



MYCOLOGY
WORKSHOP
2025

9-12TH APRIL 2025

MASTERING
ANTI-FUNGAL
THERAPY

Rapid diagnostics of fungal diseases

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10 April 2025

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Disclosure

- I received ISR grant from Pfizer on evaluation of culture and non-culture diagnostics methods for invasive candidiasis.



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Questions!!

Q1. How many deaths caused by fungal infections where the cause of deaths were not reported as “fungal infections” in your institution?

Q2. Has anyone used PCR for fungal detection in their practice?"

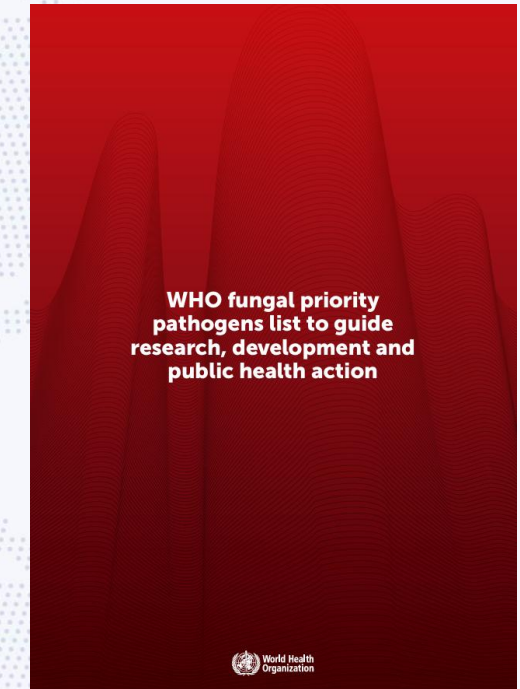
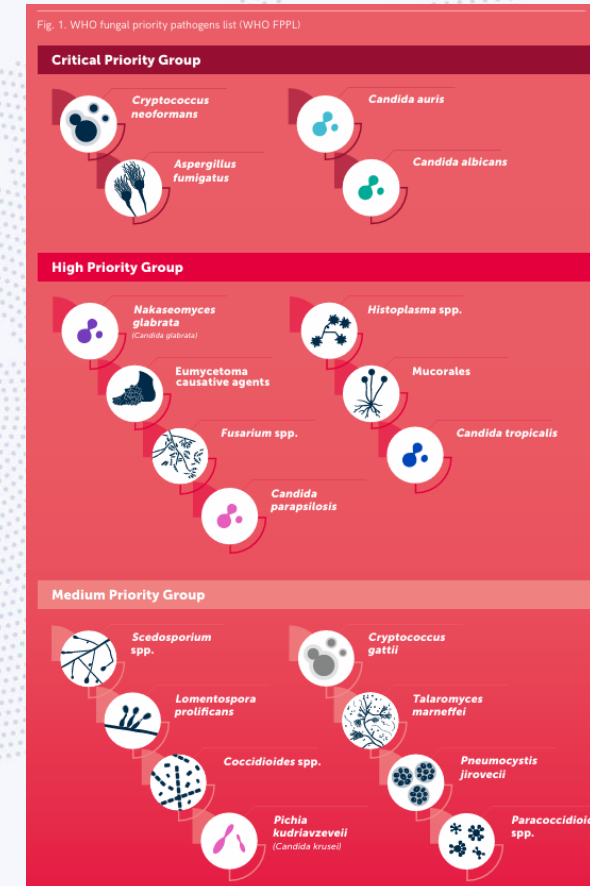
Burden of Fungal Infections in Malaysia

Table 1. Estimated annual cases and total burden of serious fungal infections in Malaysia.

Fungal Infection	Number of Infections per Underlying Disorder per Year					Total Burden	Rate/100,000
	None	HIV/AIDS	Respiratory	Cancer/Tx	ICU		
Oesophageal candidiasis	-	5850	-	-	-	5850	19
Candidaemia	-	-	-	1073	460	1533	5
<i>Candida</i> peritonitis	-	-	-	-	230	230	0.8
Recurrent vaginal candidiasis (>4x/year)	501,138	-	-	-	-	501,138	4800 *
ABPA	-	-	30,062	-	-	30,062	98
SAFS	-	-	39,682	-	-	39,682	130
Chronic pulmonary aspergillosis	-	-	7635	-	-	7635	24.9
Invasive aspergillosis	-	-	-	184	834	1018	3.3
Cryptococcal meningitis	47	700	-	108	-	855	2.8
Pneumocystis pneumonia	-	1286	-	-	-	1286	4.2
Histoplasmosis	-	175	-	-	-	175	0.6
<i>T. marneffei</i> infection	-	350	-	-	-	350	1.1
Fungal keratitis	400	-	-	-	-	400	1.3
Total burden estimated	501,585	8361	77,379	1365	1524	590,214	

Tx, transplant recipients; ICU, intensive care unit; ABPA, allergic bronchopulmonary aspergillosis; SAFS, severe asthma with fungal sensitization; HIV/AIDS, Human immunodeficiency virus infected/Acquired immunodeficiency syndrome; * Note rate of recurrent vaginal candidiasis is per 100,000 females.

Source: Velayuthan, et al. (2018). *Journal of Fungi*, 4(1), 38



Source: WHO fungal priority pathogens list to guide research, development and public health action. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO

Early Detection and Reduced Mortality

(Importance of Early detection in Fungal infections)



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- Fungal infections can rapidly progress to severe and life-threatening forms, esp. in immunocompromised individuals. The longer the delay in dx, the higher the likelihood of complications, i.e., organ failure or sepsis. **Early intervention significantly improves patient outcomes.**
- E.g., Invasive infections in ICU patients have mortality rates of 30-50% if not diagnosed early and treated promptly (Pappas et al, 2018). In contrast, early initiation of antifungal therapy can reduce mortality by over 50%

Early Detection and Reduced Mortality

(Impact of Rapid Diagnostics on Mortality)



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- Several studies have demonstrated that rapid diagnostics **reduce mortality** by allowing earlier and more accurate treatment. Rapid identification of the pathogen leads to a faster, more effective treatment regimen.
- A randomized controlled trial (RCT) by Hedayati et al. (2021) found that rapid diagnosis of *Candida* bloodstream infections reduced mortality rates by 35% compared to the standard culture-based diagnosis
- Another study showed that the use of rapid PCR for *Aspergillus* detection in ICU settings significantly reduced mortality from 70% to 48% (Riche et al., 2020)

Delayed diagnosis worsens outcomes

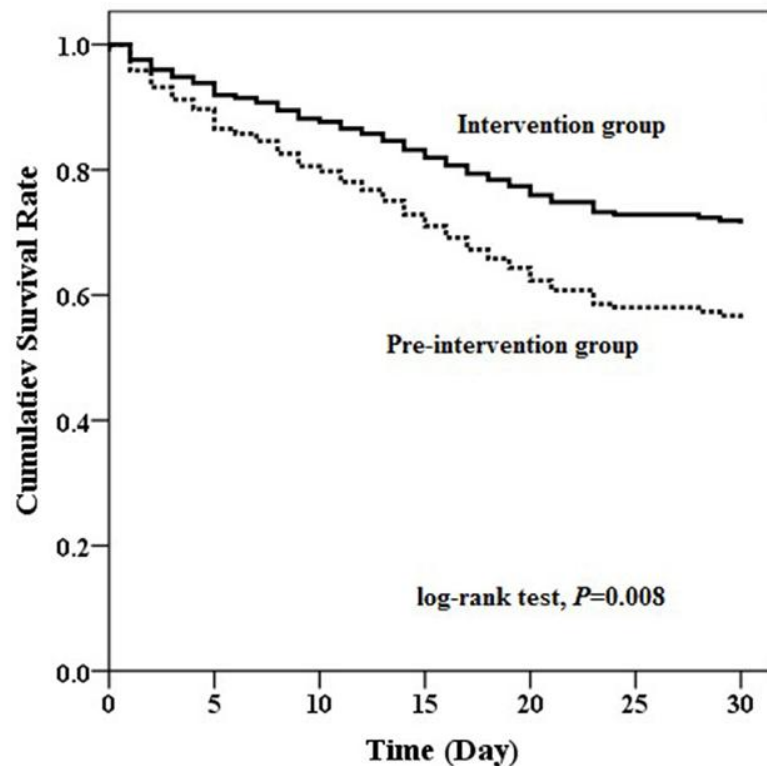


Figure 3. The survival analysis curves in the intervention case group (solid line) associated with a favorable 30-day mortality than pre-intervention control group (dot line) (log-rank test, $P = 0.008$).

Table 4

Odds ratios for mortality among patients with invasive pulmonary aspergillosis (IPA) assessed using multivariate logistic regression analyses, with an additional variable of administration history of voriconazole (model 1) and that of voriconazole or liposomal amphotericin B (model 2)

Variable	Model 1			Model 2		
	OR	95% CI	P-value	OR	95% CI	P-value
Early diagnosis	0.55	0.31-0.99	0.047	0.58	0.33-1.01	0.056
Female sex	0.64	0.30-1.36	0.24	0.69	0.33-1.45	0.33
Age ≥ 65 years	3.13	1.17-8.34	0.023	3.32	1.26-8.76	0.016
BMI, kg/m ² (referent, $18.5 \leq \text{BMI} \leq 24.9$)						
<18.5	1.38	0.76-2.53	0.29	1.40	0.78-2.52	0.26
≥ 25	0.83	0.16-4.48	0.83	0.86	0.16-4.63	0.86
COPD	1.19	0.46-3.04	0.72	1.25	0.50-3.14	0.63
Diabetes mellitus	0.88	0.45-1.74	0.72	0.90	0.47-1.75	0.77
Influenza	2.69	0.63-11.60	0.18	2.43	0.59-9.97	0.22
Liver cirrhosis	4.11	0.12-143.30	0.44	5.30	0.18-157.51	0.34
Mechanical ventilation	10.78	4.24-27.40	<0.001	10.20	4.10-25.40	<0.001
JCS ≥ 1	1.63	0.72-3.71	0.24	1.70	0.76-3.82	0.20
Administration of VRCZ	1.36	0.76-2.43	0.30			
Administration of VRCZ or L-AMB				2.06	1.15-3.71	0.016

Early diagnosis was defined as the initiation of antifungal therapy within 7 days of hospital admission.

BMI, body mass index; CI, confidence interval; COPD, chronic obstructive pulmonary disease; JCS, Japan Coma Scale; OR, odds ratio; VRCZ, voriconazole; L-AMB, liposomal amphotericin B.

Sources:

Jan et al., J Microbiol Immunol Infect. 2023 Dec;56(6):1253-1260.

Katsuhiko et al. International Journal of Infectious Diseases, Volume 122, 279 - 284

Challenges in Diagnosing Fungal Infections



Laboratory support
for mycology not
widespread



Long turn-around
time for diagnostic
tests

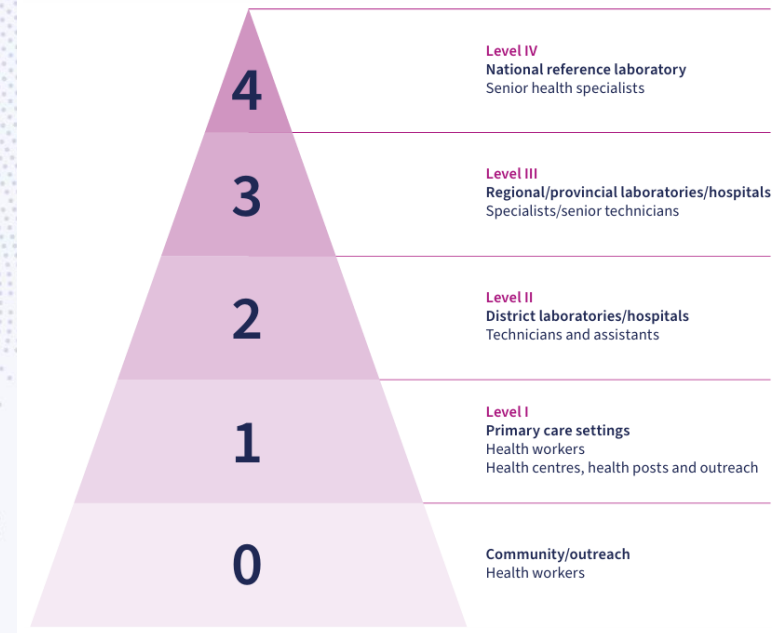


Confirmatory
diagnosis is still
based on
microscopy and
histopathology

Gaps and needs identified include the following.

- Limited knowledge of the impact of antifungal resistance in LMICs, as a consequence of very limited ability to perform fungal culture for detection and AFST below level III in LMICs; difficulty to perform microscopy/histopathology in level II facilities due to the need for highly trained personnel.
- Few commercially available, automated (or semi-automated) tests, including biochemical assays, for identification of yeasts, and lack of broad regulatory approval for identification of filamentous fungi on these systems. Inadequate sensitivity/specificity of most tests.
- Limited availability of non-culture-based diagnostic methods beyond the most commonly occurring pathogens on the WHO FPPL (e.g. *Candida* spp., *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Pneumocystis jirovecii*) and limited accessibility to these tests to higher levels of the health system.
- No multiplex tests/platforms suitable for use at level II settings to detect fungal pathogens directly from clinical specimens (no culture required) with AFST/resistance testing done on a separate platform or combined with AFST/resistance testing on the same platform.
- Few LFIA to adequately identify a broad range of IFDs and no available multiplex LFIAs for use at any level of the health system.

Fig. 2. Diagram of tiered laboratory systems in LMICs



Sources:

Iyadorai, et al., Epidemiol Rev. 2024 Sep 16;46(1):1-12.

Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

Urgency of Rapid Fungal Diagnostic

TABLE 2 Diagnostic performance of BAL fluid GM test, culture, BDG test, LFD test, and PCR for probable and proven IPA versus no IPA in patients with all test results available^a (Table view)

Test method and/or conditions ^b	Performance characteristic (%) ^c				
	Sensitivity (n = 10)	Specificity (n = 41)	PPV	NPV	DOR (95% CI) ^d
GM ODI of >1.0	70	98	88	93	93.3 (8.5-1,030)
GM ODI of >0.5	80	98	89	95	160 (12.9-1,984)
Mycological culture	50	95	71	89	19.5 (3-129)
BDG > 80 pg/ml	80	76	44	94	12.4 (2.3-68)
BDG > 200 pg/ml	60	88	55	90	10.8 (2.2-52)
LFD test	80	95	80	95	78 (9.5-639)
PCR	70	100	100	93	161 (7.5-3,445)
PCR and/or BDG > 80 pg/ml	90	76	47	97	27.9 (3.1-248)
PCR and/or LFD test	90	95	82	98	176 (14-2,154)
GM ODI of >1.0 and/or PCR	100	98	91	100	567 (21.5-14,946)
GM ODI of >1.0 and/or LFD test	90	93	75	97	114 (10.6-1,228)

^a GM, galactomannan; BDG, beta-D-glucan; LFD, lateral-flow-device.

^b ODI, optical density index.

^c PPV, positive predictive value; NPV, negative predictive value.

^d DOR, diagnostic odds ratio; 95% CI, 95% confidence interval.

Source: Hoenigl et al., 2014.. J Clin Microbiol 52:.

Table 1 Comparing patients under diagnostic-driven therapy with voriconazole and those under empiric antifungal therapy without voriconazole

Characteristics and outcomes	Diagnostic-driven therapy with voriconazole (n = 44) N (%)	Empiric antifungal therapy without voriconazole (n = 221) N (%)	p-value
Age (years), median (range)	63 (23-81)	51 (14-80)	< 0.001
Gender, male	26 (59)	131 (59)	0.98
Diagnosis of IA			< .001
Definite IA	4/43 (9)	83 (38)	
Probable IA	39/43 (91)	138 (62)	
Invasive pulmonary infection ^a	40 (91)	178 (81)	0.10
Disseminated infection ^a	2 (5)	18 (8)	0.41
Localized or sinus infection ^a	4 (9)	28 (13)	0.51
Leukemia	19 (43)	181/220 (82)	< .0001
Lymphoma	16 (36)	34/220 (15)	0.001
Myeloma	8 (18)	5/220 (2)	< .001
Transplantation within 1 year prior to infection	16 (36)	82/220 (37)	0.91
Type of transplantation within prior year			0.010
Allogeneic transplant	10/16 (63)	74/82 (90)	
Autologous transplant	6/16 (38)	8/82 (10)	
Graft vs Host Disease (GVHD)	8/10 (80)	52/74 (70)	0.72
Neutropenia (< 500 ANC) at onset of IA	8/42 (19)	120/216 (56)	< .0001
Persistent neutropenia	14/36 (39)	87/210 (41)	0.77
Received immunotherapy	10/43 (23)	154/220 (70)	< .0001
Received WBC transfusion	2/42 (5)	45 (20)	0.016
Year of IA diagnosis/treatment			< .0001
1993-2004	8 (18)	162 (73)	
2005-2016	36 (82)	59 (27)	
Prophylactic antifungal treatment prior to infection	7 (16)	78 (35)	0.012
Breakthrough	6/7 (86)	67/78 (86)	> .99
Response to therapy	32 (73)	30 (14)	< .0001
Death within 42 days of starting therapy	2 (5)	123/220 (56)	< .0001
Aspergillosis-attributable death within 42 days of starting therapy	2 (5)	107/218 (49)	< .0001

^aOne patient had all 3 types of IA infections and 3 patients had both invasive pulmonary and localized or sinus infections

Source: Dib et al. BMC Infect Dis 18, 656 (2018).

Why Rapid Diagnostics Matter

- Early detection
- Fungal resistance
- Healthcare burden

Overall, antifungal agents in the clinical pipeline combined with those approved in the past decade are still insufficient, when considering the key targets and the innovation needed, to address the therapeutically challenging fungal pathogens identified by WHO.

THE CONVERSATION
Academic rigour, journalistic flair

Q Search analysis, research, academics...



Global deaths from fungal disease have doubled in a decade – new study

Published: January 13, 2024 11:10am GMT

There are no vaccines for fungi. Severe fungal disease strikes when people are already ill, with only a few exceptions in healthy people and in those living or working in [mouldy homes](#) or work environments. That is why accurate and timely diagnosis is desperately needed, and why we need to take fungi very seriously.

Denning, David W
The Lancet Infectious Diseases,
Volume 24, Issue 7, e428 - e438



Antifungal agents in clinical and preclinical development

Overview and analysis



Source: Antifungal agents in clinical and preclinical development. Overview and analysis. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

R.E. - A.S.S.U.R.E.D. (WHO)

- **R** Real-time connectivity
- **E** Ease of specimen collection
- **A** Affordable
- **S** Sensitive
- **S** Specific
- **U** User-friendly
- **R** Rapid and robust
- **E** Equipment free or simple, Environmentally friendly
- **D** Deliverable to end-users

Table 3 | Characteristics of a REASSURED diagnostics test

Criteria	Description
R Real-time connectivity	Tests are connected and/or a reader or mobile phone is used to power the reaction and/or read test results to provide required data to decision-makers
E Ease of specimen collection	Tests should be designed for use with non-invasive specimens
A Affordable	Tests are affordable to end-users and the health system
S Sensitive	Avoid false negatives
S Specific	Avoid false positives
U User-friendly	Procedure of testing is simple — can be performed in a few steps, requiring minimum training
R Rapid and robust	Results are available to ensure treatment of patient at first visit (typically, this means results within 15 min to 2 hours); the tests can survive the supply chain without requiring additional transport and storage conditions such as refrigeration
E Equipment free or simpleEnvironmentally friendly	Ideally the test does not require any special equipment or can be operated in very simple devices that use solar or battery power Completed tests are easy to dispose and manufactured from recyclable materials
D Deliverable to end-users	Accessible to those who need the tests the most

Source: Land, et al. 2019 *Nat Microbiol* 4, 46–54

Traditional Diagnostic Methods

Table A2.1. Phenotypic test methods for the detection and identification of priority invasive fungal pathogens

Methodologies	Microorganisms	Specimen	Sensitivity	Specificity	Advantages	Disadvantages
Fungal culture	<i>Candida</i> spp.	Blood	50%–60% (30) 21–71% (22)	95% (30)	<ul style="list-style-type: none"> • Direct detection of fungal pathogen and ability to do AFST • If positive, identifies the specific etiological agent • Gold standard 	<ul style="list-style-type: none"> • Low and variable sensitivity, especially in <i>Aspergillus</i> • Inability to culture <i>Pneumocystis jirovecii</i> • Especially low sensitivity in those on antifungals • Long TAT (depending on species: 1 day to weeks) • Delays treatment • Prone to contamination
Commercial assays: BacT/ALERT® 3D BacT/ ALERT® VIRTUO® (bioMérieux, France); BD BACTEC™ FX (BD Diagnostics) VersaTREK™ (Thermo Fisher, United States)	<i>Aspergillus</i> spp.	Various, depending on infection site	30%–68% (30) 1–5% (23)	72%–100% (30)		
	<i>Cryptococcus</i> spp.	CSF	>95% (30)	100% (30)		
	<i>Histoplasma</i> <i>capsulatum</i>	Tissue, BAL fluid or other bodily fluids	85% for disseminated and acute pulmonary infections (30)	100% (30)		
	<i>Coccidioides</i> spp.	Sputum or tissue	90% (30)	100% (30)		

Source: Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

Traditional Diagnostic Methods

Table A2.1. (continued) Phenotypic test methods for the detection and identification of priority invasive fungal pathogens

Methodologies	Microorganisms	Specimen	Sensitivity	Specificity	Advantages	Disadvantages
Microscopy and histopathology	<i>Candida</i> spp. <i>Aspergillus</i> spp.	Various, depending on the infection site, includes CSF, BAL fluid, expectorated sputum, tissue biopsies, among others	Varies with the individual agent, the source and quality of the specimen, and the skills and experience of the laboratory technician		<ul style="list-style-type: none"> • Direct microscopy can rapidly detect pathogen by shape/size • Histopathology can rapidly provide a presumptive diagnosis and can detect host response 	<ul style="list-style-type: none"> • Microscopy cannot identify fungal species • Histopathology from sterile sites can require invasive procedures • Both methods require highly trained mycologist • Similar microscopic and histopathologic appearance of several fungi • Staining required
Commercially available instruments or methods for: Automated staining/streaking and inoculation of media	<i>Pneumocystis</i> spp.	Expectorated sputum, induced sputum, or BAL fluid	Histo: 33%–100%, depending on stain and specimen used (30)	Histo: 72%–100% (30)		
	<i>Cryptococcus</i> spp.	Primarily CSF	Histo: 75% (30)	Histo: 100% (30)		
	<i>Histoplasma capsulatum</i>	Tissue or BAL fluid	Low sensitivity (56)			
	<i>Coccidioides</i> spp.	Sputum or tissue	Histo: 31%–42% (30)	Histo: 100% (30)		

Source: Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

Traditional Diagnostic Methods

Table A2.1. (continued) Phenotypic test methods for the detection and identification of priority invasive fungal pathogens

Methodologies	Microorganisms	Specimen	Sensitivity	Specificity	Advantages	Disadvantages
Biochemical identification systems Commercial assays Manual: API® Test Strips (bioMérieux, France), RapID™ Yeast Plus System (Thermo Fisher, United States) Automated: VITEK® 2 YST ID card and VITEK® System 2 (bioMérieux, France) MicroScan Rapid Yeast Identification System (Beckman Coulter, United States) BD Phoenix™ automated microbiology system (Becton Dickinson, United States)	Yeasts	Culture	Commercial systems generally have high sensitivity (22)	Not found	<ul style="list-style-type: none"> • Relatively low cost • Can produce quantitative and qualitative results • Accurate identification of an unknown sample 	<ul style="list-style-type: none"> • Depends on culture • Only detect yeasts • Low sensitivity to identify and distinguish emergent fungal species, e.g. <i>Candida auris</i> (22)

Source: Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

Other culture-based Diagnostic Methods

Table A2.2. Other test methods used for the detection and identification of the most common invasive fungal infections

	Methodologies	Microorganisms	Specimen	Sensitivity	Specificity	Advantages	Disadvantages
Culture-based	MALDI-TOF MS Commercial assays: MBT Sepsityper® IVD kit and Bruker MALDI Biotyper® IVD System (Bruker Daltronics, Germany); VITEK MS® and VITEK MS® Prime (bioMérieux, France)	<i>Candida spp.</i> <i>Aspergillus spp.</i> <i>Cryptococcus</i> <i>Fusarium spp.</i> <i>Mucorales</i> <i>Histoplasma capsulatum</i> , <i>Blastomyces dermatitidis</i> , and <i>Coccidioides</i> ; can identify some uncommon fungi	Isolates cultured on solid media; subcultures generally required	92.7% in filamentous fungi (41) 96.3% in common strains of yeast (89)		<ul style="list-style-type: none"> • Rapid • Low reagent costs • Most sensitive on yeasts, but generally high sensitivity and specificity • Can be performed on early culture 	<ul style="list-style-type: none"> • Prior extraction required • Cannot quantitate • High instrument cost • Possible species limit in reference databases • Generally more difficult for moulds
	Hybridization						
	PNA-FISH Commercial assay: Accelerate Pheno™ System FISH (Accelerate Diagnostics, United States)	<i>Candida spp.</i> <i>Aspergillus fumigatus</i> (not commercialized)	Culture	96.4% (22,90)	95.8% (22,90)	<ul style="list-style-type: none"> • Accurate capture of <i>Candida spp.</i> • Relatively fast TAT (2 hrs) • High sensitivity and specificity 	<ul style="list-style-type: none"> • Requires culture
	ATR-FITR						
	Commercial assay: I-dOne (Alifax, Italy)	<i>Candida spp.</i>	Culture	N/A	N/A	<ul style="list-style-type: none"> • Very rapid • No sample preparation required • Relatively low instrument cost • Has the potential to identify a broad range of fungi 	<ul style="list-style-type: none"> • Requires culture • Currently only available for yeasts • Can be limited by available spectra library

Source: Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

Other non-culture-based Diagnostic Methods



Table A2.2. (continued) Other test methods used for the detection and identification of the most common invasive fungal infections

	Methodologies	Microorganisms	Specimen	Sensitivity	Specificity	Advantages	Disadvantages
Non-culture-based	Immunoassays – Laboratory-based assays						
	(1→3)-β-D-glucan (BDG) Commercial assays: Fungitell® Assay and Fungitell STAT™ (Associates of Cape Cod, United States); Wako β-Glucan Test (FUJIFILM, Japan); Dynamiker Fungus BDG (Dynamiker, Japan); Goldstream® Fungus (1-3)-β-D-Glucan Detection Kit (ERA Biology, China); BGSTAR β-Glucan Test (Maruha, Japan); and FungiXpert® Fungus (1-3)-beta-D-Glucan Detection Kit (ERA Biology, China).	<i>Candida spp.</i> <i>Aspergillus spp.</i> <i>Pneumocystis spp.</i> But, not, <i>Cryptococcus spp.</i> or Mucorales	Serum	Highly variable 27%–100% (53)	Highly variable 0%–100% (53)	<ul style="list-style-type: none"> • Non-invasive • Short TAT Useful for screening for multiple fungal infections (30)	<ul style="list-style-type: none"> • Not possible to estimate diagnostic accuracy (53)
	Galactomannan (GM) Commercial assays: Platelia™ <i>Aspergillus</i> Ag Assay (Bio-Rad, France); clarus <i>Aspergillus</i> GM-EIA (IMMY, United States); <i>Aspergillus</i> Antigen ELISA (EIA-GM-E) (EUROIMMUN, Germany); Dynamiker <i>Aspergillus</i> Galactomannan (Dynamiker, China); MycoMEIA® <i>Aspergillus</i> Assay (Pearl Diagnostics, United States); and clarus <i>Histoplasma</i> GM ENZYME IMMUNOASSAY (IMMY, United States).	<i>Aspergillus spp.</i> <i>Histoplasma capsulatum</i>	Serum, BAL fluid, or CSF Urine Urine	Serum: 56%–89% (91) Depends on Optical density index (ODI): At 0.5, sensitivity: 78% in all specimens (59)	Serum: 67%–99% (91) Depends on ODI: At 0.5, specificity: 85% in all specimens (59)	<ul style="list-style-type: none"> • Good biomarker for the detection of IA • Can be used to assess the response to antifungal therapy 	<ul style="list-style-type: none"> • Cross reactive with <i>Histoplasma spp.</i> and <i>Fusarium spp.</i> (22,91) • May not distinguish colonization (30)

Source: Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

Other non-culture-based Diagnostic Methods



Table A2.2. (continued) Other test methods used for the detection and identification of the most common invasive fungal infections

	Methodologies	Microorganisms	Specimen	Sensitivity	Specificity	Advantages	Disadvantages
Non-culture-based	Mannan antigen and anti-mannan antibody Commercial assays: Platelia™ <i>Candida</i> Ag/Ab Plus EIA (Bio-Rad, France; Serion ELISA Antigen <i>Candida</i> Assay (Serion, Germany); and <i>Candida</i> Mannan IgM and IgG assays (Dynamiker, China).	<i>Candida</i> spp.	Serum, plasma	Combined 83% (61)	Combined 86% (61)	<ul style="list-style-type: none"> Non invasive Short TAT 	<ul style="list-style-type: none"> Limited specificity due to colonization Best run together for improved sensitivity and specificity
	CAGTA Commercial assays: Invasive candidiasis CAGTA IFA IgG (Vircell Microbiologist S.I., Spain) and Invasive candidiasis (CAGTA). VirCLia® IgG Monotest (Vircell Microbiologist S.I., Spain).	<i>Candida albicans</i>	Serum	Pooled sensitivity 66% (63)	Pooled specificity 76% (63)	<ul style="list-style-type: none"> Rapid Easy to use 	<ul style="list-style-type: none"> Accuracy of the test is marginal and it should not be used alone (22,63)
	Lateral flow immunoassays (LFIAs)						
	Commercial assays: <i>Aspergillus</i> : IMMY soñá <i>Aspergillus</i> GM LFA (IMMY, United States); Galactomannan TECO® Fast <i>Aspergillus</i> Ag LFA (TECOmedical, Switzerland); QuickGM™ <i>Aspergillus</i> Galactomannan Ag LFA (Dynamiker, China)	<i>Aspergillus</i> spp.	Serum, BAL fluid			<ul style="list-style-type: none"> Rapid Easy-to-use CrAg® is gold standard and has good sensitivity and specificity (15, 31) 	<ul style="list-style-type: none"> Most commercially available assays are not sufficiently specific (22) Often cross-reactive with other fungal species (22)

Source: Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

Other non-culture-based Diagnostic Methods



Table A2.2. (continued) Other test methods used for the detection and identification of the most common invasive fungal infections

	Methodologies	Microorganisms	Specimen	Sensitivity	Specificity	Advantages	Disadvantages
Non-culture-based	<p><i>Cryptococcus</i>: CrAg® LFA (IMMY, United States); CryptoPS (Biosynex, France); Cryptococcal Antigen LFA (Dynamiker, Japan); and FungiXpert® Cryptococcal Capsular Polysaccharide Detection K-Set (Lateral Flow Assay) (Genobio Pharmaceutical, China).</p> <p><i>Histoplasma</i>: <i>Histoplasma</i> Urine Antigen LFA (MiraVista Diagnostics, United States).</p>	<p><i>Cryptococcus</i> spp.</p> <p><i>Histoplasma capsulatum</i></p>	<p>Serum, BAL fluid, WB</p> <p>Urine, serum</p>				
	Amplification						
	<p>NAAT-based methods</p> <p>Commercial assays: Numerous</p> <p>See Table A2.3.</p>	<p><i>Candida</i> spp.</p> <p><i>Aspergillus</i> spp.</p> <p><i>Pneumocystis jirovecii</i></p> <p>Mucorales</p> <p>Other</p>	<p>Samples directly from sterile sites (e.g. whole blood, CSF) or non-sterile sites (e.g. BAL fluid);</p> <p>Some platforms take culture isolates</p>	<p>Invasive candidiasis: 95% (65)</p> <p>Invasive aspergillosis: 76.8%–88.0% (67)</p>	<p>Invasive candidiasis: 92% (65)</p> <p>Invasive aspergillosis: 75.0% - 94.5% (67)</p>	<ul style="list-style-type: none"> • Relatively fast TAT • Can identify fungal infections at an early stage • Most do not require culture 	<ul style="list-style-type: none"> • Can be difficult to process samples (e.g. extraction) • May not allow for quantification (unless RT-PCR) • Non-closed systems prone to contamination • In absence of PCR assay that can detect and differentiate potential fungal pathogens, require a level of clinical suspicion that fungus targeted by the assay is likely causing disease in the patient

Source: Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

PCR based advanced technologies workflow

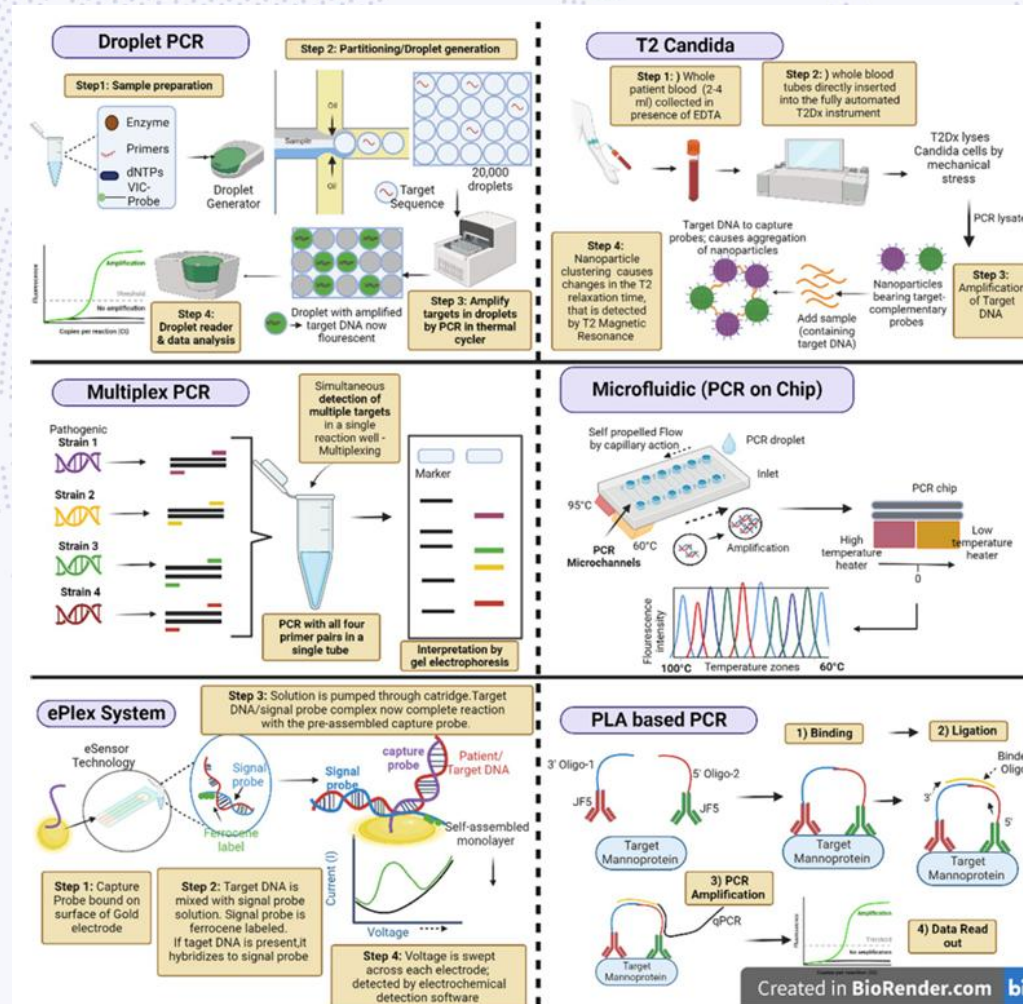


Fig. 2 Workflow of various PCR based advanced technologies that are of potential use in the field of diagnostics (PLA: Proximity ligation assay; VIC: fluorophore). [The figure has been created in Biorender.com]

Source: Fang et al., *J Biomed Sci* 30, 42 (2023).

Other non-culture-based Diagnostic Methods



Table A2.2. (continued) Other test methods used for the detection and identification of the most common invasive fungal infections

	Methodologies	Microorganisms	Specimen	Sensitivity	Specificity	Advantages	Disadvantages
Non-culture-based	Sequencing						
	NGS	Pan-fungal					
	Commercial assays for clinical use: DISQVER® (Noscendo GmbH, Germany); good number of platforms are for research use only.	<i>Pneumocystis jirovecii</i> <i>Talaromyces marneffeii</i>				<ul style="list-style-type: none"> • Breadth of pathogens that can be identified • Can ID fungal infections at an early stage • Do not require culture • No hypothesis required 	<ul style="list-style-type: none"> • prior target amplification • High cost of instrumentation • Need for specialized expertise • Lack of standardization • TAT
	mNGS						
	Karius® Inc. (United States); other platforms are for research use only.						

Source: Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

NGS workflow

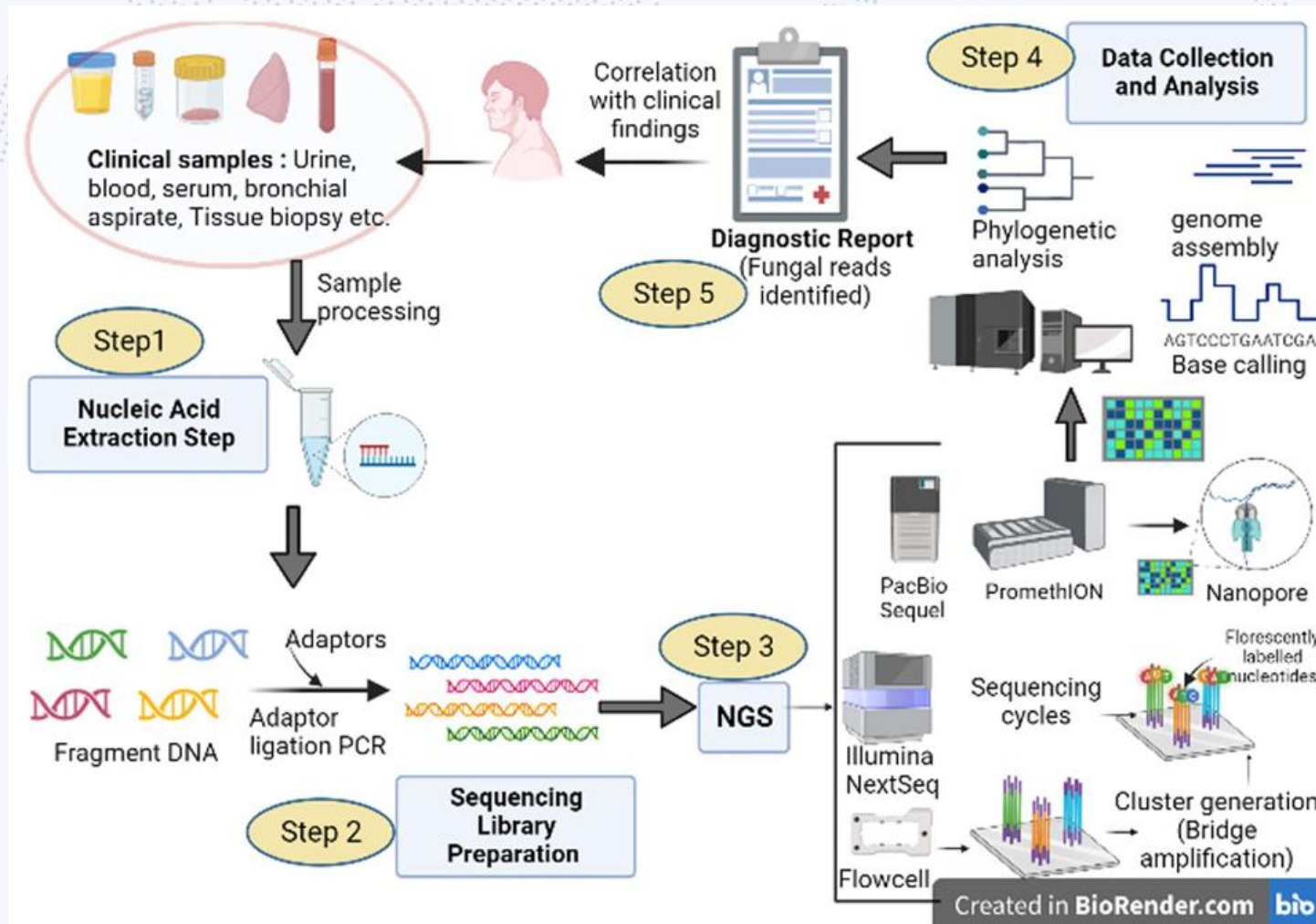


Fig. 3 Schematic diagram depicting workflow of NGS for application in fungal pathogen diagnosis. [The figure has been created in Biorender.com]

Source: Fang et al., *J Biomed Sci* **30**, 42 (2023).

Emerging technologies

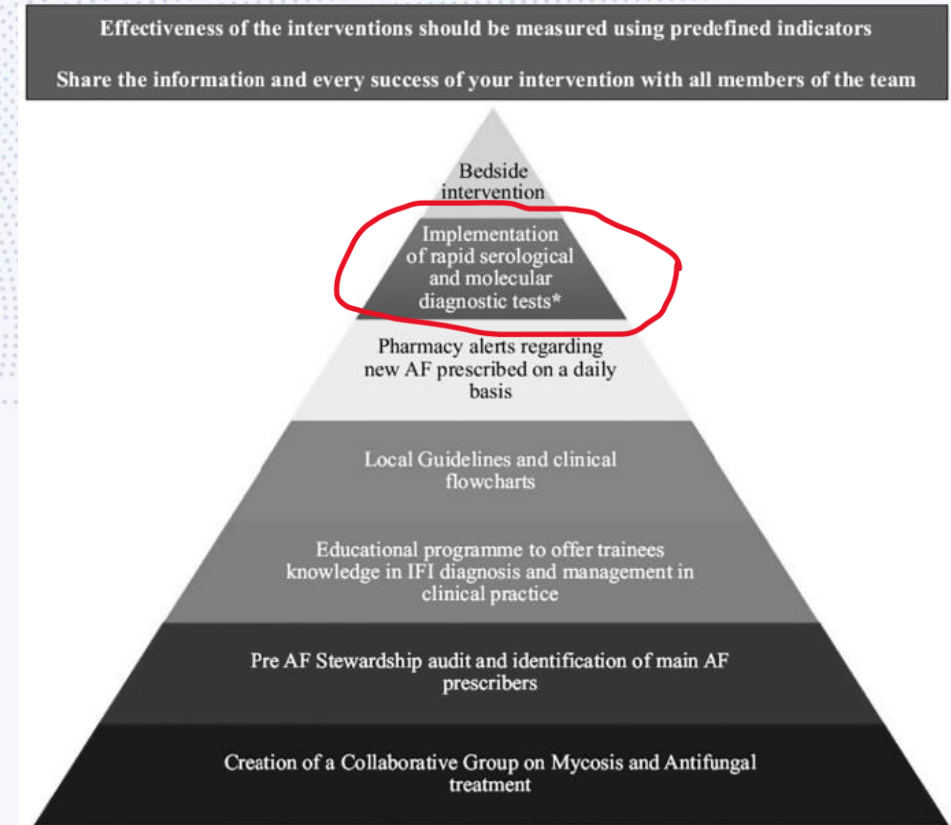
- Methods that combine existing technologies with entirely new technologies
- Examples,
 - i. PCR Multiplex testing strategy (*Candida*, *Aspergillus* and *Rhizopus*)
 - ii. Surface-enhanced Raman scattering (quant. & quali., determine severity)
 - iii. Bio-sensor based technologies
 - iv. Microfluidics, volatile organic compounds, AI/ML

Source: Landscape analysis of commercially available and pipeline in vitro diagnostics for fungal priority pathogens. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

Antifungal Stewardship

(Role of Rapid Diagnostics in Antimicrobial Stewardship)

- Antifungal stewardship refers to the optimization of antifungal therapy to ensure that patients receive the correct treatment for the shortest duration, while minimizing adverse effects and the development of resistance. Rapid diagnostics are critical in **guiding the appropriate use of antifungals**.
- Empirical treatment for invasive fungal infections often involves broad-spectrum antifungals. This can result in overuse of antifungals, which contributes to the development of resistance. Rapid diagnostics allow clinicians to **de-escalate therapy** when fungal infections are ruled out or when a more targeted antifungal can be used.



Procacci et al., (2024). Antifungal Stewardship in Invasive Fungal Infections, a Systematic Review. In: Advances in Experimental Medicine and Biology(). Springer, Cham.

Antifungal Stewardship

(Avoiding Overuse and Resistance)

- Broad-spectrum antifungals should not be used indefinitely due to the risk of resistance and toxicity. With rapid diagnostics, clinicians can **narrow their antifungal treatment spectrum**, preventing unnecessary exposure to drugs.
- A study by Lortholary et al. (2014) showed that the use of rapid fungal PCR testing resulted in a reduction of unnecessary antifungal therapy in patients with suspected fungal infections but negative results.
- A study from 2022 by Gonzalez et al. found that using PCR-based diagnostics to guide antifungal therapy reduced the duration of broad-spectrum antifungal treatment by 50% in critically ill patients.

Antifungal Stewardship

(Impact on Treatment Duration and Cost reduction)

- By reducing unnecessary antifungal use, rapid diagnostics not only improve patient outcomes but also **help reduce healthcare costs**. Shorter courses of antifungal therapy mean reduced drug costs, shorter ICU stays, and fewer adverse drug reactions.
- Example, in a study of ICU patients, the use of rapid diagnostics led to a 30% reduction in antifungal treatment costs, as unnecessary broad-spectrum treatments were avoided (Nimri et al., 2017)



Current Implementation and Limitations in Malaysia



- **Access to Technology:** Rapid diagnostic tests like PCR, MALDI-TOF, and antigen assays are still not universally accessible across all Malaysian hospitals
- **Cost and Infrastructure:** Some advanced tests (e.g., MALDI-TOF, NGS) are expensive and require well-equipped labs
- **Awareness and Training:** Need for continuous medical education and training to familiarize clinicians with the benefits and limitations of rapid diagnostics



Future Directions and Recommendations

Expanding Access to Rapid Diagnostics:

- Government and private sector collaboration to reduce costs and improve infrastructure for rapid diagnostics across Malaysia.
- Encourage research on affordable, rapid diagnostic tools suitable for resource-limited settings.

Integration with AI and Digital Health: AI-assisted diagnostic platforms to analyze test results quickly and accurately, facilitating decision-making in clinical settings

Increased Focus on Fungal AMR: Research into local patterns of resistance and the role of rapid diagnostics in combating antifungal resistance in Malaysian hospitals



Machine-learning paradigms

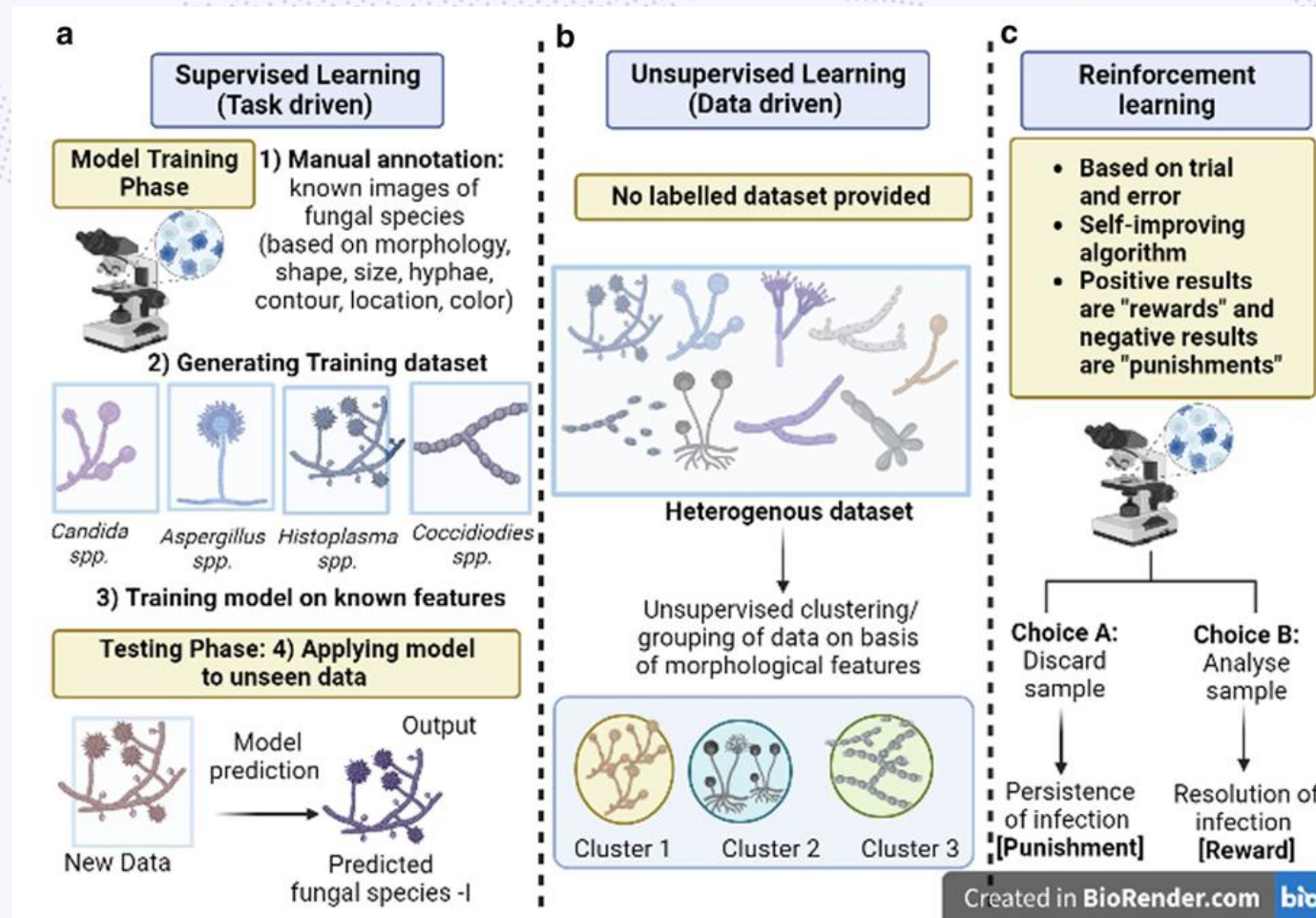


Fig. 4 Schematic diagram of the three basic machine-learning paradigms: **a** Supervised learning, **b** Unsupervised learning and **c** Reinforcement learning explained in terms of fungal species detection based on microscopic images as datasets [The figure has been created in Biorender.com]

Source: Fang et al., *J Biomed Sci* **30**, 42 (2023).

Summary

- Fungal infections are a significant health concern in Malaysia, especially for immunocompromised patients
- Rapid diagnostic techniques, can significantly reduce diagnostic time and improve patient outcomes, improve the speed and accuracy of fungal infection diagnosis, leading to quicker and more targeted antifungal treatment.
- Antifungal stewardship is enhanced by rapid diagnostics, ensuring the appropriate use of antifungals and reducing the risk of resistance.
- While some advanced techniques are emerging, challenges remain in terms of cost, accessibility, and infrastructure



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“It always seems impossible until it is done.”

- Nelson Mandela



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