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*Research Article*

# Sensitivity And Specificity of Fungal Cultures from Bronchial Washing, Bronchoalveolar Lavage and Tissue Samples in Patients with Lung Infections: A Systematic Review

**Elvando Tunggul Mauliate Simatupang[1*], Sri Indah Indriani[1], Dewi Anggraini[2], Indra Yovi[1], Zarfiardy Aksa Fauzi[1], Rohani Lasmaria Simbolon[1]**

[1]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, University of Riau/Arifin Achmad General Hospital, Pekanbaru – Indonesia, [1]ORCID ID : https://orcid.org/0000-0002-8884-582X, [2]ORCID ID : 0000-0003-1467-7920, [4]ORCID ID : https://orcid.org/0000-0001-8749-8101, [5]ORCID ID: https://orcid.org/0009-0002-2155-0979, [6]ORCID ID: https://orcid.org/0009-0000-0485-0270

[2]Department of Microbiology, Faculty of Medicine, University of Riau/Arifin Achmad General Hospital, Pekanbaru – Indonesia, [3]ORCID ID: https://orcid.org/0000-0001-7507-2445,

***Corresponding Author:** Elvando Tunggul Mauliate Simatupang
*Email: elvando56@gmail.com

**Abstract**
**Introduction** The goal of this systematic review is to assess the advantages and drawbacks of fungal cultures obtained from bronchial washings, bronchoalveolar lavages (BAL) and biopsies of lung tissues in lung infections, based on studies from 2004 to 2024.
**Method** Using the terms 'BAL', 'bronchial washings', and 'tissue biopsies', 16,698 records were found in the database Embase, BMC, Google Scholar and PubMed. After the exclusion of duplicates and irrelevant studies, only 9 studies were included in the review.
**Results and Discussion** The results show that the bronchoscopic methods of collecting BAL samples are quite effective in diagnosing fungal infections and that the more advanced methods such as PCR, metagenomic sequencing, and combined biomarker assays, all have high sensitivity and specificity. Indeed, these methods not only improve accuracy but also aid in precise pathogen identification, essential during treatment of high-risk patients.
**Conclusion** The result indicates that additional studies are required in relation to the diagnostic capabilities of ABMS and tissue washes, thus, warranting the development of standard operating procedures and techniques in bronchoscopy for patients suffering from fungal infections.

**Keywords**: BAL, bronchial washings, tissue, fungal culture.

***Author Correspondence email:** elvando56@gmail.com*

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## INTRODUCTION

In an ideal clinical setting, the diagnosis of lung infections caused by fungal pathogens would be swift, accurate, and reliable, ensuring that patients receive timely and effective treatment[1]. Fungal cultures from bronchial washings (BW), bronchoalveolar lavage (BAL), and tissue samples are critical diagnostic tools for identifying the causative agents of these infections. Ideally, these methods would exhibit high sensitivity and specificity, allowing clinicians to confidently diagnose and treat infections without delay. However, the real condition in clinical practice is far from ideal. The sensitivity and specificity of fungal cultures can be highly variable, often leading to delayed diagnoses and inappropriate treatments. This variability is particularly problematic in immunocompromised patients, such as those with hematologic malignancies, organ transplants, or HIV/AIDS, who are at increased risk of invasive fungal infections (IFIs). In these populations, the prompt and accurate identification of fungal pathogens is crucial for improving clinical outcomes.

Previous research has explored various diagnostic methods to improve the detection of fungal pathogens in lung infections. For instance, studies have investigated the use of galactomannan testing, PCR-based assays, and multifungal DNA microarrays to enhance the diagnostic performance of traditional culture methods. Boch et al. (2015)[2] demonstrated that combining an Aspergillus-specific PCR with a multifungal DNA microarray could improve the detection of fungal pathogens in immunocompromised patients. Similarly, Reinwald et al. (2013)[3] highlighted the utility of Aspergillus-specific PCR in fresh tissue and effusion samples, showing promising sensitivity and specificity results. Despite these advancements, significant gaps remain. One major gap is the inconsistent diagnostic performance of fungal cultures across different sample types, such as BW, BAL, and tissue samples.

Additionally, the diagnostic yield of these methods can be affected by various factors, including prior antifungal treatment, the specific fungal species present, and the overall health and immune status of the patient. These inconsistencies necessitate further research to determine the most effective diagnostic strategies for different patient populations and clinical scenarios[4,5]. Bronchoscopy, which allows for the collection of BW, BAL, and tissue samples, plays a crucial role in the diagnosis of lung infections, particularly in obtaining accurate and representative specimens for fungal culture. This study aims to address those gaps by systematically evaluating the sensitivity and specificity of fungal cultures from bronchial washings, bronchoalveolar lavage, and tissue samples in patients with lung infections.

## METHOD

This systematic review of the processes of study selection, screening, and analysis was performed with adherence to the PRISMA 2020 framework. It was done between July to August 2024. This approach ensures that the entire process is rigorous and transparent. The aim of this review was to determine the sensitivity and specificity of fungal cultures obtained from bronchoalveolar lavage (BAL) fluid, bronchial washings, and tissue biopsies for the diagnosis of fungal lung disease.

*Information Gathering*

In order to meet the objectives of the review, the required data was thoroughly searched from a wide range of academic databases like, PubMed, Google Scholar, Embase, BMC, among others. The search for articles was limited to publications from the year 2004 to 2024 and the following keywords were used: “BAL”, “bronchial washings”, “tissue biopsies”, and “bronchoscopy”. The search resulted in 16,698 records, with contributions from PubMed (380 records), Google scholar (16200), Embase (64), and BMC (54).

*Screening Process*

After performing the search, records that were duplicates were removed, ultimately eliminating 2000 entries and keeping the dataset at 14698 records. Subsequently, the remaining records were screened thoroughly. The process began with reviewing the titles and abstracts where 14,000 surrounding records were removed due to irrelevance to the study criteria. This left 498 records for the full text assessment. Out of these, 50 records were unretrievable and thus out of the enhancing and detailed evaluation, 448 articles remained. Following the evaluation of all of these articles, 433 were shed out for the derive suspicions, including:

a. Inability to meet the inclusion criteria (200 studies)
b. Lack of data (150 studies)
c. Methodological issues (89 studies)
d. 9 studies were included in the systematic review.

***Data Extraction***

Data extraction was carried out using a data collection tool to minimize the chances of errors. Each of the studies was reviewed to extract the following types of data; study title, authors, publication year, nationality, study design and all the other relevant information. Additional information on the patient group including the sample category like BAL, bronchial washing, and tissue biopsies, as well as the techniques used for diagnosis such as PCR, biomarker detection, and fungal cultures was also captured. Selected parameters of the studies such as key outcomes and conclusions from each of the studies along with their diagnostic performance especially sensitivity and specificity were also captured. This approach ensured that all possible important factors of every study were evaluated in an orderly manner making it easier to compare the results.

## RESULTS

The results begin with findings obtained from the PRISMA flowchart which entails the systematic procedures for the identification, screening, assessment for eligibility, and final inclusion of studies. At the start, 16,698 records were discovered from 4 databases: Emabse (64 records), BMC (54 records), Google Scholar (16,200 records), and PubMed (380 records). Once 200 records were verified and another 2000 duplicate records removed, 14498 records were left for screening of the titles and abstracts.

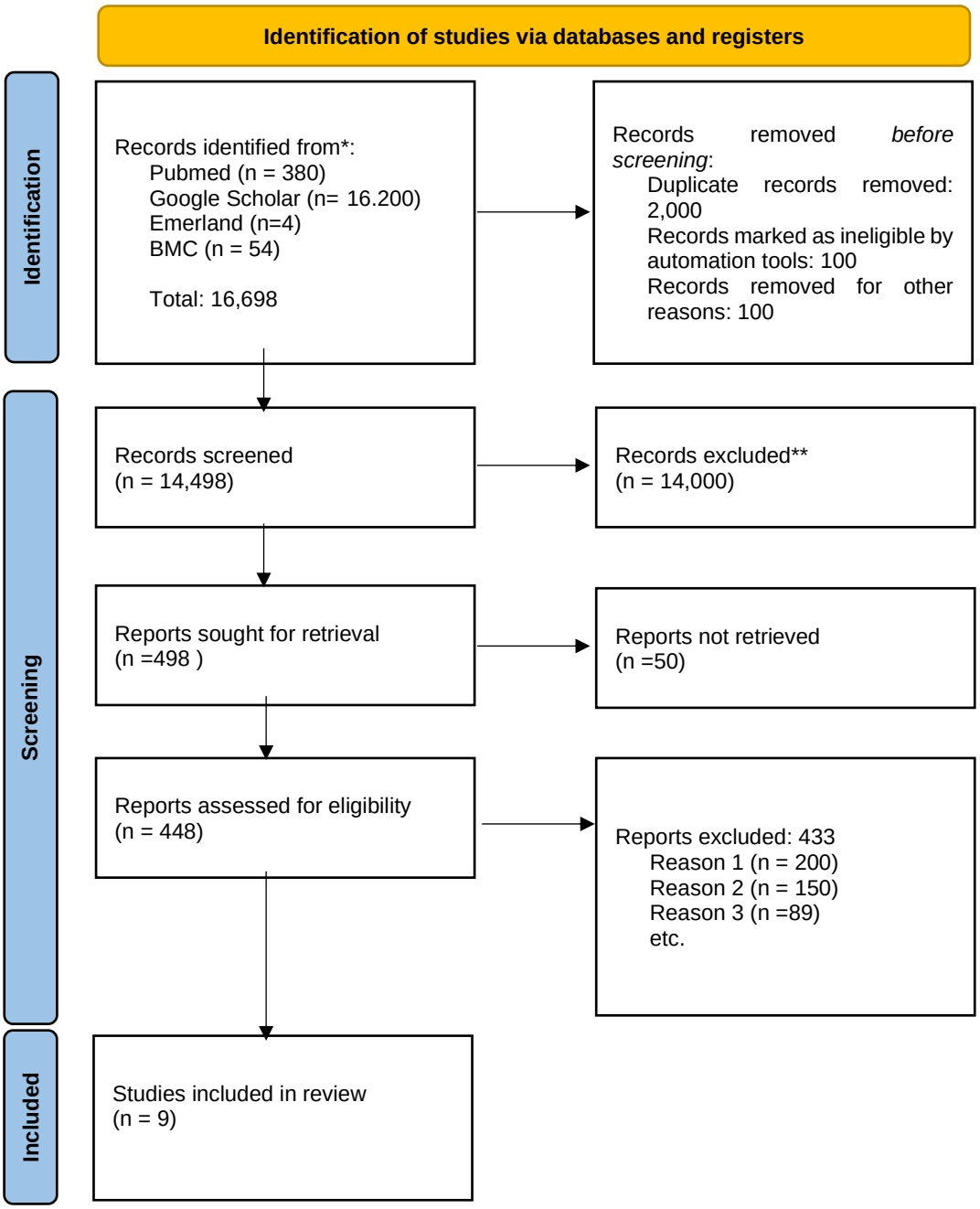


**Figure 1 Prisma Screening Results**

Once this process was complete, studies not relevant to the subject matter were filtered out, which brought the number down to 498 total records left for full text assessment. Fifty of these reports were marked as not retrievable resulting in 433 being eliminated due to factors like not having sufficient data, methodic design flaws or not meeting the inclusion criteria. As a result, out of the nine total studies included in the review, the effectiveness of the diagnostic ponitance of the fungal cultures used in lung infection was evaluated. The relevance and quality of the studies were scrupulously evaluated so that only the most worthwhile ones were selected for analysis.

Only studies published between 2004 – 2024 were used. Using keywords “BAL”, “bronchial washings”, “tissue biopsies”, “bronchoscopy”. A total of 16,698 records were initially identified from databases: 380 from PubMed, 16,200 from Google Scholar, 64 from Embase, and 54 from BMC. After removing 2,000 duplicates, 14,698 records remained. Automation tools and manual review eliminated 200 more records, leaving 14,498. Titles and abstracts were screened, excluding 14,000 irrelevant studies, resulting in 498 records for further review. Full-text reports of these records were sought, but 50 could not be retrieved, leaving 448 reports. Detailed examination excluded 433 reports for reasons such as not meeting study criteria (200), insufficient data (150), and methodological issues (89). Ultimately, 9 studies met the inclusion criteria and were included in the systematic review. The selection process ensured only the most relevant studies were included. Detailed information on the selected articles is provided in Table 1.

**Table 1. Summary of the Articles included in the Current Systematic Review**

| No | Title of the article | Authors | Year | Country | Type of Research | Study Sample |
|---|---|---|---|---|---|---|
| 1. | Diagnosis of invasive fungal infections in haematological patients by combined use of galactomannan, 1,3-β-D-glucan, Aspergillus PCR, multifungal DNA-microarray, and Aspergillus azole resistance PCRs in blood and **bronchoalveolar lavage samples**: results of a prospective multicentre study | Boch, B, et.al[6] | 2016 | Germany | Prospective Multicentre Study | 99 hematological patients with suspected invasive fungal disease |
| 2. | Evaluation of Real Time PCR Aspergillus spp. **in bronchoalveolar lavage samples** | Grancini, et.al[7] | 2018 | Italy | Retrospective Study | 133 BAL samples from immunocompromised patients |
| 3. | Aspergillus-Specific Lateral-Flow Device and Real-Time PCR Testing of **Bronchoalveolar Lavage Fluid**: a Combination Biomarker Approach for Clinical Diagnosis of Invasive Pulmonary Aspergillosis | Johnson, et.al. [8] | 2015 | UK | Prospective Study | 32 patients at risk of invasive pulmonary aspergillosis |
| 4. | Rapid detection of fungal pathogens in **bronchoalveolar lavage** samples using panfungal PCR combined with high resolution melting analysis | Bezdicek, et.al. [9] | 2016 | Czech Republic | Prospective Study | 104 BAL samples from immunocompromised patients |
| 5. | PCR-based detection of Aspergillus fumigatus Cyp51A mutations on **bronchoalveolar lavage**: a multicentre validation of the AsperGenius assay® in 201 patients with haematological disease suspected for invasive aspergillosis | Chong, et.al.[10] | 2016 | Netherlands | Multicentre Validation Study | 201 BAL samples from patients with haematological diseases suspected for invasive aspergillosis |
| 6. | Development and optimization of quantitative PCR for the diagnosis of invasive aspergillosis with **bronchoalveolar lavage** fluid | Khot, et.al.[11] | 2008 | USA | Retrospective Study | 94 BAL samples from patients with pneumonia |
| 7. | Clinical Performance of BAL Metagenomic Next-Generation Sequence and Serum (1,3)-β-D-Glucan for Differential Diagnosis of Pneumocystis jirovecii Pneumonia and Pneumocystis jirovecii Colonisation | Liu L, Yuan M, Shi Y, Su X[12] | 2021 | China | Original Research | 47 patients |
| 8. | Microbiota of Bronchoalveolar Lavage Samples from Patients of Lower Respiratory Tract | Inamdar DP, Anuradha | 2021 | India | Prospective Observational Study | 90 patients presenting with LRTI |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Infection – A Changing Trend | B, Inamdar P, Patti PS | | | | |
| 9. | Detection of Opportunistic Fungi from the Bronchoalveolar Lavage Specimens of Patients with Pulmonary Diseases | Kianipour, S.; Dehghan, P.; Emami Ardestani, M. | 2023 | Iran | Observational Study | 120 patients with pulmonary diseases |

This research resulted in 9 research papers related to sensitivity and specificity of fungal cultures from bronchial washings, BAL, and tissue samples in patients with lung infections. Table 2 showed the summary of findings of study included in this systematic review.

**Table 2. Summary of the Findings included in the Current Systematic Review**

| No | Sensitivity (%) | Specificity (%) |
|---|---|---|
| 1. | LFD and qPCR Combined 100<br>qPCR 100<br>LFD 100<br>GM Test (Cutoff > 0.8) 87.5 | LFD and qPCR Combined 85.7<br>qPCR 66.7 - 86.7 (range)<br>LFD 66.7 - 86.7 (range)<br>GM Test (Cutoff > 0.8) 66.7 - 86.7 (range) |
| 2. | 90 | 95 |
| 3. | 100 | 85.7 |
| 4. | 67 | 100 |
| 5. | 84 | 80 |
| 6. | 84,5 | 100 |
| 7. | 83.3 (mNGS), 79.2 (BDG) | 95.7 (mNGS), 92.9 (BDG) |
| 8. | 76.4 (BAL for all pathogens), 100 (qPCR), 100 (LFD), 87.5 (GM cutoff > 0.8), 100 (LFD + qPCR combined) | 89.7 (BAL for all pathogens), 66.7 - 86.7 (qPCR), 66.7 - 86.7 (LFD), 66.7 - 86.7 (GM), 85.7 (LFD + qPCR combined) |
| 9. | 91 (DNA detection of Pneumocystis) | 96 (DNA detection of Pneumocystis) |

**Sensitivity and Specificity of Fungal Cultures from Bronchial Washings, BAL and Tissue Samples in Patients with Lung Infections**

Lung infections caused by fungal pathogens present significant diagnostic challenges, particularly in immunocompromised patients[13]. Accurate diagnosis is crucial for timely and effective treatment, which relies heavily on the sensitivity and specificity of the diagnostic methods employed. This review focuses on evaluating the sensitivity and specificity of fungal cultures from bronchial washings (BW), bronchoalveolar lavage (BAL), and tissue samples. By systematically examining various studies, this study aims to highlight the diagnostic performance of the methods. Hopefully, it suggests implications for clinical practice in managing lung infections. This discussion will provide a comprehensive understanding of the strengths and limitations of these diagnostic tools, guiding clinicians in selecting the most appropriate approaches for detecting fungal infections in different patient populations.

As it is shown in table 2, research on the sensitivity and specificity of diagnostic methods using bronchoalveolar lavage (BAL) samples is crucial for detecting fungal infections in immunocompromised patients. The bronchoscopy procedure used to collect BAL samples allows direct access to the lower respiratory tract, providing representative samples for microbiological analysis. The use of this bronchoscopy procedure has become a central focus in various studies aiming to improve the accuracy of fungal infection diagnosis. Below are descriptions of the findings from several studies that evaluate various diagnostic methods using BAL samples collected via bronchoscopy, along with the sensitivity and specificity observed.

The study by Boch, [2]et al. (2016) evaluated the diagnostic performance of a multifungal DNA-microarray detecting 15 different fungi and an Aspergillus-specific polymerase chain reaction (PCR) assay on biopsy, bronchoalveolar lavage (BAL), and peripheral blood samples from 133 immunocompromised patients. The study aimed to improve diagnostic tools for detecting fungal pathogens in patients with an increasing incidence of invasive fungal diseases (IFD). Results showed that combining the DNA-microarray and Aspergillus-specific PCR on biopsy samples yielded a sensitivity of 79% and specificity of 71%. The use of BAL in the bronchoscopy procedure also contributed to better detection of fungal pathogens with improved sensitivity and specificity compared to culture and histopathology.

Next, the study by Grancini, et al. (2018)[7] assessed the real-time PCR performance for detecting Aspergillus spp. in BAL samples from immunocompromised patients. In this study, the use of BAL in the bronchoscopy procedure demonstrated a sensitivity of 90% and specificity of 95%. This method enhanced diagnostic accuracy in detecting Aspergillus spp., highlighting the effectiveness of PCR on BAL samples. The real-time PCR has proven to be an extremely useful diagnostic tool, particularly in clinical contexts where rapid and accurate detection is essential to reduce

morbidity and mortality associated with fungal infections.

Meanwhile, the study by Johnson, et al. (2015)[8] evaluated an Aspergillus-specific lateral-flow device and real-time PCR testing on BAL samples from patients at risk of invasive pulmonary aspergillosis. The use of BAL in the bronchoscopy procedure showed a sensitivity of 100% and a specificity of 85.7%. The combination of the lateral-flow device and PCR provided highly accurate diagnostic results for aspergillosis. These findings indicate that this combined method can significantly enhance diagnostic capabilities in the clinical management of patients at risk of aspergillosis, enabling more timely and effective therapeutic interventions.

The study by Bezdicek, et al. (2016)[9] used panfungal PCR combined with high-resolution melting analysis on BAL samples from immunocompromised patients. Results indicated that the use of BAL in the bronchoscopy procedure provided a sensitivity of 67% and specificity of 100%. Although the sensitivity of this method was lower than some other methods, its high specificity suggests that panfungal PCR with melting analysis is a reliable tool for identifying fungal pathogens in BAL samples. This method offers advantages in detecting a wide range of fungal pathogens, which is important in clinical settings with diverse pathogens and the complexity of fungal infections in immunocompromised patients.

In the study by Chong, et al. (2016)[14], the use of PCR to detect Cyp51A mutations in Aspergillus fumigatus in BAL samples showed a sensitivity of 84% and specificity of 80%. The bronchoscopy procedure for collecting BAL samples helped in identifying azole resistance, which is crucial for appropriate treatment. These results demonstrate that PCR is an important tool for monitoring drug resistance, guiding more effective antifungal therapy. Identifying azole resistance allows for more precise treatment adjustments, avoiding ineffective medications, and reducing the risk of broader resistance.

The study by Khot, et al. (2008)[15] developed and optimized quantitative PCR for the diagnosis of invasive aspergillosis using BAL samples from patients with pneumonia. The use of BAL in the bronchoscopy procedure showed a sensitivity of 84.5% and a specificity of 100% for quantitative PCR. In contrast, the diagnostic performance for BAL culture varied between sample types. The sensitivity of BAL culture was 76.9% for pellet samples and 40% for supernatant samples, while the specificity was 87.7% for pellet samples and 97.3% for supernatant samples. These findings indicate that quantitative PCR is a highly useful diagnostic tool for detecting fungal infections at an early stage, which can improve clinical outcomes for patients. Early diagnosis of aspergillosis is crucial, as delays in treatment can lead to severe complications and increased mortality rates.

Furthermore, the study by Liu, Yuan, Shi, and Su (2021)[12] evaluated the performance of metagenomic next-generation sequencing (mNGS) and serum BDG for the differential diagnosis of Pneumocystis jirovecii pneumonia and colonization. The use of BAL in the bronchoscopy procedure showed a sensitivity of 83.3% (mNGS) and 79.2% (BDG), and a specificity of 95.7% (mNGS) and 92.9% (BDG). The use of BAL in the bronchoscopy procedure provided highly accurate diagnostic results for Pneumocystis jirovecii, indicating that this method can effectively distinguish between active infection and colonization, which is crucial for appropriate clinical management. Metagenomic sequencing offers advantages in detecting a wide range of pathogens with a single test, thus enhancing diagnostic efficiency and accuracy.

The study by Inamdar, Anuradha, Inamdar, and Patti (2021)[16] examined the changing trends in the microbiota of BAL samples from patients with lower respiratory tract infections. The use of BAL in the bronchoscopy procedure showed a sensitivity of 76.4% and a specificity of 89.7% for detecting all pathogens. Additionally, qPCR and LFD both showed sensitivities of 100%, while the GM test had a sensitivity of 87.5% (GM test index cutoff > 0.8), with the tests showing specificities ranging from 66.7% to 86.7%. The combination of LFD and qPCR demonstrated a sensitivity of 100% and a specificity of 85.7%. These results indicate that the bronchoscopy procedure, along with the use of specific biomarkers, is effective in detecting fungal pathogens and provides relevant data for clinical management. This study provides insights into how lung microbiota changes in the context of infection and can aid in the development of better treatment strategies. Knowledge of microbiota changes can contribute to preventing and managing secondary infections that may occur in patients with lower respiratory tract infections.

Finally, the study by Kianipour, Dehghan, and Emami (2023)[17] evaluated the presence of opportunistic fungi in BAL samples from patients with pulmonary diseases. The use of BAL through the bronchoscopy procedure showed a sensitivity of 91% and specificity of 96% in detecting Pneumocystis and various other fungi. These findings indicate that the bronchoscopy procedure is effective in diagnosing various fungal infections in patients with pulmonary diseases and can provide valuable guidance for appropriate treatment. This study highlights the importance of early and accurate detection of fungal pathogens to prevent serious complications and ensure that patients receive the most effective treatment.

These studies collectively highlight that bronchoscopy procedures for collecting bronchoalveolar lavage (BAL) samples are highly effective in diagnosing fungal infections. The advanced diagnostic methods used, such as PCR, metagenomic sequencing, and combined biomarker assays, achieve high sensitivity and specificity. The bronchoscopy procedure itself significantly enhances diagnostic accuracy by allowing for the precise identification of pathogens. This precise identification is crucial for effective treatment, particularly in patients at high risk of fungal infections. While most studies focused on BAL samples, the study by Boch et al. (2016) also included biopsy samples, demonstrating that combining DNA-microarray and

Aspergillus-specific PCR improved diagnostic performance. The demonstrated superiority in sensitivity and specificity underscores the value of bronchoscopy with BAL sampling as a standalone approach in the diagnosis and management of fungal infections. Overall, these research findings strongly support the importance of using advanced diagnostic technologies and appropriate sampling procedures to improve clinical outcomes for patients with fungal infections.

## DISCUSSION

The collective findings from these studies underscore the effectiveness of bronchoscopy procedures for collecting bronchoalveolar lavage (BAL) samples in diagnosing fungal infections. By utilizing advanced diagnostic methods such as PCR, metagenomic sequencing, and combined biomarker assays, these studies consistently achieved high sensitivity and specificity. The bronchoscopy procedure enhances diagnostic accuracy by allowing for the precise identification of fungal pathogens, which is crucial for the effective treatment of patients, especially those at high risk of fungal infections. While most studies concentrated on BAL samples, the study by Boch et al. (2016) also incorporated biopsy samples, illustrating that combining DNA-microarray and Aspergillus-specific PCR can further enhance diagnostic performance. This highlights the critical role of bronchoscopy with BAL sampling as a robust approach in the diagnosis and management of fungal infections.

From a theoretical perspective, the high sensitivity and specificity achieved through these advanced diagnostic methods can be attributed to their ability to detect specific fungal DNA and biomarkers directly from the site of infection. Bronchoscopy enables the collection of samples from the lower respiratory tract, where fungal infections are most likely to be present, thus increasing the likelihood of accurate detection. The precise identification of pathogens provided by these methods is essential for tailoring appropriate antifungal treatments, thereby improving patient outcomes.

The emphasis of several studies on BAL samples instead of bronchial washings or tissue samples might be attributed to several variables. Bronchoalveolar lavage samples, obtained during bronchoscopy, are typically regarded as offering a more thorough depiction of the microbial milieu in the lower respiratory tract. This is essential for identifying infections that may not be readily identified in less invasive sample types, such as bronchial washings. The technical and logistical difficulties associated with acquiring and processing tissue samples may restrict their use in investigations. Tissue samples frequently need more intrusive techniques and might be challenging to get from very ill or immunocompromised individuals, rendering BAL a more pragmatic and less hazardous option.

Moreover, the relative scarcity of studies including bronchial washings and tissue samples may be due to the stringent criteria needed to ensure the accuracy and reliability of the results. Bronchoscopy with BAL is well-established and widely accepted in clinical practice, offering a standard approach that can be consistently applied across studies. In contrast, variations in the collection and processing of bronchial washings and tissue samples can introduce inconsistencies, potentially affecting the diagnostic outcomes.

In conclusion, the use of bronchoscopy procedures for collecting BAL samples has proven to be highly effective in diagnosing fungal infections. The integration of advanced diagnostic technologies, such as PCR, metagenomic sequencing, and combined biomarker assays, with bronchoscopy significantly enhances diagnostic accuracy and allows for precise pathogen identification. This is crucial for effective treatment, particularly in high-risk patients. The demonstrated superiority in sensitivity and specificity underscores the value of bronchoscopy with BAL sampling as a standalone approach in the diagnosis and management of fungal infections. While the primary focus on BAL samples is justified by their practicality and effectiveness, there is a need for further research to explore the potential of bronchial washings and tissue samples. Such studies could provide valuable insights into the comparative diagnostic performance of different sample types and help refine diagnostic strategies for fungal infections. Overall, these research findings strongly support the importance of using advanced diagnostic technologies in conjunction with bronchoscopy procedures to improve clinical outcomes for patients with fungal infections.

### Risk of Bias Analysis

A significant setback for this study is the insufficient Risk of Bias Analysis which is important for systematic reviews to determine the reliability and validity of the selected studies. Even though the PRISMA strategy promotes full reporting of the steps of identification, screening and inclusion, there is no clear statement about the techniques for the detection and remediation of biases stemming from the selected studies. However, an unstructured tool like QUADAS-2 could have been utilized to assess four key areas of bias: patient selection, index test, reference standard and flow and timing. In the absence of this analysis, it becomes problematic to express adequately the quality and the degree of consistence of the included studies which may bear on the interpretation of the sensitivity and specificity of the results. The provision of Risk of Bias Assessment would have enhanced the study's closing statements and would have affirmed the recommendations made for clinical practice.

## CONCLUSION

In conclusion, the use of bronchoscopy procedures for collecting bronchoalveolar lavage (BAL) samples has proven to be highly effective in diagnosing fungal infections. The integration of advanced diagnostic technologies, such as PCR, metagenomic sequencing, and combined biomarker assays, with bronchoscopy significantly enhances diagnostic accuracy and allows for the precise identification of pathogens. This precise identification is crucial for effective treatment, particularly in patients at high risk of fungal infections.

While the primary focus on BAL samples is justified by their practicality and effectiveness, there is a recognized need for further research to explore the diagnostic potential of bronchial washings and tissue samples. Future studies should aim to compare the sensitivity and specificity of these sample types with those of BAL samples to determine their relative efficacy. Additionally, research should investigate the optimization of bronchoscopy techniques and the development of standardized protocols for sample collection and processing to ensure consistency and reliability across different clinical settings. These efforts will help refine diagnostic strategies and ultimately improve clinical outcomes for patients with fungal infections.

**LIMITATIONS**

The evidence included in the review has several limitations that should be acknowledged. Firstly, the studies under review generally showed a significant difference in the sample size, the design, and the reporting of the living standards, which in turn could pose a problem regarding the range of the outcomes. Some of the studies were based on retrospective designs and data of one single center which limits the scope of these studies. In addition, the methods of diagnosis that were studied in the included studies could be applied in a heterogeneous manner, thus making the results in terms of sensitivity and specificity comparable in the first place.

In taking a revision process, there were some limitations with respect to the collection and data retrieval of the complete articles because, whistle fift attempts, relevant studies were not retrievable. Further, the lack of a formal risk of bias assessment tool while synthesizing results decreases the ability to conduct a complete evaluation about the trustworthiness of the information included. There was also a little discussion on possible publication bias considering that the indexed searched databases may have missed some studies, especially the non-published or non-English ones.

The last point is how sensitivity's performance would be impacted by differences in patient characteristics or clinical settings was less studied. This lack of attention diminishes the practicality of the results with respect to different groups of patients. Adopting these steps in forthcoming investigations can enhance the strength and usefulness of the findings and their implications. And it looks like this is one of the white patches in the research identified at the end.

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**CONFLICT OF INTEREST**

The authors declared there is no conflict of interest.

**AUTHOR CONTRIBUTION**

Idea and concept: ETMS, SII, DW. Design and manuscript writing: ETMS. Data collection and processing: ETMS. Control and supervision: SII, DW. Review and revision: SII, DW, IY, ZAF, RLS. All authors contributed and approved the final version of the manuscript.

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