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Narrative review

How to use direct microscopy for diagnosing fungal infections

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A B S T R A C T

Background: Invasive fungal infections are an important cause of morbidity and mortality in a broad range of patients. Adequate and early diagnosis is a challenge and of importance for improved survival. New molecular-based diagnostic methods are trendsetting, yet with the drawback that conventional tests receive less attention, in the laboratory as well as in the clinical setting.
Objectives: We aimed to provide a useful recommendation for direct microscopy for effectively managing numerous specimens related to fungal infections, mainly covering opportunistic pathogens.
Sources: A PubMed literature search covering direct fungal microscopy was performed with no restrictions on publication dates.
Content: Best practise recommendations targeting the role of direct microscopy in diagnosing fungal infections are given. This review highlights when to perform direct microscopy, displays the main fungal morphologies, discusses the pitfalls related to microscopy, and recommends how to best report the results to clinicians.
Implication: In many samples, the performance of direct microscopy provides an important diagnostic benefit that is greater than culture alone. Fluorescent dyes improve sensitivity and allow a fast and rapid read. Reporting includes the presence or absence of yeast forms, septate or non-septate hyphae, pigmentation, cellular location, or any other specific structures being present. The visualization of fungal elements from a sterile body site is proof of an infection, independent of other test reports. **Miriam Alisa Knoll, Clin Microbiol Infect 2023;29:1031**
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Background

The fast and proper diagnosis of fungal infections is a challenge, particularly in the immunocompromised host [1]. Clinical signs and symptoms are non-specific, and fungal colonization is difficult to distinguish from fungal diseases, specifically, when dealing with non-sterile body specimens, such as sputum [2]. Blood cultures are commonly negative (moulds), and overall, most patients are unable to undergo invasive diagnostic procedures [3]. The question of “what is the best specimen to diagnose fungal infections?” is of major clinical importance and depends mainly on the clinical situation, tests applied, and laboratory expertise provided [1]. Human samples are diverse and not equal in terms of diagnostic significance [4]. To answer this question, it is important to know which fungi may cause which infections, when, and where. For example, in the diagnosis of pulmonary fungal infections, the microscopic examination of bronchoalveolar lavage fluids (BALs) is mandatory as an addition to culture, together with blood samples, in the search for biomarkers and/or fungal DNA [1,2]. Here, we will focus on the role of direct microscopy in the medical microbiology laboratory in diagnosing mainly opportunistic infections; direct smears of patient specimens may permit the detection of fungal infections without having any culture results at hand. Light microscopy and fluorescent microscopy are the most frequently used techniques [4]. The recommendations given here do not cover histopathologic diagnosis or non-European mycoses.

Why should we go for direct microscopy?

Culture and microscopic examination remain ‘the reference standard’, but may be insensitive and depend on the patient population and specimen investigated [1]. As a consequence, there is an increased use of molecular and antigen-based methods in diagnosing fungal infections [5]. In the age of molecular diagnostics,

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culture and, to a greater extent, microscopy are no longer used when obtaining a fungal diagnosis. Without any doubt, modern tools are of utmost importance, but they also vary in sensitivity and specificity [1,6]. Caution is required in the interpretation of antigen tests, PCR, antibodies, and fungal metabolites [6–8]. This is nicely shown by means of COVID–19-associated pulmonary aspergillosis. COVID–19-associated pulmonary aspergillosis cases varied widely between hospitals and countries, and displayed an incidence ranging from 3% to 33%, respectively [9]. These differences were explained by difficulties in obtaining a reliable diagnosis; currently, positive results from culture, microscopy, and biomarkers are equated with probable definitions of invasive mould infections [10,11]. This uniform assessment of positivity and lack of further differentiation could lead to some inaccuracies in the diagnosis of fungal infections. For example, *Aspergillus* naturally exists as airborne conidia and vegetative hyphae; hyphae may present colonization or infection depending on the sample analysed [2], and the presence of conidia may cause positive cultures but is not seen on microscopy. This implies that a positive culture should not always be seen and automatically judged as an invasive infection. Fungal microscopy is mandatory for an evidence-based diagnosis and, furthermore, for the judgement of the significance of a positive culture [2,4,11]. In the case of positive microscopy (visualization of hyphae or other fungal structures), a tentative diagnosis is given, and when detected from sterile body sites, it confirms the proof of an infection, independent of culture and other molecular-based diagnostic results obtained.

What are the best methods for direct microscopy?

Several different stains and techniques are used to detect fungi in clinical specimens [12]. Clinical microbiology laboratories routinely use aqueous potassium hydroxide (KOH), India ink, fluorescent dyes, including Calcofluor white™, Uvitex 2B, or Blancophor™, and Gramme stain for direct examination [4,13]. Each method has its own advantages and disadvantages, listed in Table 1 [4,14–18]. The specimens should first be examined for necrotic, purulent, bloody, or caseous areas; these are most likely to yield fungal elements. Sample concentration of fluids using centrifugation is recommended, and the pellet should be analysed microscopically [4]. The Gramme stain is not recommended as a specific fungal staining method [14,15], but could give the first hint of an infection as fungi typically present as Gramme positive. Hence, the detection of yeasts, such as *Candida* species or hyphal elements such as *Aspergillus* and *Fusarium* is not uncommon. Gramme staining of positive blood cultures is suitable for the detection of the most prevalent yeasts. The use of fluorescent stains is broadly recommended for the microscopic examination of many samples, such as fluids and biopsies, simply because of their rapid availability, fast readability, and improved sensitivity.

Direct examination: what is the least common denominator?

As a group, fungi exhibit great diversity and polymorphism, as exemplified by the various morphologies and structures seen in pathologic tissues. Hence, the detection of fungal elements, for instance, in a lung biopsy confirms the proof of an infection, but does not allow genus or species identification [2,4]. Recognition of specific structures may only, under certain circumstances, provide a possible fungal pathogen. Typically, the morphological characteristics seen in microscopy are diverse and depend on the pathogens involved; see Table 2 [3,4,13,19–24]. In the ideal case, the examiner is able to differentiate between yeast cells, budding cells, hyphae (septate, rarely septate, and non-septate), and pseudohyphae. Typical for *Aspergillus* species are regular septate hyphae with dichotomous branching, whereas Mucorales present with broad, sparse, or non-septate hyphae with irregular branching [3]. In either case, genus- or species-based identification needs to be performed via further culture or molecular-based tools [6,19]. Anyhow, this basic information is path-breaking as it allows individual adjustment of antifungal treatment. In the case of *Aspergillus*-like or Mucorales-like hyphae being present, treatment with voriconazole or lipid amphotericin B is recommended, respectively [1]. Although the direct examination is extremely helpful, one must keep in mind that false negative results may occur. Direct examination is less sensitive than culture, and a negative result does not rule out fungal infections [4]. But the real added value lies in a positive result, as the presence of any “fungal element”

Table 1
Most important methods available for direct microscopic detection of fungal elements in the various clinical specimens

Methods	Use	Time required	Advantages	Disadvantages	References
2021	Detection of bacteria and yeasts (e.g. vaginal smear)	3 min	Commonly performed in most clinical specimens, some fungi stain well	*Cryptococcus* may stain weakly; common artefacts appear as yeast cells (human tissue debris)	[4,15]
Calcofluor white	Detection of fungi, including *Pneumocystis*	5–15 min	Can be mixed with potassium hydroxide (KOH); detects fungi rapidly owing to bright fluorescence	Requires a fluorescence microscope; background fluorescence and vaginal secretions are not suitable	[4,16]
India ink	Detection of *Cryptococcus* in cerebrospinal fluid; demonstration of the capsule (negative stain)	3 min	When positive, this is diagnostic of meningitis	Negative in many cases of meningitis; not reliable; leucocytes and fat droplets may resemble *C. neoformans*; the capsules can be small, making recognition difficult; adding a drop of 10% KOH may dissolve leucocytes and tissue cells	[17,18]
Potassium hydroxide (KOH) wet mount	Skin scales; clearing of specimens to enhance fungal visibility	5–10 min	Rapid detection of fungal elements	Experience is required because background artefacts are often confusing	[14]
Methylene blue	Detection of fungi in skin scrapings	2 min	Provides contrast for detection of fungal elements	Background staining of cells makes reading difficult	[4]
Toluidine blue	Examination of induced sputum and bronchial specimens for *Pneumocystis jirovecii*	25 min	Reveals cysts walls of *P. jirovecii* with purple colour	Background stains; stains other fungi	[4]

Table 2
Typical characteristic elements of selected fungal pathogens are given in direct microscopy; non-European pathogens are excluded. Adapted from [3,4,13,19–24]

	Characteristic fungal elements	Sizes and diameter (μm)	Specific features	Potential pathogens
	Hyaline septate hyphae	3–12	Septate hyphae, dichotomous 45°C angel branching, hyphae disturbed, may resemble those of Mucorales (even when treated)	Species of *Aspergillus*, *Scedosporium*, *Fusarium*, or others are impossible to distinguish among each other
	Dematiceous septate hyphae	2–6	Dematiceous polymorphous hyphae and budding yeasts with single septa and chains of rounded cells	Species of *Curvularia*, *Bipolaris*, or others
	Yeast forms and pseudohyphae; true hyphae	3–4 and 5–10	Usually single-budding pseudohyphae are constricted at the ends and remain attached like links of sausage; true septate hyphae have parallel walls	Various *Candida* species; C. *glabrata* does not form hyphae
	Yeast forms	2–15	Yeast cells vary in size; they are usually spherical, buds are single, capsules may or may not be present, and rare pseudohyphae	*Cryptococcus* species
	Wide non-septate hyphae	10–30	Large hyphae, ribbon-like, often fractured or twisted; occasional septates are present; branching usually at the right angles; smaller hyphae overlap those of *Aspergillus*	Representatives of the genus Mucorales
	Hyaline septate hyphae (hair and skin specimens)	3–15	Long hyaline septate filaments, rectangular chains of arthroconidia	Dermatophytes
	Cysts and trophic forms	5–12 and 2–4	Cysts are round to cup-shaped, possible with up to 8 pleomorphic intracystic bodies; no budding, extracystic trophozoites; with collapsed forms	*Pneumocystis jirovecii*
	Round and non-budding forms	3–30	With or without morula-like forms (with endospores arranged symmetrically)	Species of *Prothotheca*

supports—in primarily sterile bodily regions or infection sites—the proof of an infection. For non-sterile sites, such as skin or sputum, identified fungi may be disease-causing (pathogenic) or may be part of the normal skin flora [5]. The presence of fungal elements in correctly sampled skin scrapings points to fungal infection instead of colonization. According to best practises for the detection of dermatophytes, the skin should be decontaminated before sampling. Moreover, it is important to take note of the fact that fungi, once exposed to antifungals (treatment), may lose their typical morphology.

Which specimens should be used for direct examination?

Immediate direct examination methods depend on the nature of the specimen, which reveals gross pathology and may identify fungi. The selection of appropriate specimens for microscopy is based on clinical examination and consideration of the most likely fungal pathogen that may cause such an infection [1,4,13,19]. Here, the most important step is to take specimens whenever possible from infection sites; besides, all primarily sterile specimens obtained from patients with suspected invasive fungal infections need to undergo microscopic examination. Table 3 [4,13,19,21–25] gives an overview of which fungi are of utmost importance and their recovery in clinical specimens. BAL fluids and lung biopsy samples are the specimens of choice to diagnose invasive pulmonary infections; less representative are tracheal secretions or sputa [1]. By contrast, sputum examination is helpful in allergic bronchopulmonary aspergillosis, as hyphae can often be seen with eosinophils and Charcot-Leyden crystals [6].

What are the pitfalls? From false positive results to operator errors

The successful detection of fungal elements in any specimen depends largely on the expertise of the examiner, the anatomical site, the amount of specimen, the site of pathology, and finally, the careful preparation of the sample. Cerebrospinal fluid (CSF), for instance, needs centrifugation for fungal concentration [4]. Next, fungi are larger than bacteria but usually exist in smaller numbers and appear in clusters [4], hence the need to examine several parts of the preparation. In addition, it is impossible to differentiate between viable and non-viable fungi. One disadvantage of using KOH comes from its reaction with pus, skin, or sputum. Artefacts may occur that superficially resemble hyphae or budding yeasts; see Fig. 1. Several artefacts increase with time; passing the slide through a flame removes air bubbles but facilitates crystal formations. Common artefacts, such as mosaic fungus (cholesterol forming polygonal depositis around cells), air bubbles, lipid vesicles, textile fibres, and crystals may resemble fungal elements. Lymphocytes in an India ink preparation of CSF may be mistaken for *Cryptococcus neoformans* and collagen fibres for Mucorales. Equivocal findings should be reviewed by another experienced examiner to confirm or rebut the results obtained.

Best practise recommendations for reporting fungal elements

Depending on the specimen and type of infection, a variety of microscopic morphologies may be given, which have to be interpreted carefully. The first step is to ensure the adequacy and quality of the sample material and to select the correct method for the

Table 3
Fungi and their importance and recovery in clinical specimens. Adapted from [4,13,19,21–24]

Potential fungal pathogens	Clinical specimens								
	Blood	Bone marrow	Cerebrospinal fluid	Synovial fluid	Eye	Urine	Respiratory tract	Skin and mucous	Systemic sites
Yeasts	++++	+	+(+)	+	+	+++	+	+++	+++
Candida spp.	+++	+	++++	-(+)	+	++	+++	+	++
Cryptococcus neoformans	++++	+	++++	—	+	++	+++	++	+++
Trichosporon sp.	++++	—	—	—	—	—	+	+++	+
Malassezia sp.									
Hyaline moulds	+[b]	—	+(+)	—	+	+	+++++	++	+++
Aspergillus sp.	—	—	(+)	—	+	—	++++	++	+++
Mucorales	+++	—	—	+	+(+)	—	++	+++(+)	++(+)
Fusarium sp.									
Dematiaceous moulds	+[c/d]	—	+++	+[d]	+	—	+++	++	++
Dimorphic[a]	+++	++	+	+	+	+	+++(+)	++	++
Histoplasma capsulatum	—	—	+	+	—	+(+)	+++(+)	+++(+)	++
Blastomyces dermatitidis	++	+	+(+)	+	+	+	++++	+++	+++
Coccidioides immitis complex	—	+	+	—	—	—	+++	++++	++
Paracoccidioides brasiliensis	+++	++	+	++	—	—	++++	++	+++
Talaromyces marneffei	+	—	—	+	—	—	++	++++	+
Sporothrix schenckii									
Other	—	+	—	—	—	—	++++	—	+
Pneumocystis jiroveci									

The presence of fungi is classified from - (usually not present) to + (low frequency or less important) and +++++ (high frequency or high importance).
[a] In order to complete this overview, non-EU mycoses are listed here. To be aware that the fungal structures given in the various specimens may also be caused by these pathogens.
[b] Aspergillus terreus only.
[c] Scedosporium apiospermum.
[d] Lomentospora prolificans.

detection of the suspected fungal pathogen (see Tables 3 and 1 for the selection of clinical specimens and the respective staining methods). Reporting results should include the following fungal morphologies: whether yeasts are small or large; whether septa, buddings, or pseudohyphae are present; whether hyphae are septate or un-septate or display bizarre shapes or structures; finally, whether pigmentation is given; and whether small intracellular yeasts are present; see Table 2. Compared with yeast cells, hyphae-forming fungi are more prone to alterations due to the infected organ, the age of the hyphae, and the inflammatory response of the host. Fig. 2 gives a very simple step-by-step flow chart for the interpretation of fungal elements when using calcofluor white or native staining. We

Fig. 1. Overview of direct microscopy of various human specimens, dyes, and characteristic features of fungal pathogens *in vivo*, as well as potential pitfalls.

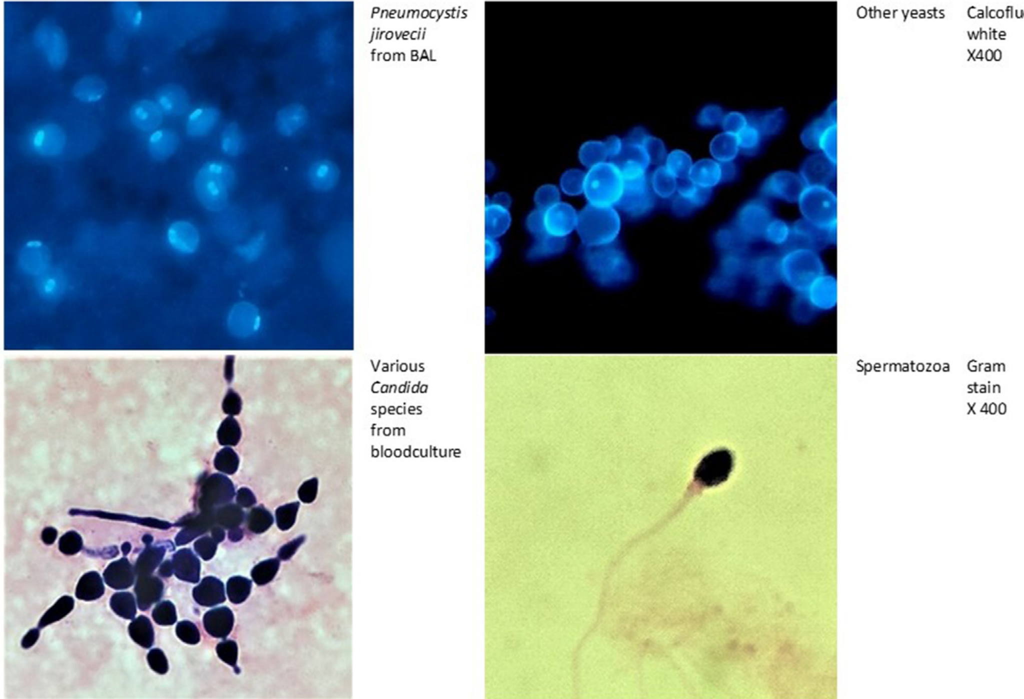


Fig. 1. (*continued*).

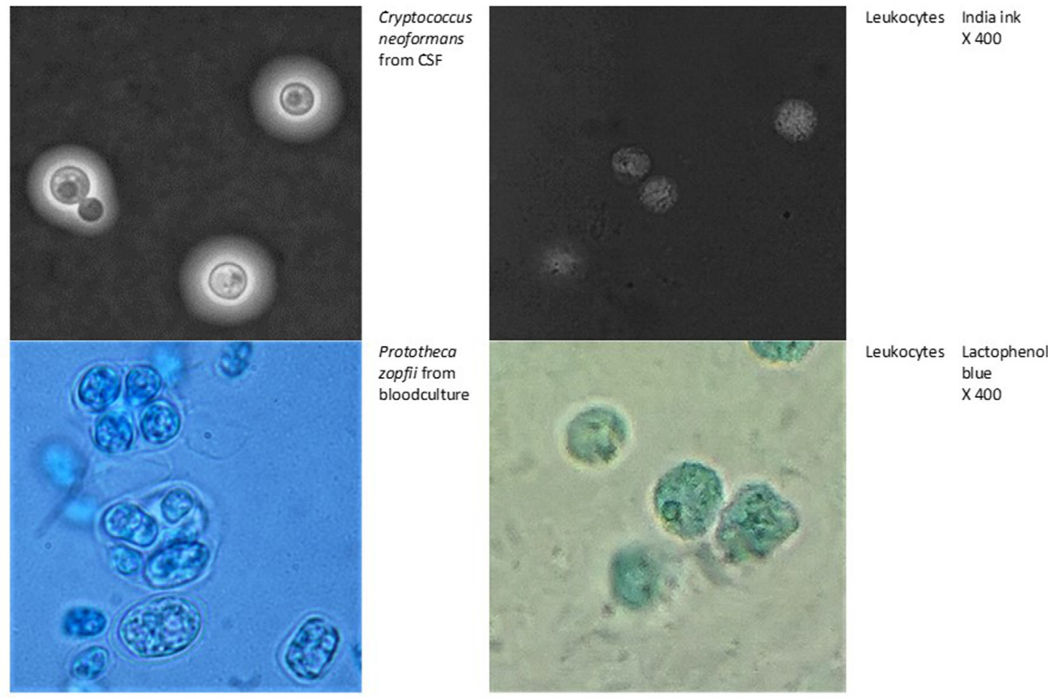
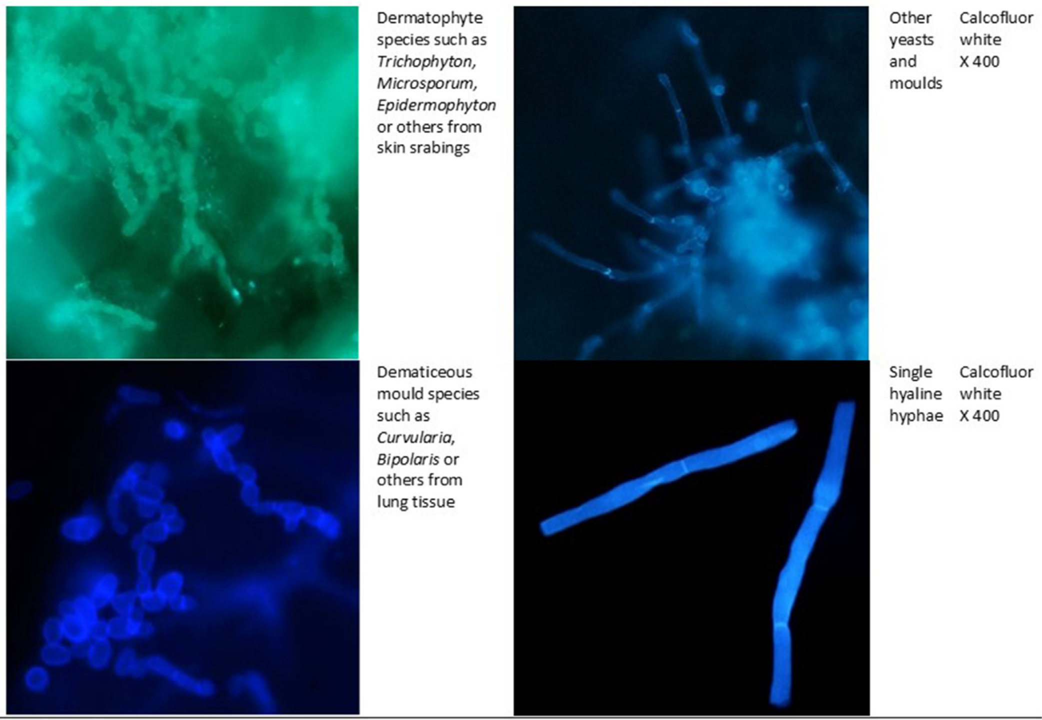


Fig. 1. (*continued*).

encourage to use this information given here for the laboratory report of specimens containing any fungal elements. The most common fungi in respiratory specimens include *Aspergillus* sp., *Candida* sp., or *Pneumocystis jirovecii* [1,2,4]. Although *Candida* sp. can also be detected by Gramme stain, the other two pathogens are usually detected by fluorescent dyes. Septate hyphae in BALs are strongly indicative of fungal infection [11] and should be immediately reported to the clinician to ensure timely antifungal treatment. Because direct microscopy cannot distinguish between moulds, such as *Aspergillus*, *Scedosporium,* and *Fusarium* species, these findings should be reported as "*Aspergillus*-like" or "septate hyphae", see Fig. 1. Molecular methods can ensure fast species identification to guide antifungal treatment based on intrinsic resistance patterns being present [5]. Microscopic findings



BAL, bronchoalveolar lavage fluid,

Fig. 1. (*continued*).

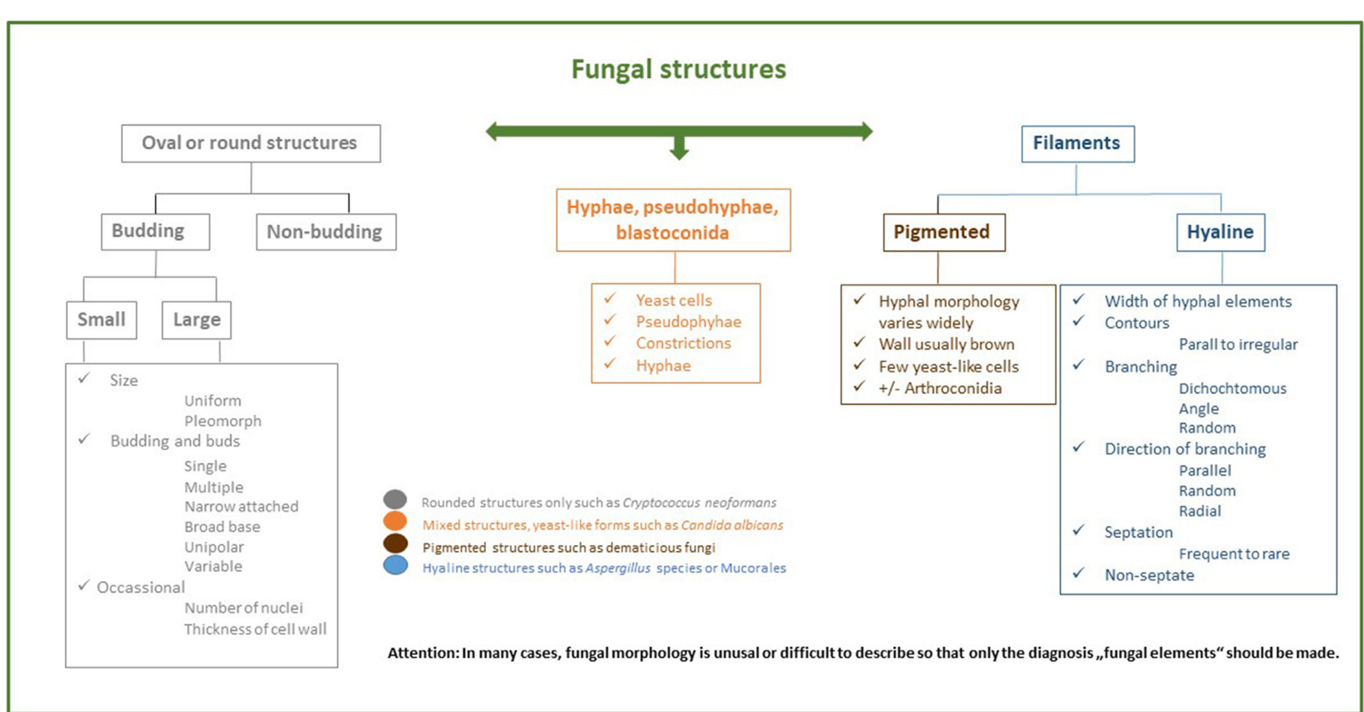


Fig. 2. Flow chart for interpretation of fungal elements seen in direct microscopy when using, for instance, Calcofluor white™ staining or native microscopy (especially for judgement of pigmentation).

suggestive of *P. jirovecii* should also be promptly reported to the clinician, and if applicable, molecular methods can additionally aid diagnosis [15]. As an exception, the presence of hyphae, pseudohyphae, or yeast cells comparable with *Candida* sp. does not represent a reason for extended diagnostic steps or immediate reporting to the clinician because *Candida* sp. are a common part of the mucosal resident microbiome and do not usually cause invasive pulmonary infections [12]. Many kinds of moulds, yeasts, and non-European pathogens can be involved in eye and sinunasal infections, and any microscopic findings suggestive of fungi should be promptly

reported to the clinician [26]. *Candida* sp. generally forms yeasts and hyphae in deep tissue, with the exception of *Candida glabrata*, for which only medium-sized yeasts are visible [4]. Microscopic identification of wide non-septate hyphae is of particular importance because infections with the genus Mucorales represent a medical emergency due to the rapid progression of the disease [27]. India ink staining of centrifuged CSF samples from immunocompromised patients is recommended in addition to *Cryptococcus* antigen testing [28]. Persistent positive CSF findings in patients undergoing treatment should be considered as evidence for failure or relapse only when they are confirmed by a deterioration in the patient's clinical condition or by positive cultures [28]. Also, direct microscopy of positive blood cultures can speed up the diagnostic workflow and treatment upon identification of fungal structures [12]. Yeasts of the *Candida* genus are most commonly found and can be detected in vaginal and other mucosal samples via Gramme stain, presenting as hyphae, pseudohyphae, or yeasts. The occurrence in samples from usually sterile regions necessitates immediate information to adapt antimicrobial therapy [20]. When the examiner is unable to identify any characteristic morphology, it is important to report at least that “fungal elements” are present. This ensures that the treating physician can make an individual decision on a case-by-case basis. A 4-eyes principle may be useful in some settings, as the quality of the microscopic reporting highly depends on experienced staff's ability to detect and distinguish different fungal elements as well as evaluate clinical situations with or without the need for immediate action.

Experienced readers may be able to describe the presence of small intracellular yeasts, typically diagnostic of either *Talaromyces* marneffei, a fission yeast with the appearance of one septum separating two halves of the yeast, and Histoplasma capsulatum, a budding yeast [4]. Another example is the differentiation between *C. neoformans* and *Blastomyces dermatitidis*, both displaying large yeast cells; *C. neoformans* presents with narrow junctions between cells, *B. dermatitidis* with broad based buds [4]. The latter section is listed only as an example, as non-European mycoses are not part of this review.

Conclusion

Microscopy is an important examination, and specimens from patients suspected of fungal infections should be treated with considerable caution. The main cornerstones should be taken into account.

1. Whenever possible, specimens should be taken from infected body sites.
2. Specimens taken from infected or primarily sterile body sites should undergo fungal microscopy (along with culture- and non–culture-based methods).
3. Any positive fungal elements should be described based on fungal morphology. As a minimum requirement, reports should include the presence of yeasts, yeast-like cells, or hyphae (with or without septate).
4. The presence of fungi in specimens obtained from infected or primarily sterile body sites is proof of an infection independent of any culture results.
5. Relevant positive fungal findings need immediate reporting to the clinicians.

Author contributions

All authors contributed equally to the concept and execution of the review.

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References

[1] Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect 2018;24:e1–38. https://doi.org/10.1016/j.cmi.2018.01.002.
[2] Lass-Flörl C. How to make a fast diagnosis in invasive aspergillosis. Med Mycol 2019;57:S155–60. https://doi.org/10.1093/mmy/myy103.
[3] Lass-Flörl C, Aigner M, Nachbaur D, Eschertzhuber S, Bucher B, Geltner C, et al. Diagnosing filamentous fungal infections in immunocompromised patients applying computed tomography-guided percutaneous lung biopsies: a 12-year experience. Infection 2017;45:867–75. https://doi.org/10.1007/s15010-017-1072-6.
[4] Schelenz S, Barnes RA, Barton RC, Cleverley JR, Lucas SB, Kibbler CC, et al. British Society for Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases. Lancet Infect Dis 2015;15:461–74. https://doi.org/10.1016/S1473-3099(15)70006-X.
[5] Dobiáš R, Stevens DA, Havlíček V. Current and future pathways in Aspergillus diagnosis. Antibiotics (Basel) 2023;12:385. https://doi.org/10.3390/antibiotics12020385.
[6] Lass-Flörl C, Samardzic E, Knoll M. Serology anno 2021-fungal infections: from invasive to chronic. Clin Microbiol Infect 2021;27:1230–41. https://doi.org/10.1016/j.cmi.2021.02.005.
[7] Lass-Flörl C, Knoll M, Posch W, Joannidis M, Mayerhöfer T, Breitkopf R, et al. A laboratory-based study on multiple biomarker testing in the diagnosis of COVID-19-associated pulmonary aspergillosis (CAPA): real-life data. Diagnostics (Basel) 2023;13:114. https://doi.org/10.3390/diagnostics13010114.
[8] Lackner N, Thomé C, Öfner D, Joannidis M, Mayerhöfer T, Arora R, et al. COVID-19 associated pulmonary aspergillosis: diagnostic performance, fungal epidemiology and antifungal susceptibility. J Fungi (Basel) 2022;8:93. https://doi.org/10.3390/jof8020093.
[9] Prattes J, Wauters J, Giacobbe DR, Salmanton- García J, Maertens J, Bourgeois M, et al. Risk factors and outcome of pulmonary aspergillosis in critically ill coronavirus disease 2019 patients-a multinational observational study by the European Confederation of Medical Mycology. Clin Microbiol Infect 2022;28:580–7. https://doi.org/10.1016/j.cmi.2021.08.014.
[10] Koehler P, Bassetti M, Chakrabarti A, Chen SCA, Colombo AL, Hoenigl M, et al. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. Lancet Infect Dis 2021;21:e149–62. https://doi.org/10.1016/S1473-3099(20)30847-1.
[11] Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and update of the consensus definitions of invasive fungal disease from the European organization for research and treatment of cancer and the mycoses study group education and research consortium. Clin Infect Dis 2020;71:1367–76. https://doi.org/10.1093/cid/ciz1008.
[12] Cuenca-Estrella M, Bassetti M, Lass-Flörl C, Rácil Z, Richardson M, Rogers TR. Detection and investigation of invasive mould disease. J Antimicrob Chemother 2011;66:i15–24. https://doi.org/10.1093/jac/dkq438.
[13] Borman AM, Mohammed S, Palmer MD, Childs N, Johnson EM. The importance of appropriate processing and direct microscopic examination for the timely diagnosis and management of invasive infections caused by filamentous fungi. Med Mycol 2022;60:myac081. https://doi.org/10.1093/mmy/myac081.
[14] Leung AK, Lam JM, Leong KF, Hon KL. Tinea corporis: an updated review. Drugs Context 2020;9:1–12. https://doi.org/10.7573/dic.2020-5-6.
[15] Mohan SK. Beyond bacteria: interpreting fungal elements in the Gram stain. Clin Microbiol Newsl 2004;26:108–12. https://doi.org/10.1016/S0196-4399(04)90208-5.
[16] Punjabi V, Patel S, Pathak J, Swain N. Comparative evaluation of staining efficacy of calcofluor white and acridine orange for detection of *Candida* species using fluorescence microscopy - a prospective microbiological study. J Oral Maxillofac Pathol 2020;24:81–6. https://doi.org/10.4103/jomfp.JOMFP_315_18.
[17] Guess T, Lai HY, Smith SE, Sircy L, Cunningham K, Nelson DE, et al. Size matters: measurement of capsule diameter in *Cryptococcus neoformans*. J Vis Exp 2018;132:57171. https://doi.org/10.3791/57171.
[18] Kwizera R, Akampurira A, Williams D, Boulware DR, Meya DB, ASTRO-CM Study Team. Acridine orange fluorescent microscopy is more sensitive than India ink light microscopy in the rapid detection of cryptococcosis among

CrAg positive HIV patients. PLOS ONE 2017;12:e0182108. https://doi.org/10.1371/journal.pone.0182108.
[19] Hoenigl M, Salmanton-Garcia J, Walsh TJ, Nucci M, Neoh CF, Jenks JD, et al. Global guideline for the diagnosis and management of rare mould infections: an initiative of the European confederation of medical mycology in cooperation with the international society for human and animal mycology and the American society for microbiology. Lancet Infect Dis 2021;21:e246–57. https://doi.org/10.1016/S1473-3099(20)30784-2.
[20] Jensen HE. Histopathology in the diagnosis of invasive fungal diseases. Curr Fungal Infect Rep 2021;15:23–31. https://doi.org/10.1007/s12281-021-00412-y.
[21] Konsoula A, Tsioutis C, Markaki I, Papadakis M, Agouridis AP, Spernovasilis N. *Lomentospora prolificans*: an emerging opportunistic fungal pathogen. Microorganisms 2022;10:1317. https://doi.org/10.3390/microorganisms10071317.
[22] Lackner N, Posch W, Lass-Flörl C. Microbiological and molecular diagnosis of mucormycosis: from old to new. Microorganisms 2021;9:1518. https://doi.org/10.3390/microorganisms9071518.
[23] Chen SC, Perfect J, Colombo AL, Cornely OA, Groll AH, Seidel D, et al. Global guideline for the diagnosis and management of rare yeast infections: an initiative of the ECMM in cooperation with ISHAM and ASM. Lancet Infect Dis 2021;21:e375–86. https://doi.org/10.1016/S1473-3099(21)00203-6.
[24] Tortorano AM, Richardson M, Roilides E, van Diepeningen A, Caira M, Munoz P, et al. ESCMID and ECMM joint guidelines on diagnosis and management of hyalohyphomycosis: *Fusarium* spp., *Scedosporium* spp. and others. Clin Microbiol Infect 2014;20:27–46. https://doi.org/10.1111/1469-0691.12465.
[25] Maschmeyer G, Helweg-Larsen J, Pagano L, Robin C, Cordonnier C, Schellongowski P, et al. ECIL guidelines for treatment of *Pneumocystis jirovecii* pneumonia in non-HIV-infected haematology patients. J Antimicrob Chemother 2016;71:2405–13. https://doi.org/10.1093/jac/dkw158.
[26] Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SCA, Dannaoui E, Hochhegger B, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European confederation of medical mycology in cooperation with the mycoses study group education and research consortium. Lancet Infect Dis 2019;19:e405–21. https://doi.org/10.1016/S1473-3099(19)30312-3.
[27] Rudramurthy SM, Hoenigl M, Meis JF, Cornely OA, Muthu V, Gangneux JP, et al. ECMM/ISHAM recommendations for clinical management of COVID-19 associated mucormycosis in low- and middle-income countries. Mycoses 2021;64:1028–37. https://doi.org/10.1111/myc.13335.
[28] Baddley JW, Forrest GN, AST Infectious Diseases Community of Practice. Cryptococcosis in solid organ transplantation-guidelines from the American society of transplantation infectious diseases community of practice. Clin Transplant 2019;33:e13543. https://doi.org/10.1111/ctr.13543.