Evidence-Based Case Report

Comparison of Diagnostic Accuracy Between Stool Staining and Polymerase Chain Reaction in Diagnosing Intestinal Microsporidiosis

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Abstract

Microsporidia are intracellular parasites that cause chronic diarrhea, particularly in immunocompromised individuals, where access to molecular diagnostics remains limited. A 27-year-old man with HIV on antiretroviral therapy presented with a 10-day history of profuse watery diarrhea. Initial stool examinations using modified Ziehl–Neelsen staining were negative, and PCR testing was not available, raising suspicion of intestinal microsporidiosis. The clinical question was whether, in immunocompromised patients with suspected intestinal microsporidiosis, stool smear staining methods are as accurate as PCR in terms of sensitivity and specificity. A structured literature review was conducted using PubMed, Cochrane, and Google Scholar for studies published up to 2025, using keywords including microsporidia, Enterocytozoon, Encephalitozoon, intestinal, diarrhea, diagnosis, detection, staining, and PCR. Eligible studies included those evaluating stool-based staining methods against PCR in immunocompromised patients. Articles were appraised using the STARD 2015 guideline and independently reviewed by two reviewers. Of 70 identified articles, 3 met the inclusion and validity criteria. Modified trichrome (MT) staining showed sensitivity ranging from 38.6% to 90.0% and specificity from 88.9% to 100%. Calcofluor white (CW) showed sensitivity ranging from 70.4% to 96.7% and specificity from 68.4% to 82.4%. Although PCR remains the reference standard, MT staining offers acceptable diagnostic performance and is a practical alternative in settings where molecular testing is not feasible.
Keywords: *Microsporidia, intestinal, diagnosis, microscopy, staining.*

Perbandingan Akurasi Diagnostik antara Pewarnaan Tinja dan Polymerase Chain Reaction dalam Diagnosis Mikrosproridiosis Usus

Abstrak

Microsporidia merupakan parasit intraseluler yang dapat menyebabkan diare kronis, terutama pada individu imunokompromais, di mana akses terhadap pemeriksaan molekuler masih terbatas. Seorang laki-laki usia 27 tahun dengan HIV yang menjalani terapi antiretroviral datang dengan keluhan diare cair lebih dari 10 hari. Pemeriksaan tinja dengan pewarnaan Ziehl–Neelsen modifikasi menunjukkan hasil negatif, sementara pemeriksaan PCR tidak tersedia, sehingga dicurigai mikrosporidiosis intestinal. Pertanyaan klinis yang diajukan adalah apakah pada pasien imunokompromais dengan kecurigaan mikrosporidiosis intestinal, pemeriksaan pewarnaan sediaan tinja dibandingkan dengan PCR memiliki akurasi diagnostik yang baik dalam hal sensitivitas dan spesifisitas. Telaah literatur terstruktur dilakukan menggunakan PubMed, Cochrane, dan Google Scholar untuk artikel yang dipublikasikan hingga tahun 2025 dengan kata kunci microsporidia, Enterocytozoon, Encephalitozoon, intestinal, diarrhea, diagnosis, detection, staining, dan PCR. Studi yang memenuhi kriteria inklusi adalah penelitian yang membandingkan metode pewarnaan tinja dengan PCR pada pasien imunokompromais. Artikel dinilai menggunakan panduan STARD 2015 dan direviu secara independen oleh dua penilai. Dari 70 artikel yang ditemukan, 3 artikel memenuhi kriteria validitas. Pewarnaan modified trichrome (MT) menunjukkan sensitivitas 38,6% hingga 90,0% dan spesifisitas 88,9% hingga 100%, sedangkan Calcofluor white (CW) menunjukkan sensitivitas 70,4% hingga 96,7% dan spesifisitas 68,4% hingga 82,4%. Meskipun PCR tetap merupakan standar rujukan, pewarnaan MT memiliki kinerja diagnostik yang cukup baik dan dapat digunakan sebagai alternatif praktis di fasilitas dengan keterbatasan pemeriksaan molekuler.
Kata kunci: *Mikrosporidia, usus, diagnosis, mikroskopis, pewarnaan.*

Introduction

Microsporidia are a group of cosmopolitan intracellular parasites belonging to the phylum Microsporidia, comprising over 1,000 species and 200 genera.[1] Historically, Microsporidia were initially classified as primitive protozoa. With advances in molecular phylogenetics, Microsporidia are now considered more closely related to the fungal kingdom.[2] These parasites can infect a wide range of vertebrates and invertebrates, as well as humans.[3] Most Microsporidia infections in humans are zoonotic or waterborne, with fecal-oral transmission as the main route.[4] Microsporidiosis has become a major public health concern in several countries, particularly due to increased reports of outbreaks and contamination of drinking water by Microsporidia.[5-7] Moreover, Microsporidia spores can survive under various environmental conditions and remain infectious for several years, especially in moist environments.[8]

In immunocompetent individuals, Microsporidia are considered part of the normal gut flora; however, they can cause disease, especially in immunocompromised individuals.[9] Human Microsporidia infection was first detected in Japan in 1959 in a pediatric patient with encephalitis.[10] With the emergence of the human immunodeficiency virus (HIV) epidemic around 1980, the incidence of Microsporidia infection in humans was reported to increase.[11,12] Opportunistic microsporidiosis has also been reported in cancer patients undergoing chemotherapy.[13] There are two main genera of Microsporidia known to infect humans: *Enterocytozoon* and *Encephalitozoon*.[14] These two genera can cause persistent watery diarrhea and malnutrition syndromes in both immunocompromised and immunocompetent individuals, particularly *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis.*[14] Estimating prevalence is challenging due to subclinical infections, non-specific symptoms, and a lack of physician awareness.[15]

Diagnosing intestinal Microsporidia infection is difficult. The parasite can be detected only by molecular examination using polymerase chain reaction (PCR) or by microscopic examination with specialized staining techniques.[16] The gold standard for diagnosing intestinal microsporidiosis is the identification of the characteristic polar filaments of Microsporidia by electron microscopy, although this method is now rarely used due to its high cost, long processing time, and the need for specialized expertise in operation and result interpretation.[8,16,17] Currently, molecular-based diagnostic methods using PCR are increasingly used, especially for species-level parasite identification.[18] Although PCR has high sensitivity and specificity, its use is often limited by the limited number of known species-specific DNA sequences, inconsistent results depending on the type of stool specimen preservative used, high testing costs, and inadequate laboratory infrastructure.[8,18] In this context, identification of Microsporidia spores in stool preparations has the potential to become the preferred diagnostic method for intestinal microsporidiosis, particularly in healthcare facilities or private laboratories with limited capacity. Therefore, a literature review is needed to evaluate the diagnostic performance of stool staining methods for intestinal microsporidiosis and their applicability in routine clinical practice.

Clinical Scenario

A 27-year-old man presented to the Internal Medicine outpatient clinic at a type A hospital with complaints of diarrhea. The diarrhea had been ongoing for approximately 10 days, characterized by watery stools occurring more than six times per day, without mucus or blood. The patient works as a rafting tour guide and has a comorbidity of HIV, for which he has been receiving antiretroviral therapy for over two years. On physical examination, signs of dehydration were noted, along with non-specific abdominal tenderness. The primary differential diagnosis considered by the physician was cryptosporidiosis. The physician requested a stool specimen examination using the modified Ziehl-Neelsen staining technique to confirm the diagnosis, but the results were negative on three separate occasions. The physician then considered other possible diseases that could present with similar symptoms in immunocompromised individuals,

such as microsporidiosis. A molecular examination of the patient's stool specimen by PCR was requested. However, the hospital did not have the necessary molecular diagnostic kits for Microsporidia. The laboratory's physician in charge suggested performing a staining method instead. The attending physician then conducted a literature review to assess the diagnostic accuracy of stool smear staining methods compared to PCR for diagnosing intestinal microsporidiosis.

Problem Formulation

"Is the accuracy of microscopic examination using stool smear staining methods as good as the PCR method?

Based on this clinical question, the following PICO framework was developed:

P : immunocompromised patients
I : stool smear staining
C : PCR examination
O : diagnostic accuracy (sensitivity, specificity)

Search Strategy and Results

Eligibility Criteria

The eligibility of articles in this study was assessed based on predefined inclusion and exclusion criteria. Articles were included if they involved immunocompromised patients presenting with diarrhea, and if the diagnostic study compared stool examination using staining methods with the gold standard PCR method. Eligible articles were limited to original research articles, meta-analyses, or systematic reviews. Articles were excluded if they used specimens other than stool, such as biopsy samples, corneal swabs, or urine. Studies conducted on animals were also excluded, as were those unrelated to Microsporidia. Additionally, articles categorized as conference proceedings, opinion pieces, editorials, brief communications, or abstracts only were not considered. Articles not written in English were also excluded from the review.

Literature Search Strategy

The literature search was conducted using three electronic databases: PubMed/MEDLINE® (https://pubmed.ncbi.nlm.nih.gov), Cochrane® (https://www.cochranelibrary.com), and Google Scholar (https://scholar.google.com) on September 21, 2020. The search was not restricted by publication year to include a wide range of staining methods from the past to the present. The search combined terms related to microsporidia, intestinal infection, diagnostic methods, staining techniques, and PCR, as shown in Table 1. Duplicate articles identified from the three databases were removed from the analysis. Articles were then screened based on their titles and abstracts in accordance with the predefined eligibility criteria. Articles that met the eligibility criteria underwent full-text review for further analysis.

Table 1. Search Strategy in PubMed, Cochrane, and Google Scholar Databases.

Database	Keyword	Hits	Selected
Pubmed	("microsporid*"[All Fields] OR ("enterocytozoon"[MeSH Terms] OR "enterocytozoon"[All Fields] OR "enterocytozoons"[All Fields]) OR ("encephalitozoon"[MeSH Terms] OR "encephalitozoon"[All Fields] OR "encephalitozoons"[All Fields])) AND ("intestinalization"[All Fields] OR "intestinalized"[All Fields] OR "intestinally"[All Fields] OR "intestinals"[All Fields] OR "intestine s"[All Fields] OR "intestines"[MeSH Terms] OR "intestines"[All Fields] OR "intestinal"[All Fields] OR "intestine"[All Fields]) AND "diagnos*"[All Fields] AND "detect*"[All Fields] AND "stain*"[All Fields] AND "PCR"[All Fields] AND ("polymerase"[All Fields] OR "polymerase s"[All Fields] OR "polymerases"[All Fields])	16	8
Cochrane	(microsporid*) AND (intestinal) AND (diagnos*)	5	0
Google Scholar	human AND microsporid* OR enterocytozoon OR encephalitozoon AND intestinal AND stool AND microscop* AND diagnosis AND detect AND stain* AND PCR AND polymerase	49	4

The validity assessment and critical appraisal of the scientific articles were conducted by two reviewers using the standardized guideline STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies,[19] which consisted of 27 key items. This includes an assessment of the appropriateness of the study background and its problem rationale, the alignment between objectives and study design, the suitability of the design with the methodology, the appropriateness of the study population and eligibility criteria, details of the diagnostic test and gold standard methods, explanation of how test results were interpreted, the diagnostic test flow in study subjects, availability of diagnostic accuracy analysis, study limitations, and the potential applicability of the study findings. Any differences in the validity assessments and critical appraisals between the two reviewers were further discussed to reach a consensus.

Results

Characteristics of Selected Studies

The literature search across three scientific databases using selected keywords yielded 70 articles potentially relevant to the clinical question and the defined PICO formulation. A total of 58 articles were excluded based on title and abstract screening for failing to meet the study eligibility criteria. These exclusions included studies on animals (13 articles), use of non-stool specimens (4 articles), non-diagnostic studies (22 articles), studies where PCR was not used as the gold standard (10 articles), studies unrelated to Microsporidia (6 articles), non-English articles (2 articles), and abstract-only publications (1 article). A full-text review of the 12 articles that met the eligibility criteria was conducted to assess study validity, focusing on the appropriateness of the study design, the representation of the study population, and the suitability and reproducibility of both the index test and the gold standard. Based on this validity assessment, 3 articles were included in the final critical appraisal phase (Figure 1).

All three studies included in the final critical appraisal phase were diagnostic studies with a cross-sectional design. Each article stated that the study was conducted in immunocompromised populations, including individuals with HIV comorbidity, hematologic malignancies undergoing chemotherapy, and/or chronic kidney disease with kidney transplantation.[20,21] One study also involved healthy participants without comorbid conditions.[22] All three studies included participants of all age groups. The index tests used were microscopic examinations with modified trichrome (MT) and calcofluor white (CW) staining methods. The characteristics of the selected studies are further described in Table 2.

The three included studies compared microscopic staining techniques with PCR for diagnosing intestinal microsporidiosis, but notable differences were observed in the staining approaches and diagnostic outcomes. In the study by Saigal et al,[22] MT detected 22 positive samples (5.6%), whereas CW detected substantially more positives (98 samples; 24.8%). However, PCR confirmed only 57 cases (14.4%) with either *E. bieneusi* or *E. intestinalis* infections. Similarly, Ghoshal et al[20] compared MT and CW staining in immunocompromised patients and reported a large discrepancy between CW-positive findings and PCR-confirmed cases. Using procedures adapted from Weber et al,[23] MT detected 28 positives (3.8%) while CW detected 250 (34.2%), although PCR confirmed only 30 infections (4.1%) of *E. bieneusi*.[20] The third study by El-Kerdany et al[21] evaluated only MT staining and reported 49 positive cases (49%) compared with 55 cases detected by real-time PCR (55%). Additionally, this study identified the most common Microsporidia species, including *E. bieneusi*, *E. intestinalis*, *E. cuniculi*, and *E. hellem*. The detailed staining procedures used in three studies are described in Table 3.

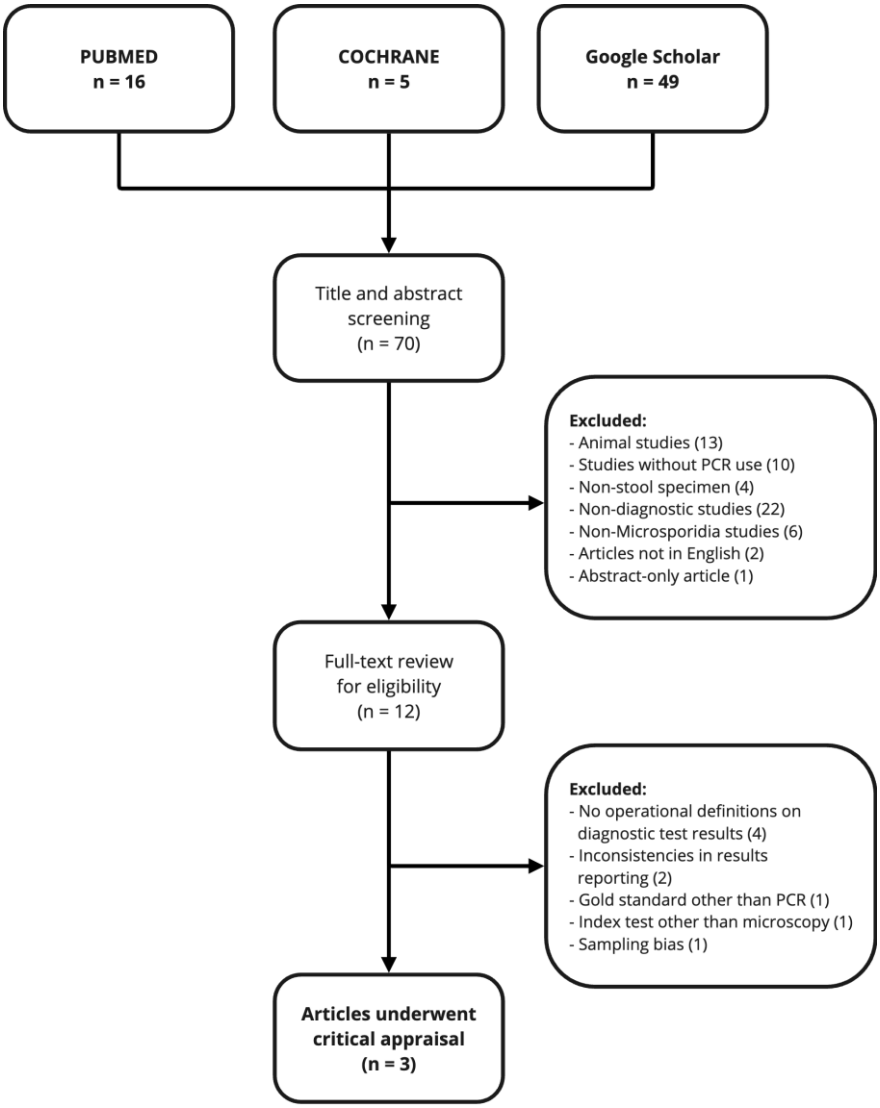

Figure 1. Scientific Literature Search Flowchart

Table 2. Characteristics Of Eligible Articles Included in The Critical Appraisal

Author And Year of Publication	Country	Study Design	Population	Specimen	Staining Technique	Gold Standard	Level of Evidence
Saigal K, et al (2013)[22]	India	Cross-sectional	• 395 patients • All age groups • HIV, non-HIV, and healthy individuals	Stool in 10% formalin	MT and CW	Nested PCR	2
Ghoshal, et al (2015)[20]	India	Cross-sectional	• 730 patients • All age groups • Immunocompromised (leukemia, transplant recipients, HIV)	Concentrated preserved stool in methanol	MT and CW	PCR	2
El-Kerdany, et al (2016)[21]	Egypt	Cross-sectional	• 100 subjects • All age groups • Immunocompromised (lymphoma, leukemia, CKD, chemotherapy)	Stool in 2.5% potassium dichromate	MT	Real-time PCR	2

HIV: human immunodeficiency virus, CKD: chronic kidney disease, MT: modified trichrome, CW: calcofluor white, PCR: polymerase chain reaction

Table 3. Differences and Similarities in Staining Procedures Among Studies

Staining Methods	Steps	Studies		
		Study 1 (Saigal, et al)[22]	Study 2 (Ghoshal, et al)[20]	Study 3 (El-Kerdany, et al)[21]
Modified trichrome (MT)	Fixation	Smear fixed with methanol for 5 minutes	Same as Study 1	Same as Study 1
	Staining	Stained with Chromotrope solution for 90 minutes: Chromotrope 2R (6 g), fast green (0.15 g), phosphotungstic acid (0.7 g) in glacial acetic acid (3 ml)	Stained with Chromotrope solution for 10-12 minutes at 50°C: Chromotrope 2R (6 g), fast green (0.15 g), phosphotungstic acid (0.7 g) in glacial acetic acid (3 ml)	Same as Study 1
	Acid wash	Smear rinsed with acid alcohol (1% glacial acetic acid in 90% ethanol) for 10 seconds	Same as Study 1	Same as Study 1
	Ethanol rinse	Smear rinsed again with 95% ethanol and 100% ethanol for 5 and 10 minutes, respectively, in sequence	Same as Study 1	Same as Study 1
Calcofluor white (CW)	Fixation	Smear fixed with methanol for 30 seconds	Same as Study 1	Not done
	Staining	Stained with Calcofluor solution 0.01% for 2–3 minutes	Same as Study 1	
	Rinse	Smear rinsed with water	Same as Study 1	

Diagnostic Accuracies between Staining Methods

When compared with PCR as the reference standard, MT and CW staining showed distinct diagnostic performance patterns across studies. MT staining consistently demonstrated higher specificity and closer agreement with PCR-confirmed infections, indicating greater reliability in identifying true positive cases. In contrast, CW staining tended to detect more suspected spores but showed lower specificity and positive predictive value, suggesting a higher likelihood of false-positive results. Detailed accuracy metrics are summarized in Table 4.

Table 4. Diagnostic Accuracy of Stool Smear Staining Methods Based on Literature Review

Author	Subjects	Stain	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR+	LR-	Pretest Probability (%)	Posttest Probability (%)
Saigal, et al[22]	395	MT	38.6	100	100	90.6	*inf*	0.6	14.4	*inf*
	395	CW	70.4	82.4	38.8	94.6	4.0	0.4	13.7	38.8
Ghoshal, et al[20]	730	MT	90.0	99.9	96.4	99.6	630	0.1	4.1	96.4
	730	CW	96.7	68.4	11.6	99.8	3.1	0.1	4.1	11.6
El-Kerdany, et al[21]	100	MT	80.0	88.9	89.8	78.4	7.2	0.2	55.0	89.8

MT: modified trichrome; CW: Calcofluor white; Sens: sensitivity; Spes: specificity; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio; inf: infinite

Clinical Applicability

According to the STARD 2015 guidelines, all three studies demonstrated good applicability, with clear descriptions of both the index test (staining) and the gold standard (PCR), allowing replication by researchers or laboratory personnel. All subjects underwent both index and reference tests. However, none of the studies explicitly stated whether the observers interpreting the index and reference tests were independent. Two studies used appropriate patient populations, which were immunocompromised individuals with diarrhea,[20,21] while one study also included immunocompetent individuals without symptoms.[22] Practical applicability was also assessed based on the availability of diagnostic tools, laboratory capacity, and cost. Detailed evaluation of diagnostic validity and applicability is summarized in Table 5.

Table 5. Validity and Applicability of Diagnostic Test Results Based on Literature Review

Author	Staining Technique	Patient Spectrum Representation	Universal Application of The Gold Standard	Independent Observer	Test Replicability	Test Applicability
Saigal, et al[22]	MT	±	+	±	+	+
	CW	±	+	±	+	+
Ghoshal, et al[20]	MT	+	+	±	+	+
	CW	+	+	±	+	+
El-Kerdany, et al[21]	MT	+	+	±	+	+

MT: *modified trichrome*; CW: *Calcofluor white*; (+): yes; (-): no; (±): not clear

Discussion

Technical Factors Affecting Diagnostic Accuracy

PCR was considered the gold standard for diagnosing microsporidiosis, with reported sensitivities of 89% to 100% and specificities of 100%. The first study by Saigal et al[22] included 395 subjects of all ages and found low sensitivity but high specificity for MT. The low sensitivity was likely due to the use of 10% formalin as a stool preservative, which interferes with spore visualization, as also noted in other studies.[24-26] Additionally, formalin preservative is not recommended for trichrome staining due to its damaging effect on parasite morphology.[27] Other possible reasons include low observer experience and low spore burden.[28] While PCR can detect as few as 10^2 spores/gram of stool, microscopy requires 10^4-10^6 spores/gram.[29] This highlights the need for multiple technicians to minimize false negatives. Conversely, the 100% specificity may reflect the low number of MT-positive samples in one study (22/395), all of which were confirmed by PCR.[22]

Microscopic examination using MT staining in the studies by El-Kerdany and Ghoshal showed similar diagnostic accuracy, with sensitivity of 80.0% and 90.0%, and specificity of 88.9% and 99.9%, respectively.[20,21] Ghoshal et al[20] used a slightly modified MT method by heating the stained smears at 50°C for 10 to 12 minutes, similar to the hot chromotrope technique. Heating the chromotrope stain to 50-55°C is known to enhance spore detection sensitivity and reduce staining time.[30] The large difference in positive likelihood ratios may be due to differences in agreement between MT and PCR results. Ghoshal's study involved 730 subjects, while El-Kerdany's included only 100, which likely affected the effect size. For diseases with low prevalence, larger sample sizes are preferred to ensure valid diagnostic accuracy estimates.[31]

Microscopic examination using the CW technique showed varying diagnostic accuracy in the two studies. Saigal et al[22] reported 70.4% sensitivity and 82.4% specificity, while Ghoshal et al[20] found 96.7% sensitivity but lower specificity at

68.4%. The discrepancy may be due to difficulty distinguishing Microsporidia spores from yeast or artifacts, leading to false positives.[8] This is supported by the high number of CW-positive specimens: 98 in Saigal's study vs 57 confirmed by PCR, and 250 in Ghoshal's vs 30 PCR positives. Additionally, both studies reported similar LR+ values, but post-test probabilities differed significantly, likely because Ghoshal's study had a higher false-positive rate.

Population Differences between Studies

The included studies differed in their study populations, which may influence both clinical presentation and diagnostic performance. Saigal et al[22] included a mixed population of HIV-positive, HIV-negative, and healthy individuals, whereas Ghoshal et al[20] and El-Kerdany et al[21] focused primarily on immunocompromised patients such as those with HIV infection, hematologic malignancies, organ transplantation, or chronic kidney disease. Immunocompromised individuals typically experience higher parasite burdens and more severe or persistent diarrhea, which may increase the likelihood of detecting microsporidial spores in stool specimens.[32,33] In contrast, mixed or immunocompetent populations may have lower parasite loads or subclinical infections, potentially reducing microscopy sensitivity and contributing to variability in laboratory detection rates across studies.[34] Because intestinal microsporidiosis occurs more frequently in immunocompromised individuals,[35] the broader population included in Saigal's study may have contributed to lower microscopy sensitivity, as low-intensity infections are more difficult to detect.

Practical Applicability in Clinical Settings

The clinical application of this evidence depends on the diagnostic validity and feasibility. All three studies showed good validity by testing all subjects with both the index test and the gold standard, minimizing partial verification bias.[36] Although the degree of immunocompromise was not clearly defined, all studies involved immunocompromised patients with diarrhea, reducing the risk of spectrum bias.[37] In the Indonesian context, PCR is less feasible for routine clinical use due to limited infrastructure and cost. PCR is more likely to be suitable for molecular epidemiology and species differentiation, especially in countries with advanced prevalence data, where the true prevalence of intestinal microsporidiosis is established.[38] In addition to these, MT requires a high-concentration chromotrope, making it costlier per slide than CW. However, CW requires a fluorescence microscope, which is more expensive than the standard light microscope used for MT. Both techniques require skilled technicians for staining preparation and Microsporidia spore identification.[8,39] Considering the estimated intestinal microsporidiosis prevalence in HIV patients in Asia (0.7−33.3%)[40] and the diagnostic accuracy reviewed, MT staining is recommended for clinical use in Indonesia, particularly based on the high validity and large sample size in the study conducted in India,[20] assuming similar patient representation in Indonesia.

Conclusion

Stool smear staining, particularly MT, offers a practical alternative to PCR for diagnosing intestinal microsporidiosis in resource-limited settings, though PCR remains the most sensitive method.

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Not applicable.

Authors' Contributions
EY and AD contributed to study design, data collection, data analysis, interpretation of results, and manuscript drafting. AK contributed to data analysis, interpretation of results, and critical revision of the manuscript. All authors have read and approved the final manuscript.

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Data and Materials Availability
All original materials are included in the article.

Consent for Publication
Consent for publication was waived as this study is a

systematic literature review using previously published articles.

Competing Interests

The authors declare no conflict of interest in relation to the research, authorship, and/or publication of this article.

AI Usage Declaration

Artificial intelligence tools were not used in the research or manuscript preparation.

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