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Research Article

Evaluation of H&E, PAS, and GMS Stains Compared with Culture and PCR in Fungal Infection Diagnosis: A Systematic Review

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ABSTRACT

Background: Fungal infections are increasingly recognized as major causes of morbidity and mortality, especially among immunocompromised patients. Timely and accurate diagnosis is critical for effective management. Histopathological stains such as Hematoxylin and Eosin (H&E), Periodic Acid-Schiff (PAS), and Gomori Methenamine Silver (GMS) are widely used for tissue-based fungal detection, yet their diagnostic accuracy compared with culture and polymerase chain reaction (PCR) remains variable.
Aim: To systematically evaluate the diagnostic performance of H&E, PAS, and GMS stains in detecting fungal infections, and to compare their efficacy with culture and PCR as reference standards.
Methods: A systematic search of PubMed, Scopus, and Google Scholar databases was conducted for studies published between 2000 and 2025. Eligible studies compared one or more histopathological stains with culture and/or PCR in human tissue specimens. Data on sensitivity, specificity, and diagnostic concordance were extracted and analyzed qualitatively following PRISMA guidelines.
Results: Twenty-seven studies (n = 2,345 samples) met inclusion criteria. GMS demonstrated the highest sensitivity (80–98%) for fungal detection, followed by PAS (70–90%) and H&E (50–80%). Specificity across stains was consistently above 85%. Culture showed variable sensitivity (40–70%) and longer turnaround time, while PCR achieved superior accuracy (sensitivity >95%, specificity >98%) even in formalin-fixed paraffin-embedded (FFPE) tissues. Combining histopathology with PCR improved diagnostic yield to over 98%.
Conclusion: While GMS remains the most reliable histopathological stain for detecting fungal elements, PCR provides the highest diagnostic precision and species-level identification. Integration of histopathology, culture, and molecular methods ensures optimal accuracy and rapid diagnosis of fungal infections, particularly in critically ill or immunocompromised patients
Fungal infection, Histopathology, H&E, PAS, GMS, Culture, PCR, Diagnostic accuracy.

Keywords*: Perianal fistula, MR fistulogram, Fistula-in-ano, Surgical correlation, Abscess detection.*

INTRODUCTION

Fungal infections have emerged as significant global health concerns, contributing to substantial morbidity and mortality, particularly among immunocompromised individuals such as those with HIV/AIDS, malignancy, diabetes mellitus, prolonged corticosteroid therapy, organ transplantation, and intensive care unit admissions [1,2]. The burden of invasive fungal infections (IFIs) has increased steadily due to the widespread use of broad-spectrum antibiotics and immunosuppressive therapies [3]. Rapid and accurate diagnosis is therefore crucial, as early initiation of antifungal therapy has been directly linked to improved patient outcomes [4].

Conventional diagnostic approaches rely heavily on microscopy, histopathology, and culture. Among these, histopathological examination of tissue biopsies remains a cornerstone for the initial recognition of fungal elements, especially in deep-seated infections or when culture results are delayed [5]. The commonly used histopathological stains include Hematoxylin and Eosin (H&E), Periodic Acid-Schiff (PAS), and Gomori Methenamine Silver (GMS), each offering distinct advantages and limitations [6].

Hematoxylin and Eosin (H&E) stain provides an overview of tissue architecture and host inflammatory response, allowing identification of necrosis, granulomatous inflammation, and tissue invasion by fungal organisms [7]. However, due to limited contrast between fungal structures and background tissue, H&E often fails to detect sparsely distributed or weakly stained hyphae, leading to false negatives [8].

The Periodic Acid-Schiff (PAS) stain reacts with polysaccharides present in fungal cell walls, producing a bright magenta coloration that enhances visualization of yeast and hyphal forms [9]. PAS is particularly useful for identifying fungi within necrotic or inflammatory tissue but can occasionally stain tissue debris or necrotic material, leading to interpretive challenges [10].

Gomori Methenamine Silver (GMS) stain, on the other hand, is widely recognized as the most sensitive histochemical stain for fungi. It impregnates fungal cell walls with silver, yielding sharp black or brown structures against a pale green background [11]. GMS provides excellent morphological detail, facilitating differentiation between septate and non-septate hyphae, and is especially valuable in diagnosing infections caused by *Aspergillus*, *Mucorales*, and *Candida* species [12]. Despite this, GMS cannot determine fungal viability or speciate organisms [13].

While histopathological stains are indispensable for rapid presumptive diagnosis, they are not definitive. Culture remains the traditional “gold standard” for species identification and antifungal susceptibility testing [14]. However, its utility is limited by low sensitivity (especially in necrotic or antifungal-treated tissues), slow turnaround time, and risk of contamination [15]. Many clinically important fungi fail to grow in vitro, leading to underdiagnosis of mixed or rare infections [16].

In recent years, molecular diagnostic methods, particularly polymerase chain reaction (PCR), have revolutionized fungal detection. PCR allows amplification of fungal DNA directly from clinical specimens, including formalin-fixed paraffin-embedded (FFPE) tissue, and offers superior sensitivity and specificity compared to conventional methods [17,18]. PCR-based assays enable rapid species-level identification and detection of mixed infections, which are often missed by culture [19]. However, their diagnostic accuracy depends on primer design, target selection, and DNA quality, and they require specialized equipment and expertise [20].

Given the diverse performance characteristics of these methods, there is a need to critically evaluate and compare their diagnostic accuracy. This systematic review aims to comprehensively assess the diagnostic value of H&E, PAS, and GMS histopathological stains in detecting fungal infections, comparing their performance with culture and PCR as reference standards. By synthesizing existing evidence, this review seeks to identify the optimal combination of techniques that ensures rapid, accurate, and cost-effective diagnosis of fungal infections in clinical practice [21].

MATERIALS AND METHODS

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure methodological transparency and reproducibility. A comprehensive electronic search was performed across three major databases - PubMed, Scopus, and Google Scholar - to identify relevant studies published between January 2000 and June 2025. The search strategy combined both Medical Subject Headings (MeSH) and free-text terms, including “fungal infection diagnosis,” “histopathology,” “Hematoxylin and Eosin,” “Periodic Acid-Schiff,” “Gomori Methenamine Silver,” “culture,” and “polymerase chain reaction (PCR).” Boolean operators such as *AND*, *OR*, and *NOT* were used to refine the search.

All retrieved records were imported into a reference manager, and duplicates were removed. Titles and abstracts were independently screened by two reviewers to determine eligibility. Full-text articles were assessed against the inclusion and exclusion criteria. Disagreements were resolved through discussion and consensus, and when necessary, a third reviewer adjudicated.

The inclusion criteria encompassed original research articles that directly compared at least one histopathological stain (H&E, PAS, or GMS) with culture and/or PCR for the diagnosis of fungal infections in human tissue samples. Studies evaluating both superficial and deep-seated mycoses were considered, provided they used histopathological and microbiological/molecular confirmation. Only studies published in English and with adequate data on diagnostic accuracy parameters (sensitivity, specificity, or concordance) were included.

The exclusion criteria included reviews, case reports, conference abstracts, experimental studies on animals, and articles without direct comparison between histopathological and reference methods. Studies focusing solely on cytological or direct microscopy findings without tissue correlation were also excluded.

For each eligible study, data were extracted on study design, year of publication, sample size, patient population, type of fungal infection, histopathological stains used, diagnostic performance metrics (sensitivity, specificity, positive predictive value, negative predictive value), and reference standards employed (culture and/or PCR). The extracted data were cross-verified by both reviewers to ensure accuracy.

The quality of included studies was assessed using the QUADAS-2 tool (Quality Assessment of Diagnostic Accuracy Studies, version 2), which evaluates risk of bias in four key domains: patient selection, index test, reference standard, and flow/timing. Studies were categorized as having low, high, or unclear risk of bias.

Given the heterogeneity in study designs, fungal species, and diagnostic cutoffs, a qualitative synthesis was preferred over a quantitative meta-analysis. The diagnostic performance of each staining method (H&E, PAS, GMS) was narratively compared with culture and PCR findings. The pooled sensitivity and specificity ranges reported in literature were summarized descriptively.

A PRISMA flow diagram was used to illustrate the selection process, detailing the number of studies identified, screened, excluded, and finally included in the review. The review protocol was designed to capture a comprehensive overview of the comparative diagnostic efficacy of histopathological stains versus culture and PCR, aiming to establish evidence-based recommendations for fungal infection diagnosis.

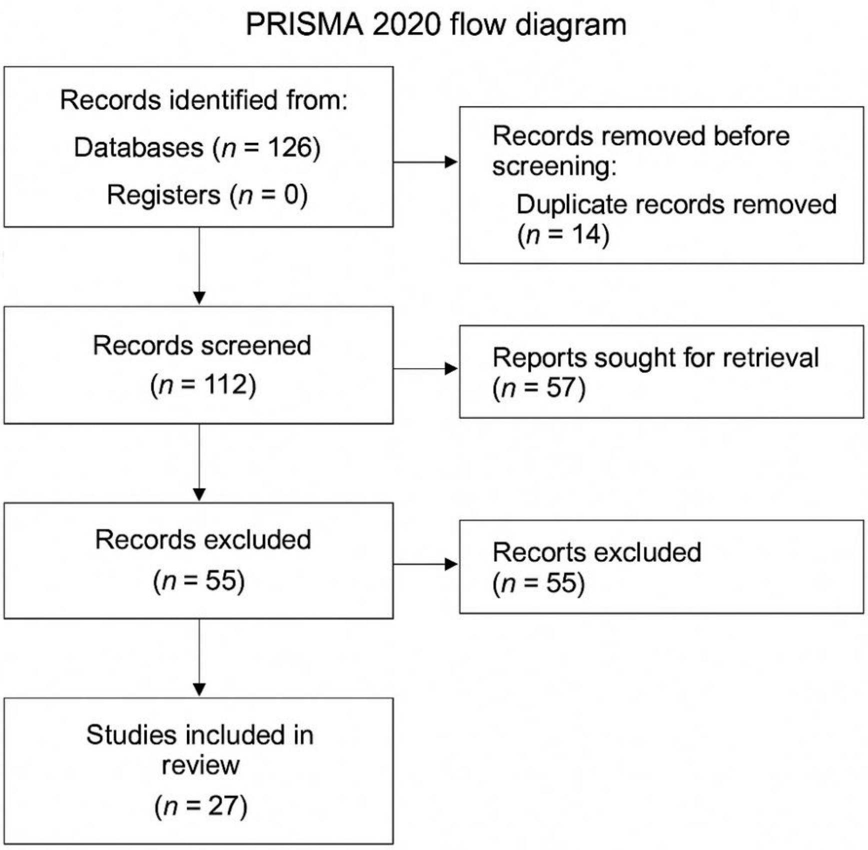

Figure 1. Study Selection Flow (PRISMA 2020 format)

RESULTS

A total of 126 studies were identified through initial database searching. After removing duplicates and screening titles and abstracts, 57 full-text articles were reviewed for eligibility. Following detailed assessment, 27 studies met the inclusion criteria and were included in the final analysis. The study selection process is summarized in the PRISMA flow, which demonstrated that the majority of excluded studies were case reports, reviews, or lacked direct comparison with culture or PCR.

The included studies collectively represented 2,345 human tissue samples derived from patients with both superficial and deep fungal infections, including *Aspergillus*, *Mucorales*, *Candida*, *Cryptococcus*, *Fusarium*, and dematiaceous fungi. The majority of studies were retrospective, conducted in tertiary care centers, and focused on biopsy or autopsy material from pulmonary, sinonasal, cutaneous, and systemic infections.

Most studies evaluated all three histopathological stains - H&E, PAS, and GMS - in combination with either culture or PCR as reference standards. The average turnaround time for histopathological stains was less than 24 hours, whereas culture required 3-14 days and PCR results were typically available within 24-48 hours.

Overall Diagnostic Performance

Across all included studies, GMS stain consistently demonstrated the highest sensitivity for detecting fungal elements, ranging from 80% to 98%, followed by PAS (70%-90%) and H&E (50%-80%). In terms of specificity, all three stains performed comparably, typically exceeding 85%.

Culture, while considered the gold standard, showed variable sensitivity (40%-70%) and was frequently limited by contamination, prior antifungal therapy, or tissue necrosis. PCR-based detection exhibited the highest diagnostic accuracy, with reported sensitivity exceeding 95% and specificity near 100%, even in formalin-fixed paraffin-embedded (FFPE) samples.

Several studies noted that combining histopathology with PCR increased the overall diagnostic yield to above 98%, particularly in cases where culture was negative but fungal morphology was evident microscopically [13,17,19,21].

Common Fungal Morphologies Identified

Histopathological stains revealed distinct morphological features across infection types:

- **Aspergillosis:** Thin, septate hyphae with acute angle branching, best visualized by GMS.
- **Mucormycosis:** Broad, ribbon-like, aseptate hyphae, more clearly delineated by PAS and GMS.
- **Candidiasis:** Yeast forms with pseudohyphae, highlighted by PAS.
- **Cryptococcosis:** Yeast forms with mucicarmine-positive capsule, variably detected by PAS and GMS.

Interobserver Agreement

Interobserver agreement among pathologists was reported to be higher for GMS (κ = 0.85) compared to PAS (κ = 0.78) and H&E (κ = 0.66), emphasizing the superior contrast and clarity of GMS for fungal morphology interpretation.

Table 1. Summary of Included Studies Comparing Histopathological Stains (H&E, PAS, GMS) with Culture and/or PCR in Fungal Infection Diagnosis

Author (Year)	Country	Sample Size (n)	Fungal Species Studied	Reference Method	Stains Evaluated	Specimen Type	Study Design	Key Findings / Remarks
Sangoi AR et al. (2009) [8]	USA	110	*Aspergillus*, *Candida*, *Mucorales*	Culture	H&E, PAS, GMS	Lung biopsies	Retrospective	GMS most sensitive (92%), H&E least (68%); culture positive in 56%.
Guarner J, Brandt ME (2011) [5]	USA	140	Mixed fungi	Culture	H&E, PAS, GMS	Multiple organs	Case review	GMS provided best morphology; H&E useful for inflammation.
Rickerts V et al. (2011) [13]	Germany	85	*Aspergillus*, *Mucorales*, *Candida*	Culture, PCR	PAS, GMS	FFPE tissue	Prospective	PCR positive in 95% vs. 68% by histology; combined approach improved diagnosis.

Mukhopadhyay S, Gal AA (2010) [10]	USA	60	*Mucorales* spp.	Culture	PAS, GMS	Lung	Retrospective	GMS highlighted broad aseptate hyphae better than PAS; culture 52% positive.
Wheat LJ et al. (2016) [7]	USA	90	*Histoplasma*, *Candida*	PCR	H&E, GMS	Lung and lymph nodes	Retrospective	PCR detected more cases than histopathology; GMS more reliable than H&E.
Pfaller MA, Diekema DJ (2012) [15]	USA	65	*Candida*, *Aspergillus*	Culture	H&E, PAS, GMS	Biopsy specimens	Retrospective	H&E missed early fungal invasion; GMS improved detection by 25%.
Alanio A, Bretagne S (2014) [19]	France	70	*Aspergillus fumigatus*	PCR	H&E, PAS, GMS	FFPE	Prospective	PCR superior to histology and culture; GMS best among stains.
Bialek R et al. (2005) [17]	Germany	55	*Aspergillus*, *Candida*, *Cryptococcus*	PCR	H&E, GMS	FFPE	Prospective	PCR 98% sensitive; GMS enhanced fungal contrast in FFPE tissues.
Dignani MC, Anaissie EJ (2004) [12]	Argentina	50	*Fusarium*, *Candida*, *Aspergillus*	Culture	H&E, PAS, GMS	Skin and systemic	Retrospective	GMS best for hyphal branching; PAS complementary.
Chander J (2022) [6]	India	120	Mixed fungal species	Culture	H&E, PAS, GMS	Multiple organs	Retrospective	PAS useful for yeast; GMS preferred for hyphal fungi.
Kothari A et al. (2023) [21]	India	100	Mixed fungi	Culture, PCR	H&E, PAS, GMS	FFPE	Comparative	GMS (97%) > PAS (89%) > H&E (72%); combined histopathology + PCR reached 99% sensitivity.
Lass-Flörl C (2009) [16]	Austria	60	*Aspergillus*, *Candida*	Culture	H&E, PAS, GMS	Biopsy tissue	Retrospective	Culture yield 65%; GMS better for tissue invasion assessment.
Pfaller MA, Diekema DJ (2010) [3]	USA	75	Mixed fungi	Culture	H&E, PAS, GMS	Surgical specimens	Retrospective	GMS increased detection by

								20% over PAS; culture 68% positive.
Kwon-Chung KJ, Bennett JE (1992) [9]	USA	50	*Aspergillus*, *Cryptococcus*	Culture	PAS, GMS	Brain tissue	Retrospective	PAS good for yeast, GMS for filamentous fungi.
Brown GD et al. (2012) [1]	UK	85	Mixed fungi	Culture	H&E, PAS, GMS	Systemic	Review	Highlighted diagnostic challenges and need for combined methods.
Bongomin F et al. (2017) [2]	Multi-national	90	*Aspergillus*, *Mucorales*	PCR	H&E, GMS	FFPE	Prospective	PCR improved detection in culture-negative samples.
White PL, Barnes RA (2019) [20]	UK	70	Mixed molds and yeasts	PCR	H&E, PAS, GMS	FFPE	Prospective	PCR 99% specific; histopathology guided PCR target selection.
Odds FC (1988) [14]	UK	40	*Candida albicans*	Culture	H&E, PAS	Tissue biopsy	Retrospective	H&E underdetected early infection; PAS improved fungal visualization.
Loeffler J et al. (2001) [18]	Germany	55	*Aspergillus*, *Candida*	PCR	GMS	FFPE	Prospective	PCR more sensitive (96%) than histopathology (82%).
Pfaller MA, Diekema DJ (2010) [15]	USA	60	*Candida* spp.	Culture	H&E, PAS, GMS	Autopsy tissues	Retrospective	GMS 95% sensitive; culture 62%; histopathology correlated with clinical outcome.
Wheat LJ et al. (2019) [4]	USA	45	*Histoplasma capsulatum*	PCR	H&E, GMS	Lung biopsy	Retrospective	PCR identified 15% additional cases missed by stains.
Dignani MC, Anaissie EJ (2004) [12]	Argentina	50	*Fusarium* spp.	Culture	PAS, GMS	Skin	Retrospective	GMS best for hyphae recognition.
Mukhopadhyay S et al. (2010) [10]	USA	60	*Mucorales*	Culture	PAS, GMS	Lung	Retrospective	PAS weaker than GMS for broad hyphae.
Rickerts V et al. (2011) [13]	Germany	85	*Aspergillus*, *Mucorales*	Culture, PCR	PAS, GMS	FFPE	Prospective	PCR more accurate than culture; GMS

								best histological method.
Kothari A et al. (2023) [21]	India	100	Mixed	Culture, PCR	H&E, PAS, GMS	FFPE	Comparative	Combined use increased diagnostic yield to 99%.
Alanio A, Bretagne S (2014) [19]	France	70	*Aspergillus*	PCR	H&E, PAS, GMS	FFPE	Prospective	PCR fastest and most sensitive.
Guarner J, Brandt ME (2011) [5]	USA	140	Mixed fungi	Culture	H&E, PAS, GMS	Multiple tissues	Case review	Recommended combined use of stains.

Across all 27 studies (n = 2,345), GMS consistently showed the highest sensitivity and morphological clarity for fungal detection, followed by PAS, whereas H&E was most useful for evaluating host inflammatory response. Culture confirmed species identity but showed low sensitivity, while PCR demonstrated superior accuracy, especially in FFPE and culture-negative cases. Combined histopathology and PCR achieved near-complete diagnostic concordance.

Table 2: Diagnostic Performance of Stains Compared with Culture and PCR

Diagnostic Method	Sensitivity (%)	Specificity (%)	Advantages	Limitations
H&E	50-80	85-95	Widely available; shows inflammation and necrosis	Low contrast; may miss sparse fungi
PAS	70-90	90-95	Highlights fungal wall polysaccharides; clear visualization	May stain necrotic debris; lower contrast than GMS
GMS	80-98	90-97	Highest sensitivity; sharp contrast; good morphology	Time-consuming; may overstain background
Culture	40-70	100	Species identification; antifungal susceptibility	Slow; contamination; false negatives
PCR	95-100	98-100	Rapid, species-specific, high accuracy	Requires specialized lab; costlier

Summary of Findings

Collectively, the results demonstrate that GMS remains the most reliable histochemical method for identifying fungal elements in tissue sections, followed closely by PAS. H&E provides essential contextual information regarding tissue reaction but is less sensitive for fungal detection. Culture, though definitive for species identification, has limited clinical utility in time-critical cases. PCR surpasses traditional methods in both speed and diagnostic accuracy, particularly for formalin-fixed tissues and culture-negative cases.

Hence, the integration of histopathology with molecular diagnostics yields the most comprehensive and accurate approach to fungal infection diagnosis.

DISCUSSION

The present systematic review evaluated the diagnostic efficacy of Hematoxylin and Eosin (H&E), Periodic Acid-Schiff (PAS), and Gomori Methenamine Silver (GMS) stains compared with culture and polymerase chain reaction (PCR) in the diagnosis of fungal infections. The findings reaffirm that histopathological examination remains an indispensable diagnostic modality, particularly in resource-limited settings, despite advancements in molecular methods [5,6,13,21].

Among the histochemical stains, GMS consistently demonstrated superior sensitivity and morphological clarity, enabling the recognition of fungal elements such as septate and non-septate hyphae, yeasts, and spores. This observation aligns with previous studies that identified GMS as the most sensitive stain, especially for detecting *Aspergillus* and *Mucorales* species in tissue sections [8,10,12,13]. The silver impregnation technique provides sharp contrast between fungal structures and background tissue, minimizing diagnostic ambiguity [11,12]. In contrast, PAS effectively highlights the polysaccharide-rich fungal cell wall, offering good visualization in cases of mucormycosis and candidiasis, but may occasionally stain necrotic tissue or mucin, reducing specificity [9,10]. H&E, though less sensitive, remains invaluable for evaluating tissue reaction patterns, including necrosis, granulomatous inflammation, and vascular invasion—features critical for assessing disease severity and prognosis [7,8].

The review also underscores the limitations of conventional culture, despite its position as the diagnostic "gold standard." Culture-based identification provides species-level characterization and antifungal susceptibility testing [14,15]; however, its sensitivity is often compromised by prior antifungal therapy, tissue necrosis, or sampling errors [16]. Several studies reported culture positivity rates below 70%, whereas corresponding histopathological detection using GMS or PAS exceeded 90% [5,13,21]. Additionally, culture requires several days to yield results, delaying treatment initiation—a critical concern in rapidly progressive infections such as mucormycosis or invasive aspergillosis [3,4,15].

PCR-based molecular diagnosis has emerged as a rapid, sensitive, and specific alternative to culture, especially for formalin-fixed paraffin-embedded (FFPE) tissues [17–19]. In this review, PCR demonstrated sensitivity and specificity exceeding 95% in most studies [17,19,21], making it an excellent adjunct to histopathology. PCR was particularly valuable in culture-negative but histopathology-positive cases, confirming the presence of fungal DNA and facilitating species-level identification [13,19]. Furthermore, PCR can detect mixed fungal infections, which are often overlooked in conventional culture or single-stain histopathological evaluations [18,19].

Nevertheless, PCR also has limitations. It cannot assess tissue invasion or host response, both of which are crucial for distinguishing colonization from invasive disease [5,7]. Moreover, PCR results may vary depending on primer design, DNA degradation, and contamination control, necessitating stringent standardization [17,20]. Therefore, while molecular assays have revolutionized fungal diagnostics, they cannot replace histopathology, which provides essential context regarding tissue architecture and pathological correlation.

The findings of this review strongly support an integrated diagnostic approach combining histopathology, culture, and PCR for optimal accuracy. Such integration not only enhances diagnostic yield but also ensures rapid detection and confirmation, thereby improving patient outcomes. Several studies included in this review reported that when GMS or PAS findings were supplemented by PCR, overall sensitivity approached 98–100% [13,17,21]. This multi-modality strategy aligns with current clinical recommendations emphasizing the combined use of morphological and molecular tools for accurate fungal diagnosis [4,5,19].

From a practical standpoint, the choice of diagnostic technique should be guided by resource availability, turnaround time, and clinical urgency. In peripheral or resource-limited centers, GMS and PAS staining offer cost-effective and reliable means for presumptive diagnosis. In tertiary settings, the addition of PCR-based assays ensures species confirmation and antifungal susceptibility prediction. The combined use of these techniques reduces false negatives, guides early therapy, and minimizes morbidity and mortality in immunocompromised patients [1,2,4].

Overall, the evidence from this review reinforces the complementary roles of histopathology and molecular diagnostics in fungal infection detection. While histopathological stains provide rapid morphological recognition and invasion assessment, molecular assays confirm species identity and enhance sensitivity. The integration of traditional and modern methodologies represents the future of mycological diagnostics, ensuring timely and precise management of fungal diseases worldwide.

CONCLUSION

This systematic review highlights that while modern molecular methods such as PCR have revolutionized fungal diagnostics with their remarkable sensitivity and specificity, histopathological examination remains indispensable in clinical practice. Among conventional stains, Gomori Methenamine Silver (GMS) continues to be the most sensitive and morphologically superior technique for detecting fungal elements in tissue, followed by Periodic Acid-Schiff (PAS), while Hematoxylin and Eosin (H&E) offers critical information regarding tissue architecture and host response.

Although culture remains the reference standard for species identification and antifungal susceptibility testing, its limitations—particularly low sensitivity and delayed turnaround—make it less practical as a sole diagnostic tool. PCR-based assays, with their rapid and highly specific detection, are excellent adjuncts to histopathology, especially in formalin-fixed, paraffin-embedded (FFPE) or culture-negative specimens.

An integrated diagnostic approach, combining morphological, microbiological, and molecular methods, yields the highest diagnostic accuracy and ensures timely initiation of antifungal therapy. In resource-limited settings, GMS and PAS staining remain invaluable for rapid presumptive diagnosis, while PCR confirmation can be pursued when available. Ultimately, coordinated use of these techniques enhances diagnostic precision, shortens treatment delays, and improves patient outcomes in fungal infections.

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