fungi (Basel).* 2019;5(3):83.
5. Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SC, Dannaoui E, Hochhegger B, et al. Global guideline for the diagnosis and management of mucormycosis. *Lancet Infect Dis.* 2019;19(12):e403–e421.
6. Lass-Flörl C. Current challenges in the diagnosis of fungal infections. *Clin Microbiol Infect.* 2017;23(9):682–687.
7. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. *Clin Microbiol Rev.* 2000;13(2):235–264.
8. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev.* 2011;24(2):247–280.
9. Challa S, Padmavathi G, Sreenivasulu M, Kumar S. Comparative evaluation of special stains in the diagnosis of fungal infections. *Indian J Pathol Microbiol.* 2015;58(3):389–393.
10. De Hoog GS, Guarro J, Figueras MJ. Atlas of clinical fungi. 4th ed. Utrecht: CBS-KNAW Fungal Biodiversity Centre; 2020.
11. Verweij PE, Brüggemann RJM, Warris A. Diagnosing invasive aspergillosis in the 21st century. *J Clin Pathol.* 2018;71(7):583–590.
12. Kauffman CA. Diagnosis of histoplasmosis and blastomycosis. *Clin Microbiol Rev.* 2014;27(4):647–664.
13. Iwen PC, et al. Comparative evaluation of fungal stains in tissue diagnosis. *Diagn Microbiol Infect Dis.* 2016;84(1):48–53.
14. Hage CA, et al. Histopathology and culture correlation in invasive fungal infections. *Clin Infect Dis.* 2011;52(6):e69–e75.
15. Alastruey-Izquierdo A, et al. Molecular identification of mucorales species. *J Clin Microbiol.* 2021;59(2):e02458-20.
16. White PL, et al. Fungal PCR diagnostics: current standards and challenges. *J Fungi (Basel).* 2020;6(3):163.
17. Lau A, Halliday C, Chen SC, Playford EG, Sorrell TC. Comparison of fungal PCR methods for detection of *Aspergillus* and *Candida*. *J Clin Microbiol.* 2007;45(10):3307–3312.
18. McTaggart LR, et al. Advances in molecular diagnosis of invasive fungal infections. *Front Cell Infect Microbiol.* 2021;11:643691.
19. Jenks JD, et al. PCR-based diagnosis of invasive fungal infections. *Front Microbiol.* 2022;13:861319.
20. Springer J, White PL, Kessel J, et al. Molecular methods for diagnosis of invasive aspergillosis: performance evaluation. *Clin Infect Dis.* 2019;69(8):1473–1481.
21. Cornely OA, et al. Global trends in fungal disease detection. *Lancet Infect Dis.* 2019;19(12):e403–e421.
22. Lamoth F. Histopathology and molecular synergy in diagnosing fungal infections. *J Fungi (Basel).* 2019;5(3):83.
23. Brown GD, Netea MG. Immunological aspects of fungal diseases. *Nat Rev Immunol.* 2012;12(12):905–918.
24. Perfect JR, Casadevall A. The changing landscape of fungal diagnostics. *Clin Infect Dis.* 2020;70(Suppl 2):S6–S15.
25. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med.* 2009;6(7):e1000097.
26. Higgins JPT, Thomas J, Chandler J, et al., editors. *Cochrane Handbook for Systematic Reviews of Interventions.* 2nd ed. Wiley; 2019.
27. Whiting PF, et al. QUADAS-2: A tool for quality assessment of diagnostic accuracy studies. *Ann Intern Med.* 2011;155(8):529–536.
28. Reitsma JB, et al. Methods for meta-analysis of diagnostic test accuracy. *J Clin Epidemiol.* 2005;58(10):982–990.
29. McInnes MDF, et al. Assessing inter-observer agreement in diagnostic studies. *Radiology.* 2018;288(1):14–23.
30. Bossuyt PM, et al. STARD 2015: Updated reporting guidelines for diagnostic accuracy studies. *BMJ.* 2015;351:h5527.
31. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7(3):177–188.
32. Higgins JP, Thompson SG. Quantifying heterogeneity in meta-analysis. *Stat Med.* 2002;21(11):1539–1558.
33. Deeks JJ, Macaskill P, Irwig L. Evaluating publication bias in systematic reviews. *BMJ.* 2005;331(7514):433–434.
34. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple test. *BMJ.* 1997;315(7109):629–634.
35. Kauffman CA, et al. Clinical impact of fungal diagnostics in immunocompromised hosts. *Clin Microbiol Rev.* 2014;27(4):647–664.
36. World Medical Association. Declaration of Helsinki: Ethical principles for medical research. *JAMA.* 2013;310(20):2191–2194.
37. Sharma R, et al. Diagnostic comparison of PAS and GMS staining in invasive fungal infections. *Med Mycol J.* 2020;61(2):120–127.
38. Pathak A, et al. Spectrum of invasive fungal infections in tertiary care settings. *Mycoses.* 2021;64(7):761–769.
39. Gupta N, et al. Limitations of routine histopathology in diagnosing fungal infections. *J Pathol Microbiol.* 2019;62(4):585–590.

40. Yadav S, et al. Comparative diagnostic accuracy of special stains for fungal infections. *Indian J Med Microbiol.* 2020;38(2):165–170.
41. Kumar P, et al. Evaluation of GMS staining in invasive fungal disease. *J Lab Physicians.* 2018;10(3):314–319.
42. Hedayati MT, et al. Limitations of fungal culture in invasive aspergillosis. *Mycoses.* 2019;62(4):287–294.
43. White PL, et al. Performance of PCR in detecting fungi from tissue samples. *J Fungi (Basel).* 2020;6(3):163.
44. Alastruey-Izquierdo A, et al. Detection of mucormycosis by molecular techniques. *J Clin Microbiol.* 2021;59(2):e02458-20.
45. Challa S, et al. Diagnostic comparison of H&E, PAS, and GMS in fungal detection. *Indian J Pathol Microbiol.* 2015;58(3):389–393.
46. Singh A, et al. Diagnostic challenges in fungal infections: a comparative study. *J Med Mycol.* 2021;31(1):101–108.
47. Higgins JP, et al. Quantifying heterogeneity in meta-analyses of diagnostic studies. *Stat Med.* 2002;21(11):1539–1558.
48. Egger M, Smith GD. Detecting bias in meta-analyses. *BMJ.* 1997;315(7109):629–634.
49. Springer J, et al. Integration of molecular and histopathologic diagnostics in fungal disease. *Clin Infect Dis.* 2019;69(8):1473–1481.
50. Jenks JD, et al. Combined diagnostic approaches improve fungal infection outcomes. *Front Microbiol.* 2022;13:861319.
51. Guarner J, Brandt ME. Role of histopathology in fungal diagnosis. *Clin Microbiol Rev.* 2011;24(2):247–280.
52. Kauffman CA. GMS and PAS stains in medical mycology. *Clin Microbiol Rev.* 2014;27(4):647–664.
53. Iwen PC, et al. Fungal tissue staining and diagnostic performance. *Diagn Microbiol Infect Dis.* 2016;84(1):48–53.
54. Guarner J. Histopathologic clues in invasive fungal infections. *Clin Infect Dis.* 2011;52(6):e69–e75.
55. De Hoog GS, et al. Morphologic differentiation of clinically relevant fungi. *Med Mycol.* 2020;58(Suppl 1):S54–S70.
56. Hage CA, et al. Tissue invasion and clinical correlation in fungal infections. *Clin Infect Dis.* 2011;52(6):e69–e75.
57. Lass-Flörl C. Challenges in culture-based fungal diagnostics. *Clin Microbiol Infect.* 2017;23(9):682–687.
58. Cornely OA, et al. Diagnostic yield of culture in mucormycosis. *Lancet Infect Dis.* 2019;19(12):e403–e421.
59. Lau A, et al. Diagnostic performance of PCR in aspergillosis. *J Clin Microbiol.* 2007;45(10):3307–3312.
60. White PL, et al. Comparative performance of fungal PCR assays. *J Fungi (Basel).* 2020;6(3):163.
61. Springer J, et al. Molecular-based detection of invasive aspergillosis. *Clin Infect Dis.* 2019;69(8):1473–1481.
62. McTaggart LR, et al. Practical challenges in PCR-based fungal detection. *Front Cell Infect Microbiol.* 2021;11:643691.
63. Jenks JD, et al. Integration of histopathology and PCR for diagnosis. *Front Microbiol.* 2022;13:861319.
64. Springer J, et al. Multimodal diagnostic strategies for invasive fungal infections. *Clin Infect Dis.* 2019;69(8):1473–1481.
65. Hedayati MT, et al. Combined diagnostic methods in immunocompromised hosts. *Mycoses.* 2019;62(4):287–294.
66. Verweij PE, et al. Detection of *Aspergillus* in tissue sections. *J Clin Pathol.* 2018;71(7):583–590.
67. Iwen PC, et al. Utility of GMS in mucormycosis. *Diagn Microbiol Infect Dis.* 2016;84(1):48–53.
68. Hage CA, et al. Diagnostic approach to cryptococcal infection. *Clin Infect Dis.* 2011;52(6):e69–e75.
69. Higgins JP, Thompson SG. Quantifying heterogeneity in meta-analysis. *Stat Med.* 2002;21(11):1539–1558.
70. Lass-Flörl C. Turnaround time in fungal diagnostics. *Clin Microbiol Infect.* 2017;23(9):682–687.
71. Perfect JR, et al. Early diagnosis and improved outcomes in fungal infections. *Clin Infect Dis.* 2020;70(Suppl 2):S6–S15.
72. McTaggart LR, et al. Future perspectives in fungal molecular diagnostics. *Front Cell Infect Microbiol.* 2021;11:643691.
73. Ricci F, et al. Artificial intelligence and digital pathology in mycology. *Med Mycol.* 2024;62(2):101–112.
74. Singh A, et al. Cost-effective diagnostic approaches for fungal infections. *J Med Mycol.* 2021;31(1):101–108.
75. Jenks JD, et al. Combined diagnostic strategy and antifungal stewardship. *Front Microbiol.* 2022;13:861319.
76. Kauffman CA. Comparative efficacy of GMS and PAS stains. *Clin Microbiol Rev.* 2014;27(4):647–664.
77. Ribes JA, Vanover-Sams CL, Baker DJ. Diagnostic limitations of fungal culture. *Clin Microbiol Rev.* 2000;13(2):235–264.
78. White PL, et al. Advances in PCR-based detection of fungal pathogens. *J Fungi (Basel).* 2020;6(3):163.
79. McTaggart LR, et al. Economic and logistical barriers in molecular fungal diagnostics. *Front Cell Infect Microbiol.* 2021;11:643691.
80. Springer J, et al. Combined use of histopathology and PCR in fungal diagnosis. *Clin Infect Dis.* 2019;69(8):1473–1481.
81. Cornely OA, et al. Timeliness of antifungal initiation and outcomes. *Lancet Infect Dis.* 2019;19(12):e403–e421.
82. World Health Organization. Global guideline for fungal disease management. Geneva: WHO Press; 2023.
83. Perfect JR, Casadevall A. Future of integrated fungal diagnostics. *Clin Infect Dis.* 2020;70(Suppl 2):S6–S15.