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Toward sustainable diagnostics for *Candida albicans*: the role of artificial intelligence in analytical chemistry from data processing to Python-based blueness and redness evaluation metrics

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Candida albicans remains one of the most clinically significant fungal pathogens, contributing substantially to morbidity and mortality among immunocompromised and hospitalized patients. In response to the growing analytical complexity of fungal diagnostics, this review presents a unified Python-based framework structured around three fundamental and interdependent axes of contemporary *C. albicans* diagnostics: (i) accurate pathogen detection, (ii) high-throughput data processing, and (iii) analytical method evaluation. Together, these three dimensions form an integrated analytical architecture, herein conceptualized as the Candida Diagnostic Triad. Within the detection and data-processing axes, recent advances in artificial intelligence, particularly convolutional neural networks, transfer-learning strategies, and hybrid machine-learning models have markedly enhanced the sensitivity, selectivity, and interpretability of analytical outputs derived from complex biological matrices. However, the most distinctive contribution of the present framework lies in the third axis, namely method evaluation, where Python-based open-source tools now enable fully automated, quantitative, and reproducible assessment of diagnostic methods within the principles of Green Analytical Chemistry (GAC) and White Analytical Chemistry (WAC). By systematically examining eighteen advanced diagnostic methodologies applied to clinically relevant matrices, including blood, urine, and vaginal samples, this review demonstrates how Python-driven analytical software tools such as the Blue Applicability Grade Index (BAGI) and the Red Analytical Performance Index (RAPI) to establish a mathematically transparent and decision-oriented workflow for comparative method assessment. This unified framework supports evidence-based selection and optimization of diagnostic strategies that are not only analytically robust, but also practically applicable and environmentally responsible. The resulting Python-enabled Candida Diagnostic Triad provides an evidence-based roadmap for selecting and optimizing diagnostic strategies that are analytically robust, practically feasible and environmentally sustainable, thereby supporting United Nations Sustainable Development Goals 3 and 9.

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1 Introduction

Severe fungal infections constitute a major and persistent global health burden, affecting more than 150 million individuals annually and contributing to approximately 1.7 million deaths worldwide.¹ Among fungal pathogens, *Candida* species

occupy a prominent position as causative agents of healthcare-associated infections, ranking as the second most common cause of catheter-associated urinary tract infections and the third leading cause of central line-associated bloodstream infections.² Within this genus, *Candida albicans* (*C. albicans*) remains the most clinically significant species owing to its remarkable adaptability, pronounced virulence, and increasing tolerance to conventional antifungal therapies.³ The pathogenic success of *C. albicans* is closely associated with its polymorphic nature causing a broad spectrum of diseases, ranging from superficial mucosal candidiasis to invasive systemic infections, particularly in immunocompromised individuals. Its virulence is largely governed by dynamic phenotypic switching between yeast, pseudohyphal, and hyphal morphologies, together with

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its well-documented ability to form highly structured biofilms on host tissues and indwelling medical devices.⁴

In the contemporary landscape of *C. albicans* diagnostics, three interrelated dimensions have emerged as being central to the development of advanced analytical strategies. The first concerns sensitive and accurate pathogen detection through modern analytical, biosensing, and imaging technologies. The second involves the computational data processing of the multidimensional datasets in order to extract diagnostically meaningful information. The third goal is comprehensive method evaluation, encompassing both analytical performance and practical applicability using software metrics. Collectively, these dimensions may be viewed as an integrated diagnostic architecture, herein referred to as the Candida Diagnostic Triad, in which detection, data processing, and rigorous evaluation converge to define the analytical value and translational utility of modern diagnostic workflows.

The present review aims to examine these three interconnected dimensions through the lens of artificial intelligence (AI), with particular emphasis on Python-based computational ecosystems that increasingly serve as a unifying tool across the diagnostic axis.⁵ Beyond its established utility in automated detection and high-throughput data handling, Python has emerged as a particularly powerful platform for the objective assessment of analytical methodologies.⁶ Recent developments in open-source Python tools have enabled quantitative, reproducible, and automated sustainability-oriented evaluation in accordance with the principles of Green Analytical Chemistry (GAC) and White Analytical Chemistry (WAC). Through the implementation of the Blue Applicability Grade Index (BAGI) and the Red Analytical Performance Index (RAPI), diagnostic methods can be compared on a standardized basis with respect to operational feasibility, analytical quality, and broader sustainability considerations.⁷ Such an approach provides a rational and evidence-based framework for the selection, optimisation, and future design of *C. albicans* diagnostic platforms that are not only analytically rigorous, but also practically deployable and environmentally responsible.

1.1 AI-driven and Python-based data processing in *Candida albicans* diagnostics

The rapid expansion of high-throughput biomedical data has rendered conventional manual microscopy and rule-based analytical approaches increasingly inadequate for handling the complexity of datasets generated by modern laboratory platforms, including digital microscopy, spectroscopy, and omics-based technologies.⁸ Consequently, AI and machine learning (ML) have emerged as valuable tools for the automated, rapid, and highly accurate interpretation of complex biological information. Within this context, Python has become one of the leading environments for scientific computing owing to its clarity, flexibility, and extensive open-source ecosystem. Libraries such as NumPy, pandas, and SciPy support efficient numerical and statistical processing, whereas TensorFlow, PyTorch, and scikit-learn provide powerful frameworks for predictive modelling and pattern recognition Fig. 1.

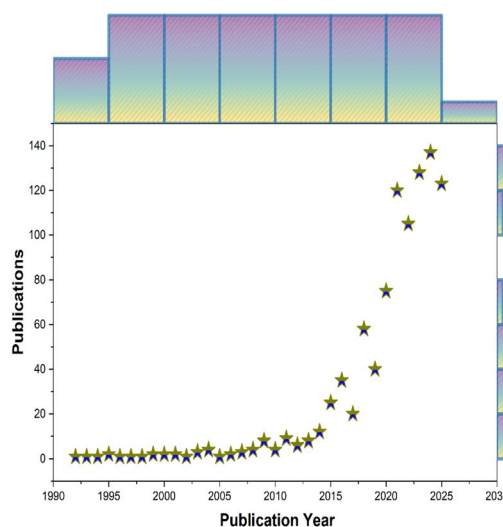


Fig. 1 Rising publication trends in chemistry incorporating Python, as determined by a SciFinder search with the keywords "Python AND chemistry" while excluding "snake", and "reptiles"; late 2025).

Collectively, these resources enable the seamless integration of data preprocessing, feature extraction, and model development within unified analytical pipelines.⁹

In clinical mycology, deep learning models, particularly convolutional neural networks (CNNs), have markedly improved the automated identification of fungal pathogens. Transfer-learning approaches based on established architectures such as VGG16, ResNet50, and InceptionV3 have demonstrated excellent performance on bright-field microscopic images of *Candida* species.¹⁰ These models can reliably distinguish yeast cells, budding forms, pseudohyphae, and true hyphae, thereby overcoming the subjectivity, time demands, and operator dependence associated with conventional microscopy. Typical implementations combine TensorFlow or Keras with OpenCV-based image preprocessing and data-augmentation strategies, enabling objective and label-free identification directly from microscopic images.¹¹

Beyond image-based analysis, Python-driven computational workflows also support spectroscopic and mass-spectrometric identification of microbial species. Libraries including pyOpenMS, maldi-learn, and RamanSPy enable machine-learning analysis of MALDI-TOF, Raman, and infrared spectral data for rapid microbial classification from complex biological matrices. Python further supports direct integration with laboratory instrumentation, chromatographic systems, and colourimetric assays, thereby improving the reproducibility, scalability, and standardisation of microbiological diagnostics. Taken together, these developments establish AI-driven data processing as a powerful analytical approach for enhancing the speed, accuracy, and reliability of *C. albicans* detection.¹²

1.2 Drug discovery and predictive Python-based modelling in clinical translation

Recent advances in AI have provided powerful computational strategies for antifungal drug repurposing, molecular



optimisation, and predictive modelling in clinical translation. In this context, graph-based and network-driven platforms, including TxGNN, deepDTnet, AtomNet, and PandaOmics, enable the integration of heterogeneous datasets such as multi-omics profiles, electronic health records, and biomedical literature in order to identify previously unrecognised therapeutic indications for existing antifungal agents. These data-driven approaches are particularly valuable in antifungal development because they facilitate the prioritisation of compounds with already established pharmacokinetic and safety profiles, thereby reducing experimental burden and translational risk.¹³

At the molecular level, AI-assisted optimisation has further expanded the analytical toolbox for lead refinement. Reinforcement-learning frameworks and generative models, commonly implemented using PyTorch, TensorFlow, and RDKit-based cheminformatics workflows, enable the *in silico* generation and rapid screening of structural analogues with improved physicochemical and pharmacological properties.¹⁴ Such computational pipelines support the prediction of antifungal potency, selectivity, and toxicity, thereby enabling the early-stage prioritisation of candidate molecules prior to synthesis and biological validation. In parallel, AI-driven predictive models have become increasingly relevant to the design and optimisation of clinical trials.¹⁵ Platforms such as IBM Watson Clinical Trial Matching, Deep 6 AI, Unlearn.AI, CURATE.AI, and Trial Pathfinder apply supervised and unsupervised learning algorithms to clinical and genomic datasets in order to improve patient stratification, biomarker selection, recruitment efficiency, and treatment-response prediction. From an analytical perspective, these frameworks enhance data interpretability and support evidence-based decision-making by reducing variability and improving the statistical robustness of trial design. Collectively, these AI-enabled computational strategies provide an integrated route towards more efficient and data-driven antifungal therapeutic development.¹⁶

1.3 The sustainability spectrum: evolution of GAC and WAC Python-based tools

The paradigm of Green Analytical Chemistry (GAC), established in the 1990s, catalyzed a fundamental shift toward minimizing the environmental footprint of laboratory processes by reducing toxic waste and energy consumption.¹⁷ In 2021, this framework was expanded into White Analytical Chemistry (WAC), an integrated approach that harmonizes environmental “greenness” with practical “blueness” (operational applicability) and analytical “redness” (performance excellence).¹⁸ The transition from qualitative assessments to high-precision computational tools has followed a distinct historical trajectory. Initial tools like NEMI (2002) and the Analytical Eco-Scale (2012) introduced structured criteria for evaluating environmental impact.¹⁹ This was followed by more sophisticated visual frameworks, such as the Green Analytical Procedure Index (GAPI) in 2018 and AGREE in 2020, the latter of which revolutionized the field by providing standardized, color-coded numeric outputs.²⁰ As the discourse extended to specialized stages like sample preparation *via* AGREEprep (2022) and SPMS (2023), the need for more

comprehensive, automated evaluation became evident.²¹ Recent advancements in 2024 and 2025 have culminated in the introduction of Python-powered software metrics: Blueness Assessment and Grading Index (BAGI) and Red Analytical Performance Index (RAPI).²² These tools mark the progression from manual scoring to automated, algorithmic evaluation, providing researchers with sophisticated methods to ensure that modern diagnostic workflows such as those used for *Candida* detection are not only scientifically robust but also operationally efficient and environmentally sustainable. *C. albicans* was selected as a representative fungal pathogen to demonstrate the versatility of the proposed Python-based GAC/WAC framework in optimizing biosensing platforms for clinical diagnostics Fig. 2.

The objective of this review is to establish a unified framework for computational decision-making by utilizing BAGI and RAPI as standardized, open-source Python metrics to evaluate the practical applicability, analytical performance, and environmental sustainability of modern *Candida* diagnostic protocols. To establish a comprehensive decision-making framework, core 18 cutting-edge methodologies were meticulously selected to represent the current vanguard of *C. albicans* diagnostics. These techniques were chosen to facilitate a rigorous comparative analysis using Python-based metrics, focusing on two critical axes: Practical Applicability (Blueness) and Analytical Performance (Redness). The selection encompasses a broad spectrum of biological matrices including urine, vaginal swabs, and blood ensuring the evaluation covers diverse clinical scenarios from biofilm monitoring to the identification of drug-resistant strains. The selection of core 18 advanced technologies reflects the comprehensive nature of modern *C. albicans* diagnostics, forming a robust framework for the accurate identification of this opportunistic pathogen as illustrated in Table 1.

1.4 Methodological summaries for *C. albicans* detection

1.4.1 Fluorescent QD-Con A labeling (at-line). Utilizes CdTe quantum dots conjugated with Concanavalin A for high-affinity glycan binding. The system enables 3D biofilm reconstruction *via* Imaris/ImageJ, providing sensitive quantification of yeast-to-hyphal transitions.²³

1.4.2 UV-vis spectrophotometry (offline). A robust, cost-effective method measuring absorbance at 540 nm. Validated against Neubauer chamber counts, it offers a high-throughput screening solution for vaginal candidiasis in resource-limited settings.²⁴

1.4.3 UV resonance Raman spectroscopy (inline). Deep-UV excitation at 244 nm targets aromatic amino acids and nucleic acids, enabling species differentiation. Integrated PCA/SVM chemometrics distinguish viable cells and drug-resistant isolates with high specificity.²⁵

1.4.4 Aptamer-AuNP colorimetric assay (offline). Employs gold nanoparticles functionalized with AD1 aptamers targeting beta-1,3-D-glucan. A visible pink-to-blue shift allows for rapid point-of-care quantification using red-channel imaging.²⁶

1.4.5 Magnetic NPT-SERS (offline). Integrates nanoparticles for fungal capture from serum with AgNP-enhanced



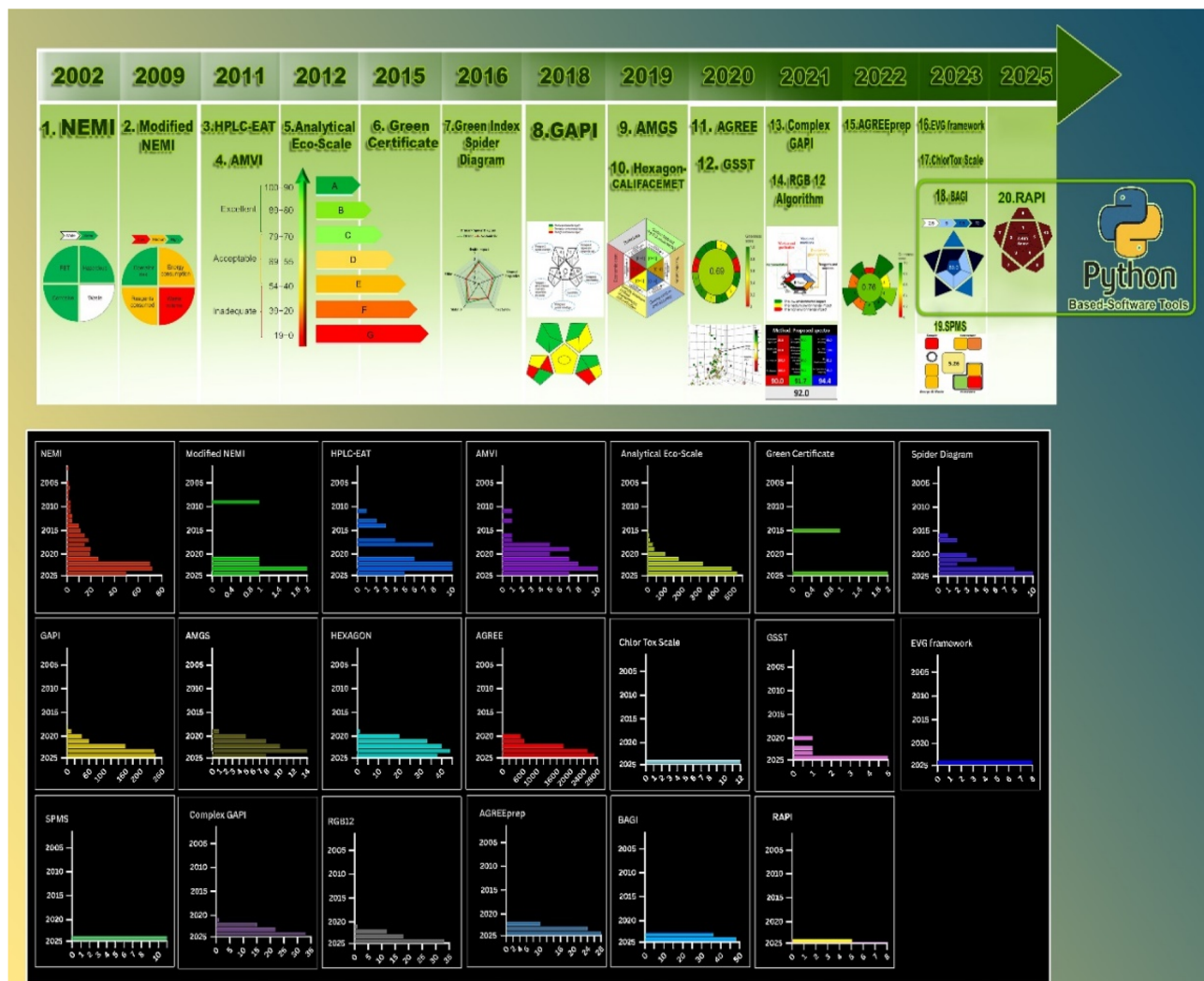


Fig. 2 Statistical growth of publications and roadmap timeline of GAC and WAC Assessment Tools from manual scoring to Python software tools.

Raman scattering. OPLS-DA multivariate analysis facilitates species-level identification within minutes without prior culture.²⁷

1.4.6 PNA-FISH (online). Employs peptide nucleic acid probes targeting the 26S rRNA of fungal cells. This technique drastically reduces turnaround times compared to traditional culturing; once a blood culture bottle flags positive, results can be obtained within 1.5–3 hours. This rapid identification directly from positive blood cultures offers a specific molecular diagnostic, enabling faster transition to targeted antifungal therapy.²⁸

1.4.7 PCR-DEIA (offline). A DNA Enzyme Immunoassay that combines PCR amplification with sequence-specific hybridization on streptavidin plates. It achieves ultra-sensitive detection down to a single fungal cell equivalent.²⁹

1.4.8 Photodynamic fluorescence spectroscopy (offline). A theranostic platform using curcumin as a photosensitizer. Fluorescence measurements assess treatment efficacy in

biofilm-associated infections, particularly in oral and bone candidiasis.³⁰

1.4.9 NMR metabolomics (offline). Utilizes 2D correlation NMR to profile metabolic signatures. Statistical classification strategies (SCS/ORS) achieve 97% accuracy in distinguishing *Candida* species with minimal sample preparation.³¹

1.4.10 CE-FISH (offline). A hybrid of capillary electrophoresis and FISH that enhances sensitivity in candidemia cases. Post-lysis labeling and electrophoretic separation improve diagnostic accuracy and reduce time-to-result.³²

1.4.11 PTR-MS (offline). Proton-transfer reaction mass spectrometry detects volatile organic metabolites (VOMs) in urine. This non-invasive method provides rapid, quantitative detection of candiduria without the need for culture.³³

1.4.12 LC-MS/MS biofilm proteomics (offline). High-resolution Q-TOF mass spectrometry provides mechanistic insights into biofilm virulence. Comparative protein profiling identifies specific markers associated with antifungal resistance.³⁴





Table 1 Analytical methods for detection of candida albicans

Title	Method	Target analysis	Pre-analysis process	Conditions	Additional setup	Data analysis	Ref.
CdTe quantum dots conjugated to concanavalin A as fluorescent probes for saccharides in <i>Candida albicans</i>	<ol style="list-style-type: none"> 1. Detect the percentage of labeled <i>Candida albicans</i> cells using fluorescence spectroscopy. 2. Bioconjugate concanavalin A to CdTe-MSA QDs with specific labeling of on hyphae and yeast <i>C. albicans</i> cells. 	<ol style="list-style-type: none"> 1. <i>Candida albicans</i> (ATCC 10231) cells. 2. Labeling with QDs-(Con A) conjugated to MSA QDs with high-resolution labeling of biofilms both <i>in vitro</i> and <i>in vivo</i>. 	<ol style="list-style-type: none"> 1. Cell culture: <i>Candida albicans</i> grown at 37 °C, 24 h in (suspension) 48 h on hydrogel slabs. 	<ol style="list-style-type: none"> 1. Fluorescence spectroscopy excitation at 488 nm and measuring emission with band pass filter 585/20 nm. 2. Conjugation: QDs: Con A ratio 1000:1, pH 8.0, 2 h incubation at room temperature with gentle stirring. 	<ol style="list-style-type: none"> 1. Circular dichroism (CD) spectroscopy: Jasco J-815 spectropolarimeter. 2. UV-vis Absorption spectroscopy: (becton dickinson) – for flow cytometry data analysis. 	<ol style="list-style-type: none"> 1. Imaris@ 7.4.2 (Bitplane) for 3D biofilm image reconstruction. 2. ImageJ 2.0 (NIH) – for confocal microscopy image processing. 3. CellQuest™ pro (becton dickinson) – for flow cytometry data analysis. 	23
Evaluating the concentration of a <i>Candida albicans</i> suspension	UV-vis spectrophotometry at 540 nm using a spectronic 2001 spectrophotometer.	Isolation from a vaginal specimen to determine concentration of a yeast cell suspension in a Neubauer's chamber for vaginal candidiasis.	<ol style="list-style-type: none"> 1. Clinical isolates: main causative agent of vaginal candidiasis, were cultured, serially diluted in 0.9% NaCl. 2. Eleven 10-fold serial dilutions analyzed spectrophotometry. 3. Cell counts in a Neubauer chamber. 	<ol style="list-style-type: none"> 1. Culture on Sabouraud's agar at 37 °C for 24 h. 2. Eleven 10-fold serial dilutions analyzed spectrophotometry. 3. Cell counts in a Neubauer chamber. 	<ol style="list-style-type: none"> 1. Nephelometry using a bhering laser automatic voltage (nephelometer), nephelometer. 	<ol style="list-style-type: none"> 1. Spectrophotometer, voltage (nephelometer). 	24
The application of UV resonance Raman spectroscopy for the differentiation of clinically relevant <i>Candida</i> species	Cell viability monitoring of metabolically active microbial bacteria and yeast to differentiate viable vs. dead cells and antibiotic resistance patterns.	Analysis of 22 strains from 12 clinically relevant <i>Candida</i> species, sourced from DSMZ, ATCC, round-robin diagnostic tests, isolates confirmed using the ID 32C system following the manufacturer's protocol (BioMérieux).	<ol style="list-style-type: none"> 1. <i>Candida</i> species were cultured in yeast medium for 24 h, biomass washed and resuspended in deionized water, then 50 µL of suspension was placed on a fused silica slide and dried before measurement. 	<ol style="list-style-type: none"> 1. UV resonance Raman (RR) spectra of <i>Candida</i> using a Horiba/Jobin-Yvon HR800 with an 800 mm focal length, excited at 244 nm from the frequency-doubled line of an argon-ion laser 2. The backscattered Raman signal was directed through a 400 µm slit to a 2400 lines/mm⁻¹ grating (2 cm⁻¹ resolution). 	<ol style="list-style-type: none"> 1. <i>Candida</i> strains were cultured and analyzed by UV resonance Raman spectroscopy (244 nm, HR800 system, CCD detection). Spectral data were preprocessed in GNU R. 	<ol style="list-style-type: none"> 1. Chemometrics with GNU R preprocessing and PCA, then classified using SVM and validated by leave-one-batch-out cross-validation. 	25
Instant <i>Candida albicans</i> detection using ultra-stable Aptamer conjugated gold nanoparticles	Colorimetric assay using AD1 aptamer-conjugated gold nanoparticles that specifically bind <i>C. albicans</i> β1,3-D-glucan; a red shift in UV-vis absorbance (solution turns pink → blue), enabling naked-eye “yes/no”.	<i>Candida albicans</i> fungal cells (yeast cells and also germ tubes/hyphae), via AD1 aptamer binding to β-1,3-d-glucan on their cellwalls; demonstrated down to ~5 × 10 ⁵ cells.	<ol style="list-style-type: none"> 1. NPTs prepared within 5 minutes using tween 20, HAuCl₄ · 3H₂O, glucose, NaOH, and AD1 aptamers. 2. Sample preparation standardized to OD₆₀₀ = 1.0 in vaginal fluid simulant (pH 4.2) and mixed directly with nanoparticles. 	<ol style="list-style-type: none"> 1. NPTs prepared within 5 minutes using tween 20, HAuCl₄ · 3H₂O, glucose, NaOH, and AD1 aptamers. 2. Sample preparation standardized to OD₆₀₀ = 1.0 in vaginal fluid simulant (pH 4.2) and mixed directly with nanoparticles. 	<ol style="list-style-type: none"> 1. Dynamic light scattering (DLS, Anton Paar LiteSizer). 2. Nanoparticle tracking analysis (NTA, NanoSight NS300, Malvern) for particle size and concentration. 	<ol style="list-style-type: none"> 1. Image analysis: ImageJ software (NIH) used for red-channel brightness intensity measurements to quantify colorimetric changes. 2. Optical density (OD₆₀₀) → semi-quantitative estimation of fungal cell concentration in suspensions. 	26



Table 1 (Contd.)

Title	Method	Target analysis	Pre-analysis process	Conditions	Additional setup	Data analysis	Ref.
Rapid detection method for pathogenic <i>Candida</i> captured by magnetic nanoparticles and identified using SERS via AgNPs ⁺	Surface-enhanced Raman scattering (SERS) using positively charged AgNPs ⁺ as the detection substrate, followed by multivariate spectral analysis (OPLS-DA with cross validation) to distinguish <i>C. albicans</i> isolates.	Detection of candidemia infection as the pathogenic <i>C. albicans</i> in serum captured by Fe ₃ O ₄ @PEI magnetic nanoparticles and identified by SERS.	1. Magnetic capture reagent: synthesis of Fe ₃ O ₄ @PEI (300–500 nm); Fe ₃ O ₄ , PEI coating by ultrasonication to yield positively charged beads for electrostatic capture. 2. Preparation of positively charged AgNPs ⁺ (CTAB-stabilized) with UV peak ~404 nm.	1. SERS detection used a 785 nm laser, 30 s integration, 10% of 275 mW power, with a 105 μm. 2. <i>Candida</i> strains were cultured on sabouraud agar at 25 °C for 72 h.	Capture involved 1 mL serum (10 ⁶ cells per mL) mixed with Fe ₃ O ₄ @PEI, magnetically enriched, and rotated with AgNPs ⁺ for 15 min.	SIMCA 14.1 (Umetrics, Umeå, Sweden), which was applied to perform orthogonal partial least squares discriminant analysis (OPLS-DA) along with 10-fold cross-validation.	27
Fluorescence in situ hybridization with peptide nucleic acid probes for rapid identification of <i>Candida albicans</i> directly from blood culture bottles from positive-bloodculture bottles	Fluorescence <i>in situ</i> hybridization (FISH) method that uses peptide nucleic acid (PNA) probes for identification of <i>Candida albicans</i> directly from positive-bloodculture bottles	Rapid identification of <i>C. albicans</i> directly from blood culture bottles with fluorescein-labeled probe target <i>C. albicans</i> 26S rRNA directly from the contents of the blood.	Clinical isolates collected from various specimens (blood, respiratory samples, cystic fibrosis patients) and confirmed by D1–D2 26S rDNA sequencing.	1. Smear fixation: heated at 55–60 °C for 20 min or flame-fixed. 2. Hybridization: performed at 55 °C for 90 min. 3. Post-hybridization wash: carried out at 55 °C for 30 min in Tris/NaCl/Triton buffe.	1. IMAGEN mounting fluid (DAKO) with coverslips applied for fluorescence microscopy. 2. Nikon Optiphot, 60×/1.4 oil immersion objective, HBO 100 W mercury lamp.	1. DNASTAR (Madison, WI, USA) for sequence processing and alignments (MegAlign v4.03 and PrimerSelect v4.03). 2. GeneMan (v3.30)	28
Fluorescence spectroscopy of <i>Candida albicans</i> biofilms in bone cavities treated with photodynamic therapy using blue LED (450 nm) and curcumin.	Fluorescence spectroscopy combined with photodynamic therapy (PDT) using 450 nm blue LED light.	Oral and bone associated candidiasis linked to persistent endodontic and bone cavity infections, and the target cells were <i>Candida albicans</i> biofilm cells (ATCC 18804).	1. Samples were incubated at 36 °C ± 1 °C for 14 days to allow biofilm formation. 2. Cavities were filled with 750 μL sabouraud dextrose broth and inoculated with 100 μL <i>Candida albicans</i> (1 × 10 ⁶ cells per mL, ATCC 18804).	Fluorescence spectroscopy was performed at 405 nm with evince system (MMOptics, São Carlos, SP, Brazil) equipped with a 400 ± 10 nm UV LED light source delivering a maximum luminous intensity of 40 mW cm ⁻² ± 20%, including a 420 nm band-pass filter, a dichroic reflector (350–475 nm), and a transmission filter spanning 475–800 nm.	1. Cell preparation THP-1 cells incubated with DOX for 48 hours, fixed in ice-cold Roti-Histofix. 2. Sample stabilization with cells immobilized on poly-L-lysine-coated CaF ₂ slides.	1. GraphPad prism 5 (GraphPad Software, San Diego, CA, USA).	29
Simple and rapid detection of <i>Candida albicans</i> DNA in serum by PCR for diagnosis of invasive candidiasis	Rapid PCR-DEIA method for the detection of <i>Candida albicans</i> DNA in serum.	Candidiasis detection in clinical samples (serum) using molecular and immunoassay.	1. Yeast isolates: twelve <i>Candida</i> strains including <i>C. albicans</i> . 2. Clinical samples: swabs, stool, blood, and sera	1. <i>Candida</i> growth: 24 h on sabouraud dextrose agar. 2. Serum processing: centrifugation of	1. DNA enzyme immunoassay (DEIA) with biotinylated <i>C. albicans</i> probe. 2. Sensitivity testing: serial	1. MegAlign (version 4.03) – for alignment of fungal rDNA sequences. 2. PrimerSelect (version	30



Table 1 (Contd.)

Title	Method	Target analysis	Pre-analysis process	Conditions	Additional setup	Data analysis	Ref.
Rapid identification of <i>Candida</i> species by using nuclear magnetic resonance (NMR) spectroscopy, specifically employing two-dimensional correlation NMR to identify major metabolites and differentiate clinically important <i>Candida</i> species based on their metabolic profiles.	Nuclear magnetic resonance (NMR) spectroscopy, specifically employing two-dimensional correlation NMR to identify major metabolites and differentiate clinically important <i>Candida</i> species based on their metabolic profiles.	<i>Candida</i> species isolates (mainly from blood and clinical samples) to improve the detection and differentiation of candidiasis, including invasive bloodstream infections.	collected from volunteers blood cultures incubated, sera separated and stored at $-20\text{ }^{\circ}\text{C}$.	clotted blood, stored at $-20\text{ }^{\circ}\text{C}$. 3. Group-specific patients: controls, mucocutaneous cases, ICU patients.	dilutions of <i>C. albicans</i> cells ($10^6 \rightarrow 1$ cell) and DNA.	4.03) – for checking probe design.	31
Rapid identification of <i>Candida albicans</i> in blood by combined capillary electrophoresis and fluorescence <i>in situ</i> hybridization	Capillary electrophoresis (CE) and fluorescence <i>in situ</i> hybridization (FISH) approach for rapid detection of <i>Candida albicans</i> in blood samples.	Candidemia (<i>Candida</i> bloodstream infection), and the target cells were <i>Candida albicans</i> cells spiked into and present in blood samples.	1. Red blood cells lysed using a hypotonic/detergent treatment (distilled water + 0.1% triton X-100). 2. <i>Candida</i> cells were fixed in a 60% formalin enabling probe penetration for FISH labeling.	1. Capillary electrophoresis (CE). Reverse polarity mode at -3 kV using fused silica capillaries (30 cm total length, 100 μm). 2. Detection fluorescence measured at 488 nm excitation and 516 nm emission.	Red blood cell (RBC) lysis performed with distilled water plus 0.1% triton X-100 to remove background interference.	Linear regression was applied to determine correlation ($R^2 \approx 0.9897$), with detection and quantitation limits calculated based on baseline noise and sensitivity.	32
Detection of <i>Candida albicans</i> by mass spectrometric fingerprinting	Proton-transfer reaction mass spectrometry (PTR-MS) for diagnosis of candiduria	Targeted <i>Candida albicans</i> yeast cells (clinical isolates cultured and suspended in urine).	<i>Candida albicans</i> was first grown on sabouraud dextrose agar (SDA) at $30\text{ }^{\circ}\text{C}$ for 3 days, then transferred into RPMI broth and incubated overnight to produce liquid cultures	1. Four fungal loads – 5×10^5 , 1.5×10^5 , 1.5×10^4 , and 1.5×10^3 CFU mL^{-1} . 2. Incubation conditions: cultures grown at $30\text{ }^{\circ}\text{C}$ (RPMI medium overnight, then urine dilutions).	Fungal suspensions mixed with fresh human urine and serially diluted to obtain four concentrations (5×10^5 , 1.5×10^5 , 1.5×10^4 , and 1.5×10^3 CFU mL^{-1}).	SPSS (SPSS Inc., Chicago, USA)	33
LC-MS analysis reveals biological and metabolic processes essential for <i>Candida albicans</i> biofilm growth	LC-MS-based proteomics for profile proteins and map biological/metabolic processes in yeast samples.	Candidiasis (biofilm-associated in immunocompromised patients) with <i>Candida albicans</i> strain SC5314 (ATCC MYA-2876).	1. Minimal medium (pH 7.0), shake 200 rpm at $37\text{ }^{\circ}\text{C}$ (12–18 h). 2. For biofilm: adjust cells to 10^7 cells per mL, seed 200 μL in 24-well plates, 1.5 h, fresh SD medium,	1. Inoculation: 200 μL into 24-well flat-bottom plates. 2. Adhesion: 1.5 h, remove non-adherent cells. 3. Biofilm growth: add fresh SD medium,	1. Agilent 1260 infinity HPLC-chip/MS with 6540 Accurate-Mass Q-TOF (positive ion mode).	1. PEAKS studio v7.5 (bioinformatics solutions, Canada) 2. (Fisher's exact test) integrated with PEAKS/CGD pipeline to	34



Table 1 (Contd.)

Title	Method	Target analysis	Pre-analysis process	Conditions	Additional setup	Data analysis	Ref.
A novel targeted/untargeted GC-Orbitrap metabolomics methodology applied to <i>Candida albicans</i>	Gas chromatography coupled with high-resolution Orbitrap mass spectrometry (GC-Orbitrap MS, Q exactive GC system).	<i>Candida albicans</i> (SC5314 strain) to investigate mixed candidiasis – staphylococcal infections associated with high mortality, and resistance.	incubate 24 h at 75 rpm; scrape biofilm.	incubate 24 h, 75 rpm shaking, 37 °C.	2. Flow 0.3 µL min ⁻¹ ; capillary pump 2.5 µL min ⁻¹ .	determine enriched categories ($p < 0.05$).	35
Selective and sensitive probe based in oligonucleotide-capped nanoporous alumina for the rapid screening of infection produced by <i>Candida albicans</i>	Monitoring rhodamine B release from the S3 support using fluorescence spectroscopy after exposure to <i>Candida albicans</i> cells with selective prope.	Detection of invasive candidiasis (IC), with or without associated candidemia s in real competitive media.	Electropolished and anodized aluminum was used to fabricate NAA films, which were then sequentially functionalized with rhodamine B/oligonucleotides.	Detection monitoring: Rhodamine B release measured by fluorescence ($\lambda_{exc} = 555$ nm, $\lambda_{em} = 585$ nm). Quantification assays: performed with serial dilutions of <i>C. albicans</i> suspensions (10^3 – 10^6 CFU mL ⁻¹).	Nanoporous anodic alumina (NAA) films prepared by twostep anodization of high-purity aluminum (0.3 M H ₂ SO ₄ , 10 V, 2 °C), and re-anodization to obtain pores ($\sim 7.5 \pm 1.7$ nm, thickness ~ 8 µm).	1. VITEK MS (bioMérieux) for proteomic profiling and species identification of <i>Candida</i> isolates. 2. API ID20C (bioMérieux) and AuxaColor™ 2 (bioRad laboratories) for biochemical and phenotypic analysis.	36
Rapid detection of <i>Candida albicans</i> in urine by an electrochemical impedance spectroscopy (EIS)-based biosensor	Electrochemical impedance spectroscopy (EIS) for rapid detection of <i>C. albicans</i> in urine.	Detection of <i>Candida albicans</i> cells in urine samples for rapid and specific diagnosis of candidiasis and urinary tract infections.	1. Flow rate & surface coverage optimized by 4 functionalization (UV activation + antibody flow + 15 min each). 2. Surface blocking BSA (50 µg mL ⁻¹ , 15 min).	Frequency range: 0.5 Hz to 10 000 Hz. Applied potential: 0.16 V (formal potential). Amplitude perturbation: 10 mV.	The gold electrode was functionalized albicans-IgG antibodies (25 µg mL ⁻¹) (0.3 W cm ⁻² , 30 s), flowed over electrode 15 m.	PSTrace v5.5 software (PalmSens, Netherlands).	37
Magnetic nanobead PaperBased biosensors for colorimetric detection of <i>Candida albicans</i>	Colorimetric <i>C. albicans</i> nanobead paper-based biosensor.	Candidiasis including vulvovaginal and invasive forms, (ATCC 10231 and clinical isolates).	Carboxylated <i>m-C. albicans</i> -specific peptide substrate using EDC/NHS chemistry, and stored at 4 °C.	Biosensing step: 100 µL of <i>C. albicans</i> culture supernatant protease; protease activity cleaved the conjugates, fragments, revealing the gold surface.	ImageJ software applied to quantify color change and construct calibration curves.	Sensor surface was photographed, processed in ImageJ (red ch), percentage of cleavage was calculated to quantify protease activity.	38
Rapid detection of point mutations in <i>Candida</i> spp. using FRET and probe melting curves in <i>Candida</i> species	Rapid detection of point mutations in <i>Candida</i> spp. using FRET hybridization probes with probe melting-curve analysis (LightCycler-style RT-PCR).	Real-time PCR assay with dual FRET hybridization probes and melting curve analysis to rapidly detect ERG11 point mutations in - resistant <i>Candida</i> isolates.	1. Cells grown on sabouraud agar → suspended in saline ($\sim 10^6$ CFU mL ⁻¹). 2. Lyticase treatment → generate spheroplasts.	1. Culture: 48 h at 30 °C on sabouraud glucose agar. 2. Cell suspension: adjusted to $\sim 10^6$ CFU mL ⁻¹ (McFarland 0.5). 3. Lyticase buffer: 10 kU L ⁻¹ , lyticase, 50 mmol L ⁻¹ tris, 1 mmol L ⁻¹ EDTA.	1. Specificity testing with DNA previously extracted from <i>C. albicans</i> . 2. Quality check by storing DNA immediately at - 20 °C.	LightCycler software (Roche molecular systems).	39

Table 1 (Contd.)

Title	Method	Target analysis	Pre-analysis process	Conditions	Additional setup	Data analysis	Ref.
Analysis of <i>Candida albicans</i> plasma membrane proteome	Online LC-MS/MS with ECL chemiluminescence and MALDI-TOF/TOF for peptide mass fingerprints and fragment spectra.	<i>Candida albicans</i> yeast cells (strain SC5314) to characterize the plasma membrane proteome to candidiasis ranging from mucocutaneous to systemic bloodstream infections.	Samples were solubilized in urea/thiourea buffer, SDS loading buffer at 37 °C, 10% SDS-PAGE gels, probed/ <i>anti</i> -Pma1 or antiGas1 antibodies, HRP-conjugated secondary antibodies.	1. Instrument: LTQ ion trap mass spectrometer (thermo electron, san Jose, CA, USA). 2. Column: BioBasic C18 PicoFrit (75 µm i.d. × 10 cm; new objective, NJ, USA). 3. Flow rate: 200 nL min ⁻¹ . 4. Mobile phases: water with 0.1% formic acid. Acetonitrile (ACN) with 0.1% formic acid.	1. Two-phase separation with triton X-114 to partition hydrophobic membrane proteins from soluble proteins. 2. Sucrose gradient ultracentrifugation to enrich plasma membrane acetylation sites. fractions and PI-PLC.	1. MS analyses with a 4700 MALDI-TOF/TOF Analyser (applied biosystems). 2. TMHMM (transmembrane domains), NetAcet 1.0 (<i>N</i> -acetylation sites). 3. FunSpec (functional enrichment of protein groups), VENNY (Venn diagram tool for common proteins).	40

1.4.13 GC-Orbitrap metabolomics (offline). Combines gas chromatography with high-resolution Orbitrap MS for untargeted profiling. It is essential for mapping the metabolic dynamics of complex polymicrobial infections and virulence factors.³⁵

1.4.14 Nanoporous oligonucleotide probe (online). Features anodic alumina membranes functionalized with Rhodamine B. Dye release triggered by molecular recognition allows for sensitive fluorescence-based detection.³⁶

1.4.15 Electrochemical impedance spectroscopy (online). Employs gold electrodes functionalized with anti-*Candida* IgG. EIS monitors real-time fungal binding *via* resistance changes, offering a label-free biosensing route for urinary infections.³⁷

1.4.16 Respiratory activity biosensor (online). This electrochemical platform monitors mitochondrial respiratory chain activity by targeting cytochrome *c* oxidase. Rather than sensing the organelle directly, the device transduces the enzymatic electron transfer into an electrochemical signal *via* Cyclic Voltammetry (CV). This approach allows for the indirect assessment of *C. albicans* viability through its metabolic signature, supporting rapid, early-stage diagnostic screening.³⁸

1.4.17 FRET-RT/PCR (inline). Dual FRET probes enable real-time melting curve analysis to detect ERG11 mutations. This tool is vital for antifungal stewardship, providing rapid genotypic profiling of azole resistance.³⁹

1.4.18 Plasma-M proteomics (offline). Utilizes SDS-PAGE fractionation and LC-MS/MS to map the plasma membrane proteome. Bioinformatics functional annotation helps identify novel therapeutic targets and transport-linked virulence proteins.⁴⁰ While reported sensitivities like 10 CFU mL⁻¹ showcase technological potential in idealized buffers, the inherent complexity of clinical matrices (blood, urine, swabs) introduces significant interference and signal attenuation, marking clinical validation as the primary bottleneck for real-world diagnostic implementation.

1.5 Benchmarking against clinical gold standards

While the 18 methodologies reviewed offer superior sensitivity, their clinical utility must be evaluated against existing gold standards: fungal culture (the benchmark for viability) and MALDI-TOF MS (the routine standard for rapid identification). Traditional culture, though slow (24–72 hours), remains the baseline for diagnostic certainty, whereas MALDI-TOF requires prior growth.⁴¹ In contrast, most AI-driven spectroscopic and electrochemical biosensors discussed here represent emerging prototypes that aim to bypass the culture step entirely. While molecular tools like PNA-FISH have successfully transitioned into clinical use due to their turnaround from positive cultures, other high-performance platforms currently face the ‘translational gap’ the need for rigorous validation in non-spiked, clinical samples to prove they can realistically displace established hospital protocols. Accordingly, this study establishes a standardized, algorithmic framework that evaluates the clinical urgency of *C. albicans* diagnostics across 18 diverse analytical technologies. Moving beyond traditional qualitative reviews, the primary novelty lies in the transition to



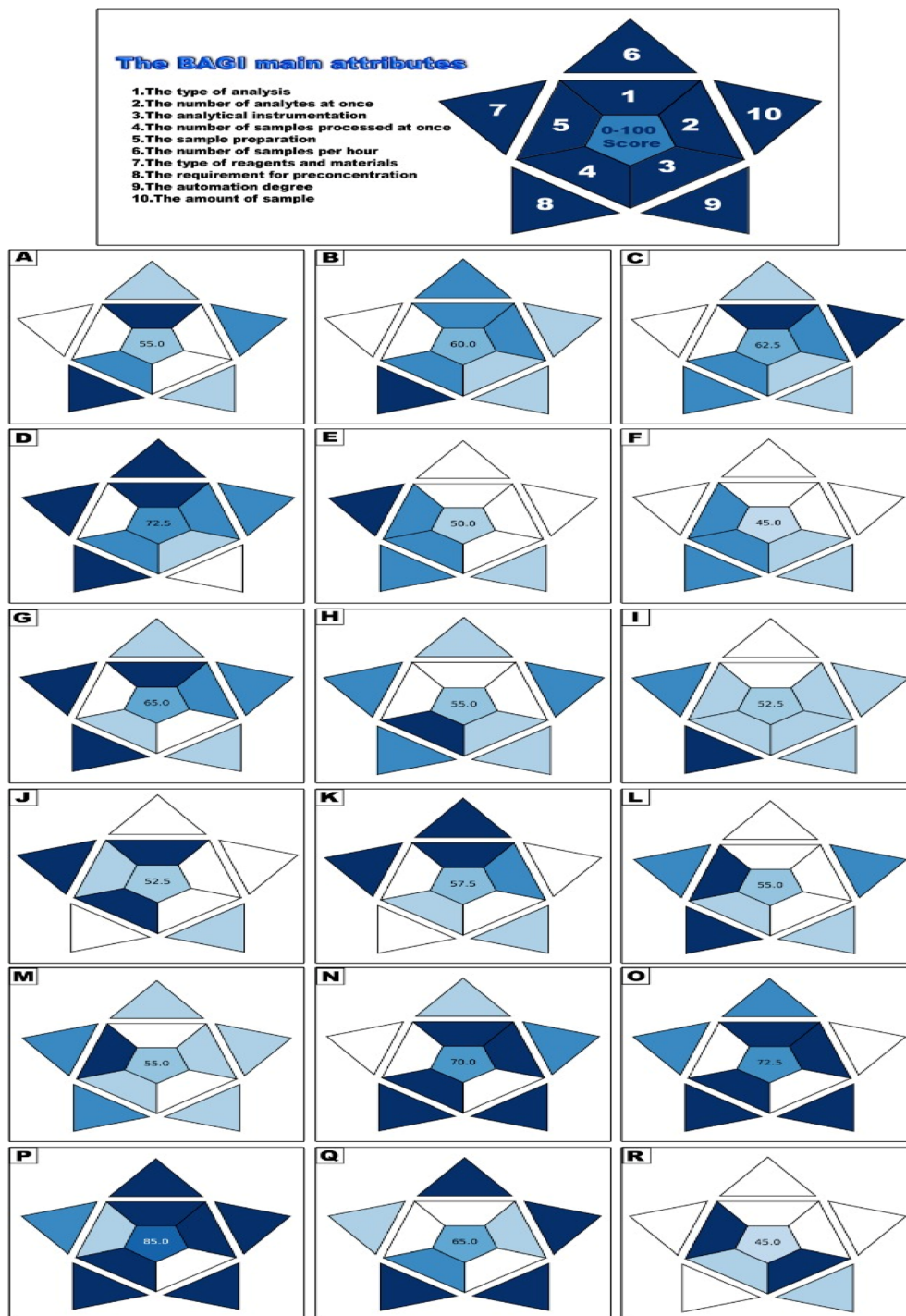


Fig. 3 The comparative BAGI evaluation encompassed eighteen analytical platforms, namely (A) atline Fluorescent quantum dot-Con A labeling method, (B) offline UV-vis spectrophotometric method, (C) inline UV resonance Raman spectroscopic method, (D) offline aptamer-gold nanoparticle colorimetric method, (E) offline SERS with magnetic NPTs method, (F) online PNA-FISH method, (G) offline PCR-DEIA method, (H) offline fluorescence spectroscopic method, (I) offline NMR metabolomics method, (J) offline capillary electrophoresis-FISH method, (K) offline PTR-MS method, (L) offline LC-MS/MS proteomics method, (M) offline GC-Orbitrap metabolomics method, (N) online nanoporous oligonucleotide probe method, (O) online impedance spectroscopic biosensing method, (P) online mitochondrial LSV bio-electrochemical sensor, (Q) inline FRET-RT/PCR method, and (R) offline plasma-M proteomics LC-MS method.

a quantitative decision-making pipeline that integrates WAC model simultaneously optimizing sustainability, clinical practicality (Blueness), and analytical rigor (Redness). A critical original contribution is the derivation of standardized BAGI and

RAPI scores, newly calculated by the authors through a rigorous meta-analysis of primary technical literature Fig. 3. By fusing these metrics within a Python-based Multi-Criteria Decision-Making (MCDM) system, this work provides the first objective,



dynamic ranking of diagnostic platforms to identify optimum techniques for clinical use. Furthermore, this review critically addresses the current limitations of automated scoring such as data heterogeneity and the subjective weighting of criteria while proposing future directions for the integration of real-time sensor data into evolving diagnostic algorithms.⁴²

2 Discussion

The diagnostic landscape for *C. albicans* is undergoing a dual transformation, shifting from traditional observation to a data-centric, sustainable paradigm. This study addresses this evolution through a two-fold analytical approach. First, we conducted a comprehensive evaluation of the role of Artificial Intelligence (AI) and computational tools in enhancing the detection accuracy of these eighteen methodologies. By mapping the transition from manual interpretation to automated pipelines such as machine vision for image-based methods and multivariate chemometrics for spectroscopic data, this work identifies how AI-driven analysis minimizes operator bias and accelerates clinical decision-making. Second, we developed a standardized comparative framework to objectively rank the 18 identified technologies. While BAGI and RAPI are established metrics within the White Analytical Chemistry (WAC) model, their integration here is unique; all scores were independently derived through a rigorous meta-analysis of primary technical data. By fusing these metrics into a novel, Python-based multi-criteria decision-making system, we have transformed static performance indicators into a dynamic ranking tool. It is important to emphasize that this framework serves as a semi-quantitative decision-support tool intended to complement, rather than supersede, absolute ICH and CLSI validation protocols.⁴³ This dual approach analyzing AI integration while simultaneously benchmarking sustainability and performance provides a reproducible roadmap for selecting the most effective and practical diagnostic platforms for modern clinical fungal diagnostics.

2.1 Computational and AI-driven data analysis in *C. albicans* detection

The detection of *C. albicans* across the eighteen reviewed methodologies reflects a paradigm shift from manual observation toward automated, AI-driven data interpretation. Rather than viewing these technologies as isolated protocols, they can be categorized by their computational requirements, which are increasingly addressed through Python-based ecosystems. Digital image processing and machine vision: several platforms, such as Methods 1, 4, and 14, rely on the quantification of fluorescence or colorimetric intensity. While initial reports utilized software like ImageJ or Imaris, these workflows are ideally suited for automation *via* Python's OpenCV and scikit-image libraries. Such integration allows for high-throughput, three-dimensional reconstruction and real-time diagnostic scaling, minimizing operator subjectivity in clinical settings. Chemometrics and multivariate modeling: spectroscopic and electrochemical techniques (Methods 3, 5, 9, 15, and 16)

demonstrate the necessity of advanced pattern recognition.⁴⁴ By employing techniques like Principal Component Analysis (PCA), Support Vector Machines (SVM), and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), these platforms can resolve complex biological signatures in clinical matrices. While proprietary tools (*e.g.*, SIMCA, MATLAB) were frequently cited, the transition to open-source libraries like scikit-learn and PyChemometrics offers a more reproducible and scriptable alternative for pathogen discrimination. Notably, the mitochondrial-based electrochemical biosensor (Method 16) aligns effectively with these algorithmic requirements, achieving high ranking in our framework due to its data-dense output and compatibility with rapid digital processing. Bioinformatics and omics integration: molecular and mass-spectrometry-based strategies (Methods 6, 8, 12, 13, 17, and 18) represent the 'Big Data' frontier of fungal diagnostics. The reliance on sequence alignment, proteomic mapping, and metabolite profiling highlights a critical role for Pyteomics. These tools allow for the seamless fusion of genomic and proteomic metadata, transforming static diagnostic readouts into predictive models for clinical mycology. Collectively, this transition toward an algorithmic diagnostic framework underscores that the 'whiteness' of a modern method is determined not only by its bench-top performance but also by its digital interoperability and the efficiency of its data analysis pipeline.

2.2 Comparative evaluation of diagnostic platforms through Python-based tools

The BAGI evaluation of eighteen diagnostic methodologies for *C. albicans* revealed marked heterogeneity in operational feasibility, analytical applicability, and sustainability. The Fluorescent Quantum Dot-ConA Labeling Method (A, 55.0) achieved labeling efficiencies exceeding 93% with excellent visualization *via* microscopy and flow cytometry, but was constrained by high reagent costs, moderate complexity, and low throughput. The UV-vis Spectrophotometric Method (B, 60.0) offered a simple, cost-effective absorbance- and nephelometry-based approach with strong agreement to manual counts, though operator-dependent handling compromised precision. The UV Resonance Raman Method (C, 62.5) enabled rapid species differentiation and resistance profiling through PCA-driven chemometrics, yet its dependence on specialized 244 nm lasers limits accessibility to advanced laboratories. In contrast, the Aptameric-Gold Nanoparticle Colorimetric Method (D, 72.5) delivered clear visual detection in just five minutes with minimal preparation and low-cost reagents, rendering it highly scalable for routine screening. The SERS with Magnetic Capture Method (E, 50.0) provided near-perfect species classification *via* nanoparticle-enhanced Raman scattering and preconcentration, but workflow complexity and reagent demands diminished overall feasibility. The PNA-FISH assay (F, 45.0) attained 100% diagnostic sensitivity and specificity without DNA extraction or subculture, yet its 2.5 hours hybridization and moderate preparation complexity reduced sustainability. Fluorescence spectroscopy with PDT (G, 65.0) demonstrated strong linear correlation with biofilm CFU counts, though limited



throughput and validation hindered broader applicability. The PCR-DEIA (H, 55.0) facilitated same-day, culture-independent detection with robust sensitivity and specificity, despite concerns over serum inhibitors and modest throughput. NMR spectroscopy (I, 52.5) offered automated, label-free species identification with good reproducibility *via* algorithmic support, but lacked validation of trueness and recovery. The CE-FISH method (J, 52.5) achieved excellent sensitivity and linearity through fluorescent hybridization combined with CE-LIF detection, although multiple preparation steps (lysis and hybridization) reduced practicality. PTR-MS volatile fingerprinting (K, 57.5) generated rapid, semi-quantitative outputs from urine samples with high automation, yet required further validation of reproducibility and robustness in clinical matrices. High-resolution omics platforms, such as LC-MS/MS proteomic mapping (L, 55.0) and GC-Orbitrap metabolomics (M, 55.0), provided deep, reproducible profiling of proteins and metabolites across planktonic and biofilm states, but were hampered by labor-intensive workflows and low throughput. Among biosensor platforms, the NAA sensing platform (N, 70.0) enabled rapid (<30 min), label-free detection with minimal sample volumes, though throughput and automation remained modest. The impedance biosensor (O, 72.5) achieved high-sensitivity candiduria detection (down to 10 CFU mL⁻¹) using microfluidic electrodes and minimal preparation, limited primarily by its single-channel design. The LSV biosensor (P, 85.0) stood out as the highest-ranked platform, combining low-cost portability, long-term stability, and user-friendliness with the unique ability to target *Candida* mitochondrial complex IV, enabling both accurate quantification/confirmation of presence and a novel pathway for integrated diagnostic-therapeutic applications. The FRET melting-curve PCR assay (Q, 65.0) supported high-throughput resistance genotyping *via* closed-tube amplification and probe-based melting analysis, with excellent reproducibility and low consumable costs. Finally, LC-MS/MS with MALDI-TOF proteomics (R, 45.0) delivered comprehensive, discovery-driven proteomic identification, but resource-intensive instrumentation and lack of quantitative validation limited its sustainability. Collectively, this BAGI assessment underscores that while omics and advanced spectroscopic platforms excel in mechanistic depth and discovery potential, their clinical sustainability is often undermined by high resource demands, complex workflows, and instrumentation costs. Biosensor-based approaches particularly electrochemical designs such as the mitochondrial-targeted sensor (P) achieve the most effective balance of diagnostic accuracy, affordability, and practical scalability.⁴⁵ By incorporating Python-driven chemometrics with sustainable analytical metrics, this framework offers a reproducible, data-informed strategy for selecting diagnostic tools that address clinical urgency while advancing environmental responsibility and alignment with the UN Sustainable Development Goals.

2.2.1 Redness assessment. The RAPI evaluation of eighteen analytical strategies for *C. albicans* detection revealed substantial variation in analytical reliability, validation completeness, and overall performance. The Fluorescent CdTe-Flow Cytometry Method (A, 43.8) exhibited high trueness across diverse

morphological states, yet was limited by absent precision and recovery validation. Similarly, the UV-vis Spectrophotometric Method (B, 40.6) provided reliable calibration for cellular counts, though manual handling introduced robustness concerns. The UV Resonance Raman Method (C, 46.9) achieved accurate species discrimination through PCA-SVM chemometric classification and resonance enhancement, but reproducibility in heterogeneous clinical matrices requires further substantiation. The aptameric-Gold Nanoparticle Colorimetric Method (D, 43.8) demonstrated strong trueness and environmental robustness (*e.g.*, under varying pH and temperature), despite lacking formal precision data. The SERS with Magnetic Capture Method (E, 37.5) delivered excellent classification *via* OPLS-DA and high capture efficiency, but was penalized for missing recovery studies. The Fluorescence-ISH Method (F, 40.6) attained 100% sensitivity in blood cultures with robust performance under hybridization conditions, even without reported precision metrics. Several platforms including Fluorescence spectroscopy with PDT (G, 28.1), PCR-DEIA (H, 28.1), NMR spectroscopy (I, 28.1), and CE-FISH (J, 28.1) showed promising analytical signatures but received lower RAPI scores due to incomplete validation of trueness, recovery, and related parameters. Advanced mapping techniques, such as PTR-MS volatile fingerprinting (K, 40.0) and LC-MS proteomics (L, 40.0), enabled reproducible biomarker identification, though comprehensive quantitative assessment and robustness validation remain incomplete. GC-Orbitrap metabolomics (M, 45.0) offered high precision in metabolic profiling, yet trueness evaluation was insufficient. In the biosensing category, the NAA platform (N, 37.5) supported rapid screening but lacked validation across large clinical cohorts. The impedance biosensor (O, 50.0) achieved outstanding sensitivity (10 CFU mL⁻¹) and reproducibility *via* microfluidic normalization, although recovery data were unreported. The LSV biosensor (P, 59.4) emerged as the highest-ranked platform under the RAPI framework, providing specific detection with confirmed robustness through long-term stability trials. By contrast, molecular and discovery-oriented platforms such as FRET melting-curve PCR (Q, 34.4) and LC-MS/MS with MALDI-TOF (R, 32.5) offered high sensitivity and broad identification capabilities, but were constrained by absent formal precision and recovery testing. Collectively, this RAPI assessment highlights that while “omics” and spectroscopic approaches excel in discovery and mechanistic insight, their analytical performance is frequently undermined by gaps in rigorous validation. Biosensor platforms particularly those incorporating electrochemical transducers currently deliver superior validated reliability and robustness.⁴⁶ This emphasis on core analytical parameters, as illustrated in the WAC Model (Fig. 4), is critical for advancing laboratory prototypes toward robust, dependable clinical diagnostics.

2.3 Integrating NQS evaluation with sustainable development goals for fungal detection

In the framework of sustainability, the Need, Quality, and Sustainability (NQS) Index provides a percentage-based metric to evaluate analytical methods, integrating necessity (Need), methodological performance (Quality), and environmental-



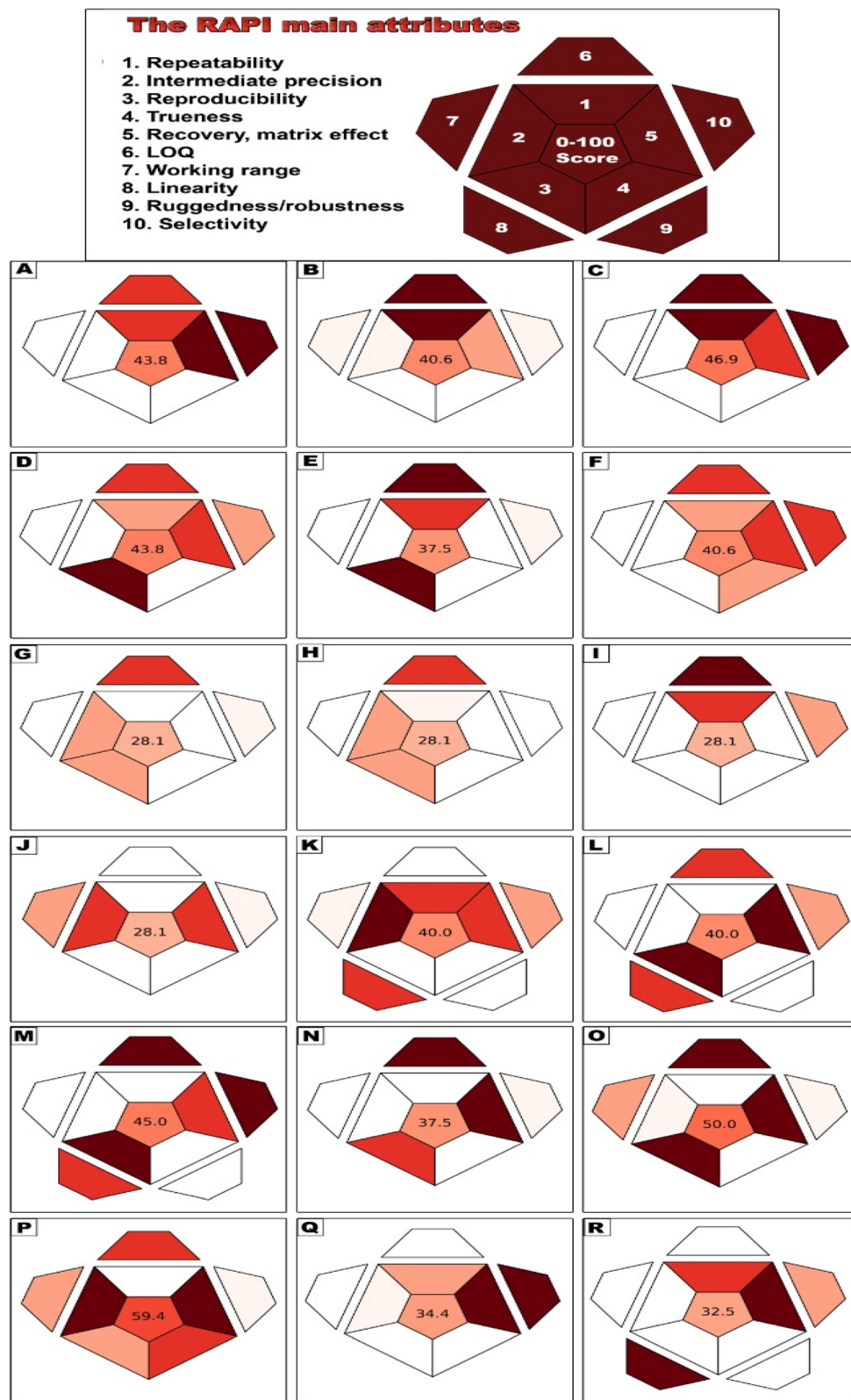


Fig. 4 The comparative RAPI evaluation encompassed eighteen analytical platforms, namely (A) atline fluorescent quantum dot-Con A labeling method, (B) offline UV-vis spectrophotometric method, (C) inline UV resonance Raman spectroscopic method, (D) offline aptamer-gold nanoparticle colorimetric method, (E) offline SERS with magnetic NPTs method, (F) online PNA-FISH method, (G) offline PCR-DEIA method, (H) offline fluorescence spectroscopic method, (I) offline NMR metabolomics method, (J) offline capillary electrophoresis-FISH method, (K) offline PTR-MS method, (L) offline LC-MS/MS proteomics method, (M) offline GC-Orbitrap metabolomics method, (N) online nanoporous oligonucleotide probe method, (O) online impedance spectroscopic biosensing method, (P) online mitochondrial LSV bio-electrochemical sensor, (Q) inline FRET-RT/PCR Method, and (R) offline plasma-M proteomics LC-MS method.



operational responsibility (Sustainability).⁴⁷ In the framework of sustainability, the NQS index provides a comprehensive, percentage-based metric that has evolved significantly. Initially rooted in traditional analytical methodologies,^{48,49} its scope has progressively extended through environmental, agricultural, and industrial applications.⁵⁰ Today, it culminates in the evaluation of modern, high-tech diagnostic platforms, ensuring that contemporary analytical practices are fully aligned with the United Nations Sustainable Development Goals (SDGs) by balancing methodological excellence with global environmental responsibility. The NQS index serves as a transformative benchmarking tool, providing a standardized framework to evaluate the evolution of analytical chemistry from foundational methods to cutting-edge diagnostic platforms.

The Need dimension quantifies the indispensability of a method using Koel's pyramid, the Quality percentage is determined by assessing the individual elements of the WAC framework where the %Quality is calculated by taking the average of the summed percentages of redness (R), greenness (G), and blueness (B), as follows:

$$\% \text{ Quality} = \left(\sum_{i=1}^4 R_i + \sum_{i=1}^4 G_i + \sum_{i=1}^4 B_i \right) / 3$$

As summarized in Table S1, the application of this framework to diagnostic innovations for *C. albicans* illustrates direct contributions to SDG 3 (Good Health and Well-Being) through rapid interventions, SDG 9 (Industry, Innovation, and Infrastructure) via advanced platforms, SDG 12 (Responsible Consumption and Production) through rational antifungal stewardship, and SDG 17 (Partnerships for the Goals) by fostering multidisciplinary and international collaborations. The resulting single score enables holistic, sustainability-driven method selection as follow:

$$\text{NQS index (\%)} = (\% \text{ Need} + \% \text{ Quality} + \% \text{ Sustainability})/3.$$

Together, the NQS index and SDG mapping as shown in Fig. S1 ensure that diagnostic advances are not only analytically rigorous but also societally and environmentally sustainable.

2.4 Limitations and decision-making in multi-parameter assessment

Decision-making for selecting analytical methods in *C. albicans* detection benefits substantially from integrated multi-parameter evaluation frameworks, such as those combining greenness, blueness (practicality), and redness (performance) within the White Analytical Chemistry model. When Python-based scoring software identifies sustainability gaps, targeted interventions such as substituting conventional solvents with eco-friendly alternatives or incorporating green surfactants can improve environmental performance without compromising diagnostic accuracy.⁵¹ This approach empowers clinical settings to adopt high-quality assays that align with green analytical principles while supporting reliable biomarker detection. The selection of

the eighteen core techniques prioritized widespread adoption and diverse operational modes (online, offline, inline, and at-line). However, this review focuses predominantly on established conventional approaches, currently excluding emerging AI-assisted strategies such as capacitance spectroscopy and machine learning-driven automated image analysis.⁵² This omission underscores a persistent divide between traditional diagnostics and next-generation intelligent methodologies. To bridge this gap, we recommend incorporating a novel factorial metric into assessment frameworks to quantify the extent of machine learning integration in biomarker decision processes, effectively linking conventional assays with advanced automated platforms. In high-stakes clinical scenarios, such as the management of immunocompromised patients, sustainability metrics must be judiciously balanced against diagnostic priorities. Parameters of clinical applicability, sensitivity, and specificity take precedence over environmental considerations in these contexts. For instance, resource-intensive techniques like LC-MS remain essential due to their superior specificity in identifying fungal biomarkers, despite lower greenness scores.⁵³ Finally, even the most optimized platforms within this framework are subject to technical trade-offs. While the mitochondrial-based biosensor exhibits an optimum balance of sustainability and performance, its clinical implementation remains subject to specific constraints. These include the long-term stability of biological recognition elements and potential matrix interference from complex clinical samples (*e.g.*, blood or serum).⁵⁴ The multi-parameter evaluation serves as a realistic guide for method selection, ensuring that analytical precision supports real-time therapeutic decisions where diagnostic imperatives are paramount.

2.5 Strategic scope and computational boundaries of Python's tools

While Python's dominance in analytical chemistry is driven by its ecosystem, it is essential to acknowledge its inherent computational limitations in standard builds as shown in Fig. S2. Unlike natively compiled languages such as C++ or Julia, Python's execution speed in the classic CPython interpreter is often hindered by the Global Interpreter Lock (GIL), which prevents true multi-threaded execution of Python bytecodes.⁵⁵ This remains a significant bottleneck in extreme High-Performance Computing (HPC) scenarios, such as large-scale molecular dynamics or real-time high-frequency signal processing. However, the analytical chemistry community effectively bypasses these constraints through libraries like NumPy and SciPy (implemented in C/Fortran), achieving near-native speeds for most numerical tasks. Recent advancements particularly free-threaded (no-GIL) builds in Python enable genuine multi-threading parallelism in many CPU-bound workloads, with single-threaded overhead typically reduced to 5–10% and ongoing ecosystem maturation lowering adoption barriers. Integration with high-level parallel computing interfaces (*e.g.*, Dask or PySpark) further scales data workflows, while hybrid approaches (Python wrappers around Julia/C++/Fortran kernels) erode strict boundaries.⁵⁶ This makes Python a highly



pragmatic choice for the diverse analytical chemistry applications (Table S2), from sustainability assessment to point-of-care diagnostics. Ultimately, this computational flexibility has facilitated the development of open-source, Python-based tools such as BAGI and RAPI. By making these methodologies accessible, the framework ensures a standardized and unbiased platform for multi-criteria decision-making.⁵⁷ This open-access approach is critical when comparing diverse analytical methodologies, as it allows researchers to evaluate the trade-offs between performance and sustainability through a verifiable, reproducible lens, ensuring that the selection of the optimal diagnostic method is driven strictly by objective data rather than proprietary constraints.

3 Conclusion

This WAC-driven meta-analysis and Python-MCDM framework objectively ranks 18 analytical methods for *C. albicans* detection in clinical matrices. By fusing recalculated BAGI (blueness: practicality, portability, speed, minimal prep) and RAPI (redness: sensitivity, selectivity, validation strength) scores, electrochemical biosensors consistently outperform alternatives including spectroscopic, molecular, and chromatographic techniques in overall balance of performance, clinical feasibility, and sustainability. These designs deliver rapid, point-of-care-capable detection with low reagent/waste footprint, aligning strongly with UN SDGs 3 (health access & timely therapy) and 9 (sustainable innovation). They surpass traditional culture methods in speed and practicality while rivaling or exceeding advanced techniques in WAC compliance. The open-source Python workflow offers a reproducible, extensible tool for future pathogen diagnostics. Electrochemical biosensors stand out as the preferred choice for sustainable, high-performance *Candida* diagnostics in resource-variable settings. Prospective clinical validation and cost-integration studies will further strengthen adoption, accelerating greener infectious disease management. Future directions will emphasize AI-based diagnostics, critical transition toward sustainable reagents, waste-minimized workflows, and renewable energy-powered instrumentation, in addition to advanced smart materials enabling precise and eco-friendly detection of *C. albicans*.

Author contributions

Ahmed M. Saleh: writing – original draft, methodology, investigation, data curation, conceptualization. Rabeay Y.A. Hassan: writing – review & editing, visualization, supervision, conceptualization. Amr M. Badawey: writing – review & editing, supervision, conceptualization. Hoda M. Marzouk: writing – review & editing, visualization, supervision, methodology, investigation, conceptualization.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Supplementary information (SI) is available. See DOI: <https://doi.org/10.1039/d6ra00286b>.

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