Science Forum (Journal of Pure and Applied Sciences)

Journal Homepage: https://atbuscienceforum.com.ng/index.php/jpas
https://doi.org/10.70882/edtfms31

# Rapid fungal diagnostics utilizing genomic techniques and artificial intelligence

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Abstract

Despite its high morbidity and mortality rates, invasive fungal infections are one of the most common infections in clinical care today, in part due to the lack of rapid and sensitive diagnostic techniques. Over the past few years, new genomic technologies such as next-generation sequencing, metagenomic sequencing, and CRISPR-based assays, paired with artificial intelligence (AI), have revolutionized fungal diagnostics by introducing culture-independent, rapid, and accurate pathogen detection. Automated data analysis, species identification, antifungal resistance prediction, and clinical decision support are additional strengths of AI in the field of diagnostics. This review highlights the latest developments in genomic and AI-driven fungal diagnostics, their clinical uses and the challenges that still lie ahead, as well as their future prospects for precision diagnostics and tailored treatment for individual patients.

Keywords
Invasive Fungal Infections;
Genomic Diagnostics;
Next-Generation Sequencing;
Metagenomic Sequencing;
CRISPR;
Artificial Intelligence;
Machine Learning;
Precision Medicine;
Fungal Diagnostics;
Antifungal Resistance.

ISSN 1119-4618 JPAS
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## 1. Introduction

### 1.1 Global Burden of Invasive Fungal Infections

IFIs are now a significant worldwide problem especially for patients in hospital or who are immunocompromised. New medicines have led to more people being vulnerable, such as those having chemotherapy, an organ transplant, long-term corticosteroid treatment or intensive care. Furthermore, the increased incidence of diabetes mellitus and chronic respiratory diseases has also increased the vulnerable population. Morbidity and mortality associated with clinically important pathogens (Candida spp., Aspergillus spp., Cryptococcus spp., and those of the order Mucorales) are high, particularly if the diagnosis is delayed (Hoenigl et al., 2021).

In addition to mortality, IFIs cause significant clinical and economic burden with prolonged hospital stay, intensive antifungal therapy and higher healthcare costs. They are not easy to diagnose early because their clinical symptoms are not specific, and they are resembling symptoms of bacteria or virus infection. The timely and accurate identification of a pathogen is crucial to ensure timely appropriate antifungal treatment, which adversely affects patient outcomes, and is why rapid and accurate diagnostic techniques are important. (Jenks et al., 2021).

### 1.2 Limitations of Conventional Diagnostic Methods

Progress has been achieved in clinical microbiology, but only a handful of "old fashion" techniques are available for diagnosis of the fungus. Culture-based diagnosis is regarded as the traditional reference standard, but is often a time-consuming process, as culture of the fungus is a slow process and can lead to false-negative results due to previous antifungal therapy or low fungal burden. In the same way, the results of microscopy and antigen tests are rapid but often lack sensitivity, specificity, and require experience in operation. However, biomarkers like galactomannan and β-D-glucan can also lead to false-positive or false-negative results and therefore are not reliable as single diagnostic tests (Jenks et al., 2021). These restrictions can lead to delayed diagnosis, delaying targeted antifungal treatment, and to disease progression, treatment failure and death. Accordingly, molecular diagnostic methods are urgently needed in clinical practice in order to get a rapid and highly sensitive diagnosis.

### 1.3 Emergence of Genomics and Artificial Intelligence

With the emergence of genomic technologies and artificial intelligence (AI), fungal diagnosis is undergoing a radical shift in which the ability to quickly identify a pathogen with culture independence is changing the landscape. Next generation sequencing (NGS), especially metagenomic sequencing, has the ability to detect multiple fungal pathogens, mixed infections, and identify rare and emerging fungal pathogens directly from clinical isolates.

These advances are helpful for precision diagnosis by providing a full picture of the genome for clinical decisions. These are complemented by an AI's ability to use machine learning algorithms to interpret complex clinical and genomic data to better identify pathogens, predict antifungal resistance and support clinical decision making. Genomics and AI can be used together to enhance the accuracy of diagnosis, reduce the time to diagnosis and enable personalized treatment for invasive fungal infections. This review, therefore, highlights recent developments in genomic technologies and tools for AI-assisted fungal diagnosis, addresses clinical use and challenges as well as future directions for integration into clinical practice.

## 2. Current Challenges in Rapid Fungal Diagnosis

### 2.1 Diagnostic Challenges in Emerging Mycoses

The epidemiology of invasive fungal infections has undergone significant changes in the last decade, due to increased geographic distribution and the emergence of new pathogens, as well as the increasing number of immunocompromised patients. The clinical features of many emerging fungi are non-specific and the resistance to various antifungal agents is variable, so it is very important to identify the fungi to species level quickly in order to provide appropriate treatment.

Among all emerging mycoses, mucormycosis is one of the most serious ones, mostly seen in diabetes, haematological malignancy, organ transplant or long-term immunosuppression. The high mortality rate and the ability to invade the tissues rapidly make early diagnosis imperative; however, conventional culture is not very sensitive and histopathological confirmation requires invasive sampling, which could delay the appropriate therapy (Liang et al., 2024).

Likewise, Candida auris has emerged as a significant HAI due to its environmental stability, a high capacity for fast spread in hospitals, and resistance to multiple drugs. Conventional laboratory procedures have failed to provide the necessary information for correct identification because they do not detect genetic changes, and in fact, genomic surveillance has shown the global dissemination of genetically distinct clades, indicating the need for accurate molecular identification (Bing et al., 2024). Candida tropicalis is another emerging pathogen that is causing invasive candidiasis and is becoming more resistant to azoles. Tandem gene duplication and other adaptive mutations have been found to be critical to resistance and it is important that forewarning of emerging resistant strains is possible by using rapid molecular diagnostics before resistance occurs or treatment fails (Fan et al., 2023).

**2.2 Antifungal Resistance**

Clinical mycology is facing a huge problem today because of the emergence of antifungal resistance due to the excessive use of antifungal agents in medicine and agriculture. Treatment efficacy is compromised by resistance mechanisms which include target site mutations, overexpression of efflux pumps, altered sterol biosynthesis and genomic adaptation, and these mechanisms make patient management more challenging.

Clinicians are forced to prescribe empirical therapy before the susceptibility testing results because the conventional susceptibility testing has strict requirements and takes a long period to be completed. Resistant fungal infections are linked to death, longer hospital stays, greater healthcare expenditure and HA outbreaks, highlighting the need for fast and accurate diagnostics for both fungi and resistance. The dermatophytes are a good example of this new problem. Terbinafine resistant Trichophyton indotineae has become widespread, causing suboptimal treatment with persistent infection, which often necessitate molecular diagnosis and genomic characterization for effective management (Gupta et al., 2024a). The increasing global spread of dermatophyte resistance further underscores the need for molecular surveillance, standardized laboratory testing, and antifungal stewardship efforts, in order to maintain the current therapeutic options (Gupta et al., 2024b).

**2.3 Need for Earlier and More Accurate Detection**

Novel fungal pathogens as well as rising antifungal resistance have led to a dependence on outdated diagnostics. Species identification and resistance testing are typically delayed, leading to an empirical approach to antifungal treatment that can result in treatment failures and exposure to broad-spectrum drugs. Thus, rapid molecular diagnostics are crucial to precision medicine. Ideally these platforms will be able to correctly identify the fungi type, identify mixed infections, identify resistance associated genetic markers and provide clinically actionable results in hours. This would enable earlier targeted therapy, help in controlling infections and help in the improvement of patient outcomes.

The new technologies of genomic sequencing, molecular diagnostics and artificial intelligence offer potential solutions by allowing for fast, precise and highly sensitive identification of pathogens and species-specific identification, resistance prediction and personalized therapeutic decision making. Fungal diagnostics has progressed from basic culture and microscopy to PCR, NGS, metagenomic sequencing and the introduction of AI-driven platforms, with continuous advances made in terms of: (1) Diagnostic speed, (2) Diagnostic accuracy, and (3) Clinical usefulness.


Figure 1. Evolution of fungal diagnostic technologies

## 3. Genomic Technologies Transforming Fungal Diagnostics

### 3.1 Next-Generation Sequencing (NGS)

Next-Generation Sequencing has changed the diagnostics of infectious diseases because of the technology's high throughput sequencing capability within a relatively short period. Unlike conventional DNA sequencing, NGS sequences millions of DNA molecules at once. This enables the identification of the infecting organism, variations, and resistance-associated genes from a single clinical specimen. Advantage of NGS has overcome the limitations of conventional microbiological diagnosis which often results in inconclusive results, and has improved precision diagnostics of invasive fungal infections. It is a powerful technology that involves a number of steps: nucleic nucleic acid extraction from clinical samples, preparation of a library, massively parallel sequencing, then

downstream sequence bioinformatics analysis by reference databases for identification. Sequencing can be done on either a specific genomic region or a whole genome, and can give in-depth information about pathogen identity, strain diversity, virulence factors, and resistance-associated mutations (as described by Rehder et al., 2021).

Due to the ease of use of NGS, the technology has emerged as a valuable clinical diagnostic tool for fungal identification, enabling rapid identification at species level directly from clinical samples with reduced culture times. It has particular value for the diagnosis of rare, emerging, or fastidious fungal pathogens, and can be used for diagnosis of mixed fungal, bacterial and viral infections, which is more thorough than the traditional methods (Rehder et al., 2021). NGS is more sensitive, broader spectrum, and culture independent than traditional methods. Moreover, genomic information is useful for antifungal resistance prediction, epidemiological surveillance and precision medicine. The advent of NGS is likely to have a greater impact in the field of routine fungal diagnosis as sequencing costs decrease and the tools to analyse the resulting data become more widely available (Rehder et al., 2021).

**3.2 Technical Challenges and Quality Assurance**

Although NGS is clinically very promising, its widespread adoption is hindered by technical, operational and regulatory issues. To ensure reliable diagnostic performance, all aspects of the workflow from sample collection to nucleic acid extraction, library preparation, sequencing, bioinformatics analysis, to data interpretation must be optimized. Each of these steps could introduce variability that influences the diagnosis, so it is crucial to standardise laboratory procedures and to ensure an extensive quality assurance program is implemented (Yin et al., 2021).

One of the main problems is the lack of a standardisation of sequencing platforms, sample preparation protocols, sequencing depth, and bioinformatics pipelines among laboratories, which hampers comparisons between them and limits the reproducibility of results. International harmonisation of protocols and reference standards are therefore crucial for consistent clinical implementation (Yin et al., 2021). For NGS to be used routinely in the clinic, it is also important to validate its clinical performance before it becomes a universal tool for diagnosis.

Laboratories should define the assay sensitivity, specificity, accuracy, reproducibility and detection limit for all different types of specimens and fungal pathogens, and maintain continuous quality monitoring by using internal control samples and external proficiency testing (Ménard et al., 2021). The implementation into the clinic must also meet regulatory requirements for assay validation, quality management, data integrity and patient safety. Furthermore, a range of specialized sequencing equipment, computational power, secure data storage, and multi-disciplinary knowledge is required to identify and interpret the complex genomic data from samples correctly and to differentiate between true pathogens and contaminants. Presently, sequencing technologies keep improving in terms of speed and cost, but clinical adoption depends on good clinical workflows, quality control (quality management) systems, and robust laboratory infrastructure (Yin et al., 2021; Ménard et al., 2021).

**3.3 Clinical Metagenomic Sequencing**

One of the biggest breakthroughs in infectious disease diagnosis is the introduction of clinical metagenomic sequencing: a method that allows the detection of pathogens directly from clinical samples without making any hypotheses. Metagenomic next-generation sequencing (mNGS) is a global analysis of all the nucleic acids in a sample, which means it can detect all fungal, bacterial, viral and parasitic pathogens in a single test. This unbiased approach is especially useful for culture negative diseases, polymicrobial infections, atypical and rare fungal infections, which are hard to diagnose with standard techniques (Govender et al., 2021). The high diagnostic yield is one of the benefits of mNGS. It is not only organism-specific primerless, culture enrichment-free, but also

contributes to greater detection of pathogens and reduces diagnostic uncertainty.

This approach is useful inachieving rapid speciation and ensuring appropriate antifungal therapy there by improving the management of criticallyill patient s with immunosuppression in invasive fungal infections (Govender et al., 2021). Sample preparation is a key factor for the performance of a metagenomic sequencing strategy. Efficient extraction, contamination control and standardized library preparation are important to maximize sensitivity, and ensure reproducible diagnostic results, as the amount of fungal DNA is often low and there is significant host nucleic acid present in clinical specimens (Carbo et al., 2021). One of the big technical problems is host DNA contamination that may be present in blood, tissue and respiratory samples and may constitute the bulk of the nucleic acid.

Targeted depletion of human DNA can specifically deplete human DNA, thereby improving the depth of the sequencing, the sensitivity of the analysis and the overall diagnostic value in detecting fungi (Kim et al., 2024). To conclude, the mNGS could be a useful overall method for pathogen detection, identification of co-infections and genomic analysis of a single sample. The further development of sequencing chemistry, host DNA depletion, bioinformatics, and workflow automation will further enhance precision fungal diagnostics and shorten turnaround times for metagenomic sequencing in the future (Govender et al., 2021; Carbo et al., 2021; Kim et al., 2024).

**3.4 Emerging Sequencing Applications**

New applications of sequencing are expanding the possibilities of genomic diagnostics, making it more efficient, bioinformatics capable and clinically available. The developments will help push towards the establishment of accurate fungal diagnostics. Clinical metagenomic workflows have become standardized, bringing together automated sample processing, library preparation, high throughput sequencing and computational analysis. The optimized workflows help to minimize turnaround time and enhance the efficiency of the laboratory, while also enabling the routine use in clinical microbiology laboratories (Tan et al., 2024). Sequencing has also come into cytology, allowing genomic analysis of very small biopsy samples, with the major benefit of leaving behind a precious diagnostic sample. The successful application of NGS in the context of cytopathology suggests its use in the fungal diagnosis of fine needle aspirates, bronchoalveolar lavage, and other cytological samples (Pisapia et al., 2021).

Genome annotation is also very important in the process of genome characterization as this aspect enables meaningful analysis of the genome. This automatic workflow identifies coding sequences, estimates their function and normalizes genomic data for subsequent efficient analysis of pathogen-related genomic data and identification of relevant genetic determinants (Schwengers et al., 2021). Genomic sequencing has been progressing along to become a clinical tool, as seen in advances in precision genomic diagnostics.

Sequencing is becoming increasingly utilized in the clinic as seen in Precision Genomic Diagnostics. The specific sequencing and computational analysis will help to develop personalized diagnostic strategies and eventually, the identification of pathogen-specific sequences in genome and susceptible factors in the host will be used for personalized diagnosis (Lee & Abraham, 2021). Together, these new applications show that genomic technologies are far from just identifying pathogens. Their optimized sequencing workflow, cutting-edge bioinformatics, constant genome annotation, and precision genomic analysis is laying the groundwork for fast, all-encompassing, and personalized diagnostic platforms for fungi. As genomic technologies have developed and evolved, the number of diagnostic options for fungal infections has increased and each platform has its own set of benefits and drawbacks.

The choice of technology is dependent on the clinical setting, necessary diagnostic resolution, laboratory capacity and turnaround time. Table 1

summarizes the main principle, advantage, disadvantage, and major clinical use of the main genomic technologies that are currently available for rapid fungal diagnosis.

**Table 1. Comparison of Genomic Technologies Used for Rapid Fungal Diagnosis**

| Technology | Principle | Turnaround Time | Advantages | Limitations | Clinical Application |
|---|---|---|---|---|---|
| **Targeted NGS** | Sequencing of predefined fungal genetic targets | 24–48 h | High sensitivity, species-level identification, detection of resistance-associated mutations | Requires prior target selection; limited detection of unexpected pathogens | Identification of suspected fungal pathogens and resistance-associated variants |
| **Whole Genome Sequencing (WGS)** | Sequencing of the complete fungal genome | 2–5 days | Highest genomic resolution, outbreak investigation, evolutionary analysis, resistance characterization | High computational demand, greater cost, longer analysis time | Epidemiological surveillance, strain typing, comprehensive genomic characterization |
| **Metagenomic Sequencing (mNGS)** | Unbiased sequencing of all nucleic acids within a clinical specimen | 24–72 h | Simultaneous detection of multiple pathogens without prior assumptions; identification of mixed infections | High host DNA background, complex bioinformatics, relatively high cost | Diagnosis of culture-negative, polymicrobial, or rare fungal infections |
| **CRISPR-based Detection** | CRISPR/Cas-mediated recognition of pathogen-specific nucleic acid sequences | <2 h | Rapid, highly specific, simple workflow, potential point-of-care application | Limited multiplexing and dependence on target-specific guide RNAs | Rapid molecular detection of specific fungal pathogens |
| **Single-cell Technologies** | Analysis of individual microbial cells using advanced spectroscopic or sequencing methods | Several hours | High-resolution cellular characterization, rapid species identification, minimal sample preparation | Specialized instrumentation, limited clinical availability | AI-assisted fungal identification, research, and precision diagnostics |

## 4. Artificial Intelligence in Rapid Fungal Diagnostics

Artificial intelligence (AI) is one of the most revolutionary technologies in clinical microbiology, revolutionizing the diagnosis, monitoring and management of infectious disease. As a result of the increasing number of high-dimensional clinical, proteomic, microbiological and genomic datasets, computational algorithms that can extract meaningful biological patterns, which are frequently too complex to analyse by hand, have emerged. In fungal diagnostics, AI can be used alongside sophisticated molecular technologies to streamline pathogen identification, enhance diagnostic accuracy, forecast antifungal resistance, and aid in making evidence-based clinical decisions. AI does not replace laboratory analysis; it is used as a clever analytical layer over the vast amount of increasingly complex biological information, allowing diagnosis of invasive fungal infections to be quicker and more personalized for patients.

### 4.1 Artificial Intelligence in Clinical Microbiology

Artificial Intelligence is a broad term for numerous computing techniques that enable a computer program to simulate human intelligence to interpret patterns, categorize, predict, and make decisions. AI is being used more and more in clinical microbiology to boost lab efficiency, automate data analysis and support clinicians in diagnosing and treating infectious diseases. The adoption of AI technologies such as molecular diagnostics, digital pathology, medical imaging and genomic sequencing has greatly contributed to the transition from the laboratory approach to precision microbiology, where decisions regarding diagnosis and treatment are taken with the support of advanced computational analysis. Machine learning (ML), one of the key areas of AI, is the backbone of many contemporary diagnostic applications. ML algorithms can identify statistical relationships between input variables and diagnostic outcomes by training on a large amount of data. Supervised learning algorithms can be used for classification of known pathogens, and unsupervised learning algorithms can be used to search for patterns within the large microbial datasets that have never been discovered before. They can be employed to help determine the species of fungi, to predict antimicrobial susceptibility, and to help monitor and detect outbreaks, and to improve laboratory workflows.

The more diverse the training data are, the more likely the algorithm will be able to recognize the patterns and adapt to new variations of fungal organisms and resistance mechanisms (Mairi et al., 2025). Deep learning is one of the machine learning techniques based on a multi-layered artificial neural network which simulates the working mechanism of the human brain for learning and knowledge processing.

DL can learn features from raw unstructured data without the need for data cleaning and engineering, unlike traditional ML algorithms. The application of DL algorithms in medical microbiology includes the discovery of patterns in the genomic DNA, histopathology data, protein mass spectrometry data, etc. These algorithms have enabled species identification to be automated and minimised human error when handling large numbers of images. Moreover, this approach has improved the diagnosis and identification of fungal biomarkers with greater accuracy in fungal

infections. Moreover, algorithms can be trained to analyse the genome sequence and predict virulence-associated traits and mechanisms of resistance that can be targeted in treatment and counter-measures, in clinical microbiology. AI also has a potential use in clinical microbiology that is the design of clinical decision support systems (CDSSs) for diagnosis of infectious diseases. Such computer programs may combine laboratory data and patient details, with medical histories, radiology and pathology reports and genetic data to treat patients suffering from infectious diseases. AI can predict the likelihood of a fungal infection, narrow down the differential diagnosis, recommend which lab tests to order to confirm a diagnosis, estimate a fungus's reaction to anti-fungal treatments, and make a treatment recommendation.

These systems can help improve patient care and management by providing faster diagnosis and treatment of patients with fungal infections, as well as relieving doctors of the burden of making complex diagnoses. This is especially relevant when caring for the patient in the intensive care unit (ICU), where the ability to diagnose and treat patients quickly can have a beneficial effect on patient care and outcomes (Mairi et al., 2025).

**4.2 AI-Assisted Fungal Identification**

One of the most promising directions for the use of AI is the development of automated diagnostic tools using intricate spectroscopic and molecular techniques for fast fungal diagnosis. The traditional identification of fungi are carried out through culture and biochemical testing, or through microscopic morphology, both of which are time consuming and requires expertise. AI-powered analytical platforms have the capability to identify fungal species with high accuracy and little human effort, by recognizing complex biological patterns automatically. The new technology in this field is the fusion of Artificial intelligence with single-cell Raman spectroscopy (SCRS). Raman is a form of label-free spectroscopy that depends on the interaction of photons with biological organisms to obtain the spectral fingerprint of individual cells from microbial cells. The Raman spectra are a very specific molecular fingerprint, depending on the species of fungi, and can be analysed computationally to identify the fungi rapidly. Compared with culture-dependent methods (Xu et al. 2023), SCRS is a minimally sample processing, and can yield diagnostic information in relatively short time, an appealing approach for time sensitive clinical applications.

Raman spectroscopy produces the extremely complex spectra, which are interpreted with the help of artificial intelligence. The algorithms of machine learning are trained with thousands of reference spectra from known fungal species to allow automated classification of unknown clinical isolates with a high degree of accuracy. Spectral differences between lesions that would be difficult for the operator to identify can be detected by AI, and will improve the sensitivity and specificity of diagnosis, while minimizing operator-dependent variation.

This computational approach has been proven valuable in the speedy identification of microorganisms and to address some of the limitations of traditional microbiological methods for detecting priority fungal organisms, like the closely related Candida species (Xu et al., 2023). Besides species

identification, there are other clinical applications that AI-aided Raman spectroscopy has great potential. Computational analysis may help with discrimination between susceptible and resistant fungal isolates, identification of metabolically active fungi, and monitoring of dynamic cell response when exposed to antifungal therapy.

Therefore, a combination of fast spectroscopic measurement with advanced machine learning is a promising tool to conduct real-time diagnosis of fungus, which could significantly shorten lab turnaround time and achieve more accurate diagnosis. The routine clinical microbiology laboratory is expected to see more and more applications of AI-assisted spectroscopic technologies over time with the continued development of instrumentation and computational algorithms.The use of AI in clinical microbiology laboratories is growing, and, with the maturing of these technologies, this trend will likely continue in the future (Xu et al., 2023).

**4.3 Machine Learning Coupled with MALDI-TOF Mass Spectrometry**

The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a widely popular technique for microbial identification in a clinical microbiology laboratory due to its speed, accuracy and relatively low cost of operation. The method is used to detect microorganisms through the generation of characteristic protein mass spectra which acts as a unique molecular fingerprint for a particular species. While the bulk of conventional MALDI-TOF analyses are made by comparison with spectral libraries of reference materials, recent innovations in the field of artificial intelligence have significantly broadened the diagnostic capabilities of MALDI-TOF, with the use of powerful machine learning algorithms (Weis et al., 2022).

The machine learning approach allows for the computational analysis of very complex MALDI-TOF spectra that can go beyond just species matching. AI algorithms are not only based on pre-defined reference databases, but are also looking at the subtle spectral features linked to the taxonomy, the phenotypic traits and the antimicrobial susceptibility of the microbes. This analytical approach can enhance the accuracy of identification of organisms, especially for microbial species that are very similar in amino acid profile, as determined by conventional analysis methods. In addition, further algorithm training based on continuously growing spectral databases can result in continuous improvements in diagnostic ability over time (Weis, et al., 2022).

The ability to predict the antimicrobial resistance from protein spectral data directly is one of the most clinically important applications of the AI power of MALDI-TOF technology. Spectral signatures can be associated with certain resistance mechanisms, which can be recognized by a machine learning algorithm before the conventional susceptibility testing is done. The ability to make such predictions may greatly decrease the amount of time needed for therapeutic decision making, as resistance profiles can be predicted soon after organism identification. While most of the available evidence has concentrated on bacterial pathogens, the same is increasingly true of fungal diagnostics, with the aim of predicting

resistance in emergent Candida strains, and other clinically important fungal pathogens (Weis et al., 2022).

MALDI-TOF mass spectrometry combined with AI is thus a significant step towards complete automation of clinical microbiology. The AI-driven MALDI-TOF platforms could significantly streamline laboratory workflows, expedite antimicrobial treatment, minimize unnecessary empirical antimicrobial use, and enhance patient outcomes by identifying organisms quickly and predicting their resistance.

**4.4 Future AI-Driven Immune Signature Diagnostics**

The potential of future advances in fungal diagnostics is likely to go beyond the identification of the pathogen(s) to include a more complete understanding of host immune responses. The emerging diagnostic strategies involve AI and aim to characterise the adaptive immune repertoire that is generated during infection rather than merely identify the infecting organism, and thus also offer information about the disease status, host-pathogen interaction and individual host immune responses. These methods could be used to complement genomic sequencing and traditional microbiological methods and could include host-derived biological information into the diagnosis-based decision-making process.

One of the most promising innovations is immune receptor sequencing: the vast array of B-cell receptor (BCR) and T-cell receptor (TCR) sequences generated during adaptive immune responses are analysed. Sequencing of immune receptor repertoires offers indirect molecular signatures of pathogen exposure, as specific pathogens elicit specific immune receptor repertoires.

With the advent of high throughput sequencing (HTS) technologies and the help of artificial intelligence (AI) helping to interpret the huge sequence diversity these technologies generate, there is increased demand for this type of research (Zaslavsky et al., 2025). There are many machine learning algorithms including predictive ones, which can be used to glean clinically relevant information from immune receptor datasets. They can be taught by millions of receptor sequences of disease-detected patients and the models can detect the patterns of a particular infectious agent, the activation state of the immune system and the stage of a disease.

With these computational methods, the status of a disease can be predicted from host immune signatures alone, even when it is technically difficult to detect the pathogen or there is little pathogen burden.These computational methods can be used to predict the status of a disease based on host immune signatures alone, even when it is technically difficult to detect the pathogen or there is little burden of the pathogen.

The combination of immune receptor sequencing and AI could be a major advancement in precision diagnostics of infectious diseases. In the future, pathogen genomics, host transcriptomics, immune repertoire studies, clinical metadata and machine learning can be integrated in a unified computational platform which can provide comprehensive diagnostic assessment. These multi-dimensional approaches can enhance the sensitivity of the diagnosis, allow early detection of the disease, allow for

personalised treatment and allow for monitoring of the response to treatment in real-time.

AI-based immune signature diagnostics are a glimpse into the future of precision medicine and could be the defining change in the diagnosis and management of invasive fungal infections (IFIs) (Zaslavsky et al., 2025), although still awaiting wider clinical implementation.

## 5. Emerging Molecular Platforms Beyond Conventional NGS

The molecular biology advances in recent years have led to the development of molecular tools to go beyond the traditional next-generation sequencing (NGS) in fungal diagnostics, such as fast detection, high analytical sensitivity and diagnostic precision. These platforms, which combine molecular engineering, structural biology and genomics, complement NGS and enable more comprehensive pathogen diagnostics, genomic surveillance and precision fungal diagnostics.

### 5.1 CRISPR-Based Diagnostics

Capable of rapid molecular detection of fungal pathogens, the CRISPR-based diagnostic technique has been identified as a promising option. These assays involve the use of highly specific guide RNAs and CRISPR-associated (Cas) enzymes to specifically recognize and detect predefined nucleic acid targets and have very high sensitivity and specificity, but require very few laboratory facilities compared with conventional sequencing. The advent of the CRISPR system in recent years has created an opportunity to combine with isothermal amplification methods, such as recombinase polymerase amplification (RPA), to enable molecular testing at the point of care. a chitin-based fungal capture combined with RPA-CRISPR/Cas12a was used for detecting Candida albicans, and it was found to be an extremely sensitive detection method, which suggests the clinical potential of the use of a CAS12a-based diagnostic for early detection of the fungus (Shen et al., 2024).

### 5.2 Genomic Surveillance of Emerging Fungal Pathogens

Genomic surveillance is an indispensable tool for pathogen evolution, transmission and antifungal resistance monitoring. High throughput sequencing can be used to support outbreak investigations and public health decision making, as it provides population structure and genetic diversity of emerging fungal pathogens. The rapid global dissemination and multidrug resistance of Candida auris make it an important target of the genomic surveillance program. Genomics can help in the monitoring of pathogen evolution, and thus in strengthening the global surveillance network, as has been demonstrated by population genomic studies that revealed genetically distinct lineages, including a newly identified sixth phylogenetic clade, for SARS-CoV-2 (Suphavilai et al., 2024).

### 5.3 Novel Molecular Targets for Precision Diagnosis

As structural biology and functional genomics continues to yield new molecular targets, these could help in the diagnosis of fungi and generation of drugs against fungal infections. The structures of fungal glycosylphosphatidylinositol (GPI)-anchor biosynthesis enzymes have revealed the mechanism of GWT1 binding to its target enzyme, offering potential biomarkers for the development of precision diagnostic assays

(Dai et al., 2024). A new area of application is the genomic risk stratification, which also involves the incorporation of genomic sequencing data in clinical decision making.

The approach, developed for hematological diseases, is readily transferable to fungal infections and is able to apply pathogen genomic signatures and host molecular profiles for the personalized diagnosis and therapy of fungal disease, as shown by Simonin et al., 2024. These novel molecular platforms are all emerging as a means to move fungal diagnostics beyond the traditional sequencing approach. Combination of these technologies such as CRISPR detection, genomic surveillance, structural genomics, and precision molecular profiling are expected to enhance the accuracy and rapidity of diagnosis, the analytical power, and the personalized approach to the management of invasive fungal infections. The most important clinical value of these technologies is when used in an integrated diagnostic workflow.

The conceptual framework of AI-aided genomic diagnostics of fungi is shown in figure 2, which illustrates the steps from clinical specimen to nucleic acid extraction, host DNA depletion, the use of molecular detection technologies (NGS, CRISPR, Raman spectroscopy), bioinformatics and finally artificial intelligence, which allows for the identification of the pathogen in a short period, prediction of resistance to antifungals, and patient-specific clinical decision support.

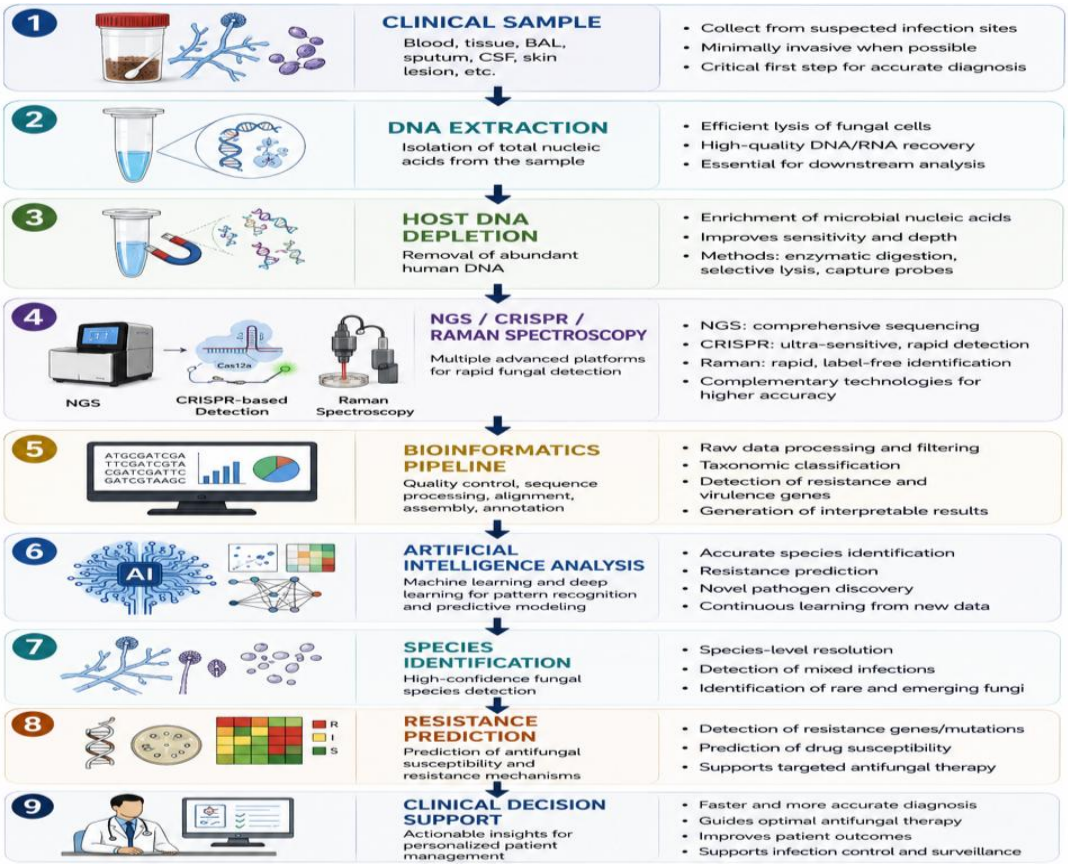


Figure 2. Integrated workflow of AI-assisted genomic fungal diagnostics

## 6. Future Perspectives and Remaining Challenges

Although significant advances have been made in genomic sequencing and artificial intelligence, there are a number of scientific and practical issues to overcome in order to make these technologies widely available for routine clinical mycology. Going forward, it is anticipated that more research will be conducted to facilitate the incorporation of several sources of biological information, enhance the transparency of artificial intelligence algorithms, establish uniform clinical workflows and develop enhanced translational research that connects laboratory discoveries with their use in the clinic.

### 6.1 Integration of Multi-Omics Technologies

In the future, the "precision" diagnostics of fungal diseases is likely to rely on the combination of several complementary omics technologies, not just genomic sequencing. A holistic understanding of fungal pathogenesis and host-pathogen interactions can be gained by integrating genomics, transcriptomics, proteomics, metabolomics with immune profiling of the host. These multi-omics methods can enhance the diagnostic sensitivity, discovery of new biomarkers, prognosis, and personalized treatment approaches. Combining multiple pieces of biological data with sophisticated computational tools may thus allow for more precise and comprehensive evaluations of invasive fungal diseases than any single diagnostic test.

### 6.2 Explainable Artificial Intelligence

The rise in AI's clinical use will be contingent upon the creation of explainable AI (XAI), as AI has shown remarkable diagnostic abilities. A large part of the existing machine learning and deep learning algorithms are so-called "black-box" systems that are hard to interpret. In clinical use, physicians and laboratory experts need clear algorithms that offer explanations that are easy to comprehend for making diagnostic predictions. Explainable AI would boost clinicians' trust in the AI's accuracy, help meet regulatory standards, and enable the validation of computational decisions, thereby enhancing the transparency and reliability of AI-driven diagnostic systems.

### 6.3 Clinical Translation and Regulatory Considerations

Laboratory standardisation, robust analytical validation, quality assurance measures and regulatory compliance are essential for successful integration of genomic and AI technologies into clinical practice. To achieve broad uptake, diagnostic platforms need to be reproducible, clinically valid, cost-effective, and have interoperability with existing LIS. Furthermore, the ability to work as a multidisciplinary team, with microbiologists, bioinformaticians, clinicians and regulatory experts, will be vital to create and define commonly accepted parameters for how to incorporate the sophisticated technologies into daily clinical practice.

### 6.4 Future Research Directions

Prospective multicenter clinical trials of the real-world effectiveness of integrated genomic and AI-based diagnostic platforms should be emphasized in the future. Special emphasis should be placed on the enhancement of rapid point of care diagnostics, the creation of more powerful computational algorithms to predict antifungal resistance and the expansion of multi-omics techniques that

simultaneously analyse fungal pathogens and host immune responses. In addition, it is important to have experimental models that reflect host–fungus interactions that will continue to be needed for the validation of new biomarkers and disease mechanisms before they are applied to humans. This translational research will help shorten the pipeline for innovative precision diagnostics of fungi and inform more personalized patient care. (Last et al., 2021).

**7. Conclusion**

With an astonishing growth in genomic technologies and artificial intelligence, rapid diagnostics of fungi are now in a new era. The ability to identify fungal pathogens rapidly, sensitively and accurately has greatly been accelerated by the development of high throughput sequencing platforms, metagenomic sequencing, CRISPR-based diagnostics and new molecular tools, and new fungal pathogens and fungal resistance have been detected earlier.

Artificial Intelligence (AI) has also contributed to improved diagnostic capabilities by enabling automated data analysis, machine learning species recognition and clinical decision support, thus offering opportunities for more personalized and efficient patient management.

While these strides have been made, there is still work to be done, such as assay standardisation, regulatory approval, laboratory implementation, and the need for explainable AI systems that foster clinician trust and safe clinical use. As multi-omics technologies are increasingly combined with more powerful computational tools, the accuracy of diagnosis should increase, and the ability to make personalized therapeutic decisions should be further enhanced. Taken together, these innovations are the building blocks of future precision diagnostics of fungal infections and can have a pronounced impact on patient care for people with invasive fungal infections.

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