Detection of the 'Big Five' Mould Killers of Humans: *Aspergillus*, *Fusarium*, *Lomentospora*, *Scedosporium* and Mucormycetes

Christopher R. Thornton

Biosciences, Hatherly Building, University of Exeter, Prince of Wales Road, Exeter, EX4 4PS.

Abstract

Fungi are an important but frequently overlooked cause of morbidity and mortality in humans. Life-threatening fungal infections mainly occur in immunocompromised patients, and are typically caused by environmental opportunists that take advantage of a weakened immune system. The filamentous fungus *Aspergillus fumigatus* is the most important and well-documented mould pathogen of humans, causing a number of complex respiratory diseases, including invasive pulmonary aspergillosis, an often fatal disease in patients with acute leukemia or in immunosuppressed bone marrow or solid organ transplant recipients. However, non-*Aspergillus* moulds are increasingly reported as agents of disseminated diseases, with *Fusarium*, *Scedosporium*, *Lomentospora* and mucormycete species now firmly established as pathogens of immunosuppressed and immunocompetent individuals. Despite well-documented risk factors for invasive fungal diseases, and increased awareness of the risk factors for life-threatening infections, the number of deaths attributable to moulds is likely to be severely underestimated driven, to a large extent, by the lack of readily accessible, cheap, and accurate tests that allow detection and differentiation of infecting species. Early diagnosis is critical to patient survival but, unlike *Aspergillus* diseases, where a number of CE-marked or FDA-approved biomarker tests are now available for clinical diagnosis, similar tests for fusariosis, scedosporiosis and mucormycosis remain experimental, with detection reliant on insensitive and slow culture of pathogens from invasive bronchoalveolar lavage fluid, tissue biopsy, or from blood. This review examines the ecology, epidemiology, and contemporary methods of detection of these mould pathogens, and the obstacles to diagnostic test development and translation of novel biomarkers to the clinical setting.

Key words: *Aspergillus*, aspergillosis, *Fusarium*, *Lomentospora*, *Scedosporium*, mucormycosis, monoclonal antibody, lateral-flow device, fungal diagnostics, mycoses

Abbreviations

AFB (acid-fast bacillus); AIDS (acquired immune deficiency syndrome); ABPA (allergic bronchopulmonary aspergillosis); ABPM (allergic bronchopulmonary mycosis); AML (acute myeloid leukaemia); AB (*Aspergillus* bronchitis); AS (*Aspergillus* sensitisation); BALf (bronchoalveolar lavage fluid); CCPA (chronic cavitary pulmonary aspergillosis); CF (cystic fibrosis); CFPA (chronic fibrosing pulmonary aspergillosis); CGD (chronic granulomatous disease); CNPA (chronic necrotising pulmonary aspergillosis); CNS (central nervous system); CPA (chronic pulmonary aspergillosis); COPD (chronic obstructive pulmonary disease); CT (computed tomography); ELISA (Enzyme-Linked Immunosorbent Assay); EORTC/MSG (European Organisation for Research and Treatment of Cancer/Mycology Study Group); EPS (extracellular polysaccharide); FDSC (*Fusarium dimerum* species complex); FOSC (*Fusarium*

47 *oxysporum* species complex); FSSC (*Fusarium solani* species complex); GM (galactomannan);
48 HSCT (haematopoietic stem-cell transplant); IPA (invasive pulmonary aspergillosis); ISHAM
49 (International Society for Human and Animal Mycology); LFA (lateral-flow assay); LFD
50 (lateral-flow device); MRI (magnetic resonance imaging); MAb (monoclonal antibody); MDS
51 (myelodysplastic syndrome); NPV (negative predictive value); NTM (non-tuberculosis
52 mycobacteriosis); PCR (polymerase chain reaction); PET (positron emission tomography);
53 POCT (point-of-care test); PPV (positive predictive value); PSC
54 (*Pseudallescheria*/*Scedosporium* species complex); PTB (pulmonary tuberculosis); SAIA (sub-
55 acute invasive pulmonary aspergillosis); TAFC (triacetylfusarinine C).
56

57 Introduction

58
59 In 1991, it was estimated that there were 1.5 million species of fungi on the planet
60 (Hawksworth, 1991). With the advent of high throughput sequencing, that estimate was up-
61 graded to as many as 5.1 million species (Blackwell, 2011). Despite this enormous number,
62 in which fungi outnumber plants by at least 6 to 1, only ~700 species have been reported as
63 causing infections in humans (Brown *et al*., 2012a), while the number that are regularly
64 reported as disease-causing agents in the clinical setting is limited to an even smaller
65 number of species. Nevertheless, that handful of species, which includes yeasts, yeast-like
66 fungi, and moulds, collectively cause enormous but largely unrecognised damage to human
67 health and well-being in the form of superficial infections of the mucosa, skin and nails
68 (Schwartz, 2004), deep infections of the bones and joints (Taj-Aldeen *et al*., 2015), asthma
69 and allergies (Denning *et al*., 2014), and life-threatening disseminated infections in already
70 seriously ill patients, amounting to billions of affected people each year (Brown *et al*.,
71 2012a,b).
72 Fungi that cause fatal disseminated infections are typically opportunistic, taking
73 advantage of immune systems weakened caused by primary immunodeficiences (Lanternier
74 *et al*., 2013) or AIDS (Limper *et al*., 2017), haematological malignancies (Pergam, 2017), or
75 the use of potent immuno-suppressive therapies that deplete microbial immunity
76 (Gundacker and Baddley, 2015). The fungi regarded as the most important medically are
77 *Aspergillus*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Paracoccidioides*,
78 *Pneumocystis*, and *Talaromyces*. Only one of these, *Aspergillus*, is a true filamentous fungus
79 or mould, the remainder being yeasts, yeast-like, or thermally dimorphic fungi. It is
80 estimated that *Aspergillus* diseases, caused principally by the single species *Aspergillus*
81 *fumigatus*, collectively affect around 9 million people worldwide, with a further 10 million
82 people estimated to be at risk of *Aspergillus* infection. However, because of under-diagnosis
83 and under-reporting of *Aspergillus* diseases, these figures are likely to be under-estimates
84 (Denning *et al*., 2013). Also under-diagnosed or under-reported, are the diseases caused by
85 other mould pathogens (*Fusarium* spp., species in the *Pseudallescheria*/*Scedosporium*
86 complex (PSC), *Lomentospora prolificans*, and the mucormycetes comprising, amongst
87 others, *Mucor*, *Rhizomucor*, and *Rhizopus* spp.), despite their increasing frequency as
88 infectious etiologies in the ever-expanding populations of immunocompromised and
89 diabetic patients, and their involvement in polymicrobial diseases following traumatic injury.
90 The aim of this review is to examine the ecology, epidemiology, and methods of
91 detection of these under-reported moulds in the context of human disease. It aims to
92 highlight the paucity of diagnostic tests for these pathogens that, despite repeated calls by

learned societies, opinion leaders, and action groups for better access to cheap diagnostics, remain pitifully inadequate, adding to the unacceptably high rates of mordity and mortality caused by these fungi, and the added financial burden to healthcare providers consequent of delayed or incorrect diagnosis. The reasons for our repeated failure to develop cheap, accurate, and user-friendly diagnostic tests are discussed, as are the steps that might be taken to 'release the brakes' on bench-to-bedside translation of diagnostics to the clinical setting, enabling access to point-of-care tests, particularly in poor and developing countries that lack access to well-resourced and well-equipped diagnostic facilities.

Aspergillus species pathogenic to humans

The genus *Aspergillus* comprises between 260 and 837 species of ascomycete fungi, making it one of the most abundant groups of fungi on the planet (Hawksworth, 2011). Despite their abundance, only a small number of species are known to cause diseases in humans, with a single anamorphic species *Aspergillus fumigatus* (teleomorph *Neosartorya fischeri*) in the section *Fumigati* responsible for >80% of recorded infections in humans (Latgé, 1999). Other well-established species capable of causing infections include *A. flavus* (section *Flavi*), *A. terreus* (section *Terrei*), *A. niger* (section *Nigri*) and *A. nidulans* (section *Nidulante*)(Sugui *et al*., 2015), while a number of sibling species of *A. fumigatus* have emerged as human pathogens over recent years. These rarer species in the section *Fumigati* bare morphological similarities to *A. fumigatus*, but worryingly also exhibit intrinsic resistance to anti-fungal drugs (Sugui *et al*., 2015; Van der Linden *et al*., 2011), further complicating detection and treatment with the advent of azole-resistance in clinical strains of *A. fumigatus* (Abdolrasouli *et al*., 2018). Of these emerging species, *A. udagawae* (*Neosartorya udagawae*), *A. lentulus*, and *A. pseudofischeri* (*Neosartorya pseudofischeri*) are the most frequently reported as causing disease in humans. Notwithstanding these, *A. fumigatus* remains the most important pathogen in the *Aspergillus* genus in terms of prevalence and capacity to cause disease in humans. It is almost the most important mould pathogen of humans compared to all other mould species capable of causing life-threatening disseminated infections.

Aspergillus diseases

While *Aspergillus* spp. have recently emerged as the cause of deep-seated tissue infections following combat-related injuries in military personnel (Paolino *et al*., 2012; Warkentien *et al*., 2012; Tribble and Rodriguez, 2014), they are best known as opportunistic pathogens which cause life-threatening infections in immuno-compromised individuals whose impaired immunity is unable to eliminate the infectious propagules (air-borne spores) inhaled into the lungs. In patients with defective cell-mediated immunity due to cytotoxic chemotherapy or T-cell dysfunction due to corticosteroid or other immunosuppressive therapy, *Aspergillus* spores germinate to produce invasive hyphae which proliferate, leading to the often-fatal disseminated disease invasive pulmonary aspergillosis (IPA). These fungi can also cause allergic and chronic respiratory diseases as a result of an over-zealous host response to saprotrophic colonisation of the lung following inhalation of air-borne spores. The clinical consequences of this are most severe in the context of underlying respiratory disorders such as chronic obstructive pulmonary disease (COPD), post pulmonary tuberculosis (PTB), non-

tuberculosis mycobacteriosis (NTM), bronchiectasis, and cystic fibrosis (CF). The role of *Aspergillus* spp. in bronchiectasis in the non-CF lung is less well understood, but *Aspergillus* sensitisation and/or allergic bronchopulmonary aspergillosis (ABPA) is a cause and also a consequence of bronchiectasis (Chotirmall and McElvaney, 2014; Chotirmall and Martin-Gomez, 2018). The sequel to saprotrophic colonisation is infection and the development of chronic pulmonary aspergillosis (CPA), a slowly progressive lung disease, with poor clinical outcome (Denning *et al*., 2011 and 2013; Takazono and Izumikawa, 2018).

The global burden of *Aspergillus* lung diseases is vast (Bongomin *et al*., 2017). There are an estimated 300,000 cases of IPA per year with approximately 10 million at risk annually (Brown *et al*., 2012b; Bongomin *et al*., 2017). The global burden of ABPA in asthma is approximately 4.8 million, ABPA in cystic fibrosis approximately 6,675, CPA approximately 3 million (including 1.6 million cases post-tuberculosis), with CPA complicating ABPA approximately 400,000 (Denning *et al*., 2013). In the United Kingdom, there are estimated to be around 4,000 cases of IPA each year, 178,000 cases of ABPA in people with asthma, and 3600 patients with CPA, based on burden estimates post PTB and in sarcoidosis (Pegorie *et al*., 2017).

Invasive Pulmonary Aspergillosis

A fatality due to disseminated invasive pulmonary aspergillosis was first described in 1953 following autopsy of a patient with agranulocytosis disease (Rankin, 1953). At that time, IPA was a rare, albeit poorly recognised, disease of immunocompromised patients. It is perhaps ironic that modern advances in the treatment of haematological malignancies, especially remission-induction therapy for acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS), and the use of highly-effective immune modulating drugs (Kyi *et al*., 2014; Reinwald *et al*., 2016), have led to a dramatic increase in the incidence of IPA in immunodeficient patients, such that the mortality rate of IPA exceeds 50% in neutropenic patients, and reaches 90% in haematopoietic stem-cell transplant (HSCT) recipients (Segal, 2009; Kousha *et al*., 2011). The major risk factors for the disease include neutropenia, HSCT and solid organ transplantation, high-dose corticosteroids, haematological malignancy, cytotoxic therapy, advanced AIDS, and chronic granulomatous disease (CGD)(Latgé, 1999; Dutkiewicz and Hage, 2010).

Allergic Bronchopulmonary Aspergillosis

Of the different yeasts and filamentous fungi able to cause allergic bronchopulmonary mycosis (ABPM) in humans (Chowdary *et al*., 2014), *A. fumigatus* and allergic bronchopulmonary aspergillosis (ABPA) are the most extensively studied. Allergic bronchopulmonary aspergillosis is one of the most severe *Aspergillus*-related diseases, with the potential to progress into pleuropulmonary fibrosis and respiratory failure (Carsin *et al*., 2017). It occurs almost exclusively in cystic fibrosis or asthmatic patients, and is characterised by a hypersensitivity reaction to *Aspergillus* spp., with diverse clinical and radiological manifestations.

185 ## Bronchiectasis
186
187 Bronchiectasis is a chronic respiratory disease characterised by progressive and irreversible
188 dilation of the bronchial lumen, with impaired mucociliary clearance, recurrent infection,
189 and chronic inflammation. *Aspergillus* species are the most common filamentous fungi
190 cultured from respiratory secretions of CF, non-CF, and bronchiectasis patients (Aogáin *et*
191 *al*., 2018; Máiz *et al*., 2015, 2018). *Aspergillus fumigatus* is the most frequently recovered
192 species and the species most likely to cause infections, followed by *A. flavus*, *A. niger* and *A.*
193 *terreus* which tend to colonise, rather than infect, the lung (Máiz *et al*., 2018). These species
194 also differ in their geographical distributions, with a higher prevalence of *A. niger* and *A.*
195 *terreus* in Japan, and *A. flavus* in India and China (Máiz *et al*., 2018).
196
197 ## Chronic Pulmonary Aspergillosis
198
199 Chronic pulmonary aspergillosis (CPA) is a semi-invasive disease (Chan *et al*., 2016), lying
200 somewhere in between allergic and invasive forms of aspergillosis (Sehgal *et al*., 2018). It
201 comprises a number of lung diseases that are long-term sequelae to pulmonary tuberculosis
202 (PTB), namely *Aspergillus* nodule, simple pulmonary aspergilloma, chronic cavitary
203 pulmonary aspergillosis (CCPA; presence of one or more cavities with or without
204 aspergilloma, accompanied by pulmonary and systemic symptoms), chronic fibrosing
205 pulmonary aspergillosis (CFPA; cavitation and fibrosis involving two or more lobes), and sub-
206 acute invasive pulmonary aspergillosis (SAIA)(Denning *et al*., 2016) also called chronic
207 necrotising pulmonary aspergillosis (CNPA)(Kaymaz *et al*., 2016) leading to progressive
208 destruction of the lung (Ohba *et al*., 2012). CCPA is the most common form of CPA (Denning
209 *et al*., 2003), with most cases of CPA occurring in South-East Asia, Western Pacific, and
210 Africa, where prevalence of PTB is the highest. In high-income settings, COPD appears to be
211 the most important pre-disposing condition for the disease (Smith and Denning, 2011).
212 Unlike IPA, CPA occurs in immunocompetent patients after treatment for TB, leading to
213 weight loss, intense fatigue, severe shortness of breath and life-threatening haemoptysis.
214 *Aspergillus fumigatus* is the most frequently identified species in negative acid-fast bacillus
215 (AFB) sputum smears from sub-Saharan Africans following treatment for PTB. Indeed, CPA is
216 an important differential diagnosis for what appears to be smear-negative tuberculosis
217 (Denning *et al*., 2011).
218
219 # Detection of *Aspergillus* diseases
220
221 Detection of *Aspergillus* lung diseases remains a significant clinical challenge. Clinical
222 manifestations of *Aspergillus* lung infection or colonisation are non-specific, as are
223 radiological abnormalities seen on X-rays or on computed tomograms of the chest (chest-
224 CT). Despite this, anomalies on a chest-CT are used in some centres to initiate antifungal
225 drug treatment in at-risk patients. However, the burgeoning costs of antifungal treatments
226 (Schumock *et al*., 2016) and prolonged hospitalisation for patients with suspected, but often
227 unproven, pulmonary mycosis, has turned the spotlight on diagnostics and the need for
228 improved detection methods that enable the implementation of antifungal stewardship
229 programmes which have the potential deliver significant savings in antifungal drug
230 expenditure (Nwankwo *et al*., 2018).

Diagnostic-driven approaches to antifungal treatment have been shown to be more effective than empirical treatment, driving down costs, and improving outcomes for patients (Barnes, 2013). The emergence of triazole resistance in clinical strains of *A. fumigatus* and other *Aspergillus* spp. (Rivero-Menendez *et al*., 2016; Abdolrasouli *et al*., 2018; Zoran *et al*., 2018), driven by a multiplicity of factors including widespread use of azole fungicides in agriculture (Fisher *et al*., 2018; Mortensen *et al*., 2010; Verweij *et al*., 2009), anti-fungal prophylaxis (Fisher *et al*., 2018), evolution of resistance in infecting strains in the lung during or after long-term (sometimes life-long) therapy with the same antifungal drug (Howard *et al*., 2009; Verweij *et al*., 2016), and sub-therapeutic dosing of patients (van der Elst *et al*., 2015), is a serious cause for concern. Up to 12% of *Aspergillus* infections are estimated to be resistant to antifungal drugs (Rivero-Menendez *et al*., 2016), with up to 7% of *Aspergillus* strains from stem cell and solid organ transplant patients found to be resistant (Baddley *et al*., 2009; Kontoyiannis *et al*., 2010; Pappas *et al*., 2010).

Diagnostics has an important part to play in guiding patient treatment, by restricting the use of antifungal drugs to those that truly need them. It is important to note that a combination approach to diagnostics is more likely to be effective than a single test approach, despite pressures to cut healthcare costs. No single test fits all, especially where an organism such as *A. fumigatus* is capable of causing a number of different diseases of the lung. For this reason, abnormalities in a chest-CT that raise the suspicion of a fungal lung infection in an at-risk patient should be accompanied by complementary biomarker tests that add additional layers of proof. Biomarker tests such as 'pan-fungal' assays for fungal (1→3)-β-D-glucan, and the Bio-Rad *Platelia*® ELISA for *Aspergillus* galactomannan (GM-ELISA) in serum are FDA-approved and are included in the EORTC/MSG and ESCMID-ECMM-ERS guidelines for disease detection (De Pauw *et al*., 2008; Ullmann *et al*., 2018), while others such as the point-of-care *Aspergillus* lateral-flow device (*Asp*LFD) have been recently CE-marked and are now available commercially for IPA detection. These assays, and other commercial or experimental tests currently in development, are discussed in the context of allergic (ABPA), semi-invasive (CPA), and invasive (IPA) *Aspergillus* lung diseases.

Invasive Pulmonary Aspergillosis

Invasive diagnostic procedures

Bronchoscopy and culture

Diagnosis of IPA at the onset of disease is of paramount importance as prognosis worsens significantly over time. Detection of circulating biomarkers in blood such as *Aspergillus* galactomannan, fungal (1→3)-β-D-glucan, and *Aspergillus* DNA would indicate that the disease is already well progressed. Early detection of *Aspergillus* lung infection is only achievable by using tissue biopsy or bronchoalveolar lavage fluid (BALf) recovered during invasive bronchoscopy, which allow culture of the pathogen or detection of biomarkers of infection, respectively. The 'gold standard' test for diagnosis of IPA is culture of *Aspergillus* from a sterile lung biopsy, with associated necrosis of tissues due to presence of hyphal elements. There are a number of problems with culture, including the need for invasive tissue sampling, slow growth or no growth of isolates from cultured material, and difficulties in differentiating *Aspergillus* spp. from other agents of hyalohyphomycoses (e.g. other hyaline septate moulds such as *Fusarium* and *Scedosporium* species) in the absence of

characteristic spore-bearing structures. This leads to lengthy turnaround times, and overall poor sensitivity of culture-based detection compared to biomarker tests (Prattes *et al.*, 2014). A major benefit of culture is the recovery of isolates for antifungal susceptibility testing and molecular detection of resistance mutations (Postina *et al*., 2018), especially given the increasing prevalence of triazole resistance in *A. fumigatus* isolates (Abdolrasouli *et al*., 2018).

Biomarker detection in BALf

Lateral-flow technology

The advantage of assaying BALf for *Aspergillus* biomarkers, is that is allows increased sensitivity and speed of detection compared to culture. The *Aspergillus* lateral-flow device (*Asp*LFD), a point-of-care test (POCT) that incorporates a monoclonal antibody (mAb), JF5, which detects a biomarker of active growth (Thornton, 2008; Thornton, 2014), allows rapid near-patient diagnosis of IPA, with confirmatory detection of disease using laboratory-based GM-ELISA, (1→3)-β-D-glucan, or polymerase chain reaction (PCR) tests of BALf samples. Combination biomarker testing adds considerable power to IPA diagnosis, with a number of studies demonstrating improvements in diagnostic sensitivity when these assays are used in combination (Hoenigl *et al*., 2014; Johnson *et al*., 2015; Eigl *et al.*, 2017). There have been numerous studies conducted with the prototype *Asp*LFD test, with two meta-analysis studies (Pan *et al.*, 2015; Zhang *et al*., 2019) evaluating its diagnostic performance in combination with GM-ELISA or (1→3)-β-D-glucan tests. The more recent study by Zhang *et al*. (2019), which undertook a meta-analysis of 13 previously-published studies of the prototype *Asp*LFD, showed that a positive GM-ELISA and a positive (1→3)-β-D-glucan or *Asp*LFD test provided a confirmatory diagnosis of IPA.

The *Asp*LFD was CE-marked in May 2018 for use with both serum and BALf, with Mercier *et al*. (2019a) reporting the performance of the CE-marked test in a retrospective evaluation of BALf samples from haematology patients across four centres in the Netherlands and Belgium. In this multicentre study, the CE-marked test had a good performance for the diagnosis of proven IPA versus controls (no IPA), with a specificity of 86% and sensitivity of 82%. Two follow-up studies (Jenks *et al.*, 2019; Mercier *et al*., 2019b) have compared the *Asp*LFD with the newly developed sōna galactomannan lateral-flow assay (GM-LFA) produced by IMMY. In the study by Jenks *et al*. (2019), both the *Asp*LFD and GM-LFA were highly sensitive and specific for probable/proven IPA versus no IPA in patients with haematological malignancies, with specificities of close to 90% for both the *Asp*LFD and GM-LFA when read at 15 min, and with 100% sensitivity for the *Asp*LFD when read at 25 min. In patients at risk for IPA but without neutropenia or underlying haematological malignancy, the GM-LFA and *Asp*LFD tests showed sensitivities ranging from 58% to 69%, with specificities between 68% and 75% (Jenks *et al*., 2018). When the two tests were combined, sensitivity increased to 81% while specificity remained around 60%, making this a reasonable approach for IPA diagnosis in non-neutropenic patients.

In the study by Mercier *et al*. (2019b), which retrospectively tested 235 BALf samples of adult haematology patients from four centres on behalf of the Dutch-Belgian Mycosis Study Group (DB-MSG), performance of the *Asp*LFD and GM-LFA for proven IPA were similar. In cases of proven and probable IPA, the tests had identical specificity, but a higher sensitivity and a better NPV for the GM-LFA. However, a very faint test line is discernible in the GM-

325 LFA with BALf samples from patients with no IPA, and is also present with the sample
326 running buffer (i.e. strictly negative tests). This means that a comparator is required in cases
327 where a weak test line is evident in order to determine whether this is due to non-specific
328 binding (NSB), or due to an actual but weak line. Because of this ambiguity, a reading card is
329 included in the IMMY test against which test line intensities are compared. Any test line
330 considered fainter than a line of semi-quantitative intensity 1 is considered to be negative.
331 Alternatively, plain running buffer can be used to create a negative control LFA against
332 which clinical samples can be compared. Any test line more intense than the negative
333 control can be considered positive. However, this procedure introduces additional
334 uncertainty, variability, and cost, since a negative control must be included for every sample
335 run.
336 While the GM-LFA and *Asp*LFD are both relatively easy to use, the *Asp*LFD is somewhat
337 simpler, and does not suffer from NSB unlike the IMMY test. For the *Asp*LFD, no sample pre-
338 treatment is required with non-bloody, non-viscous BALf, which can be applied directly to
339 the test, with results available within 15 minutes. In the case of bloody or viscous BALf,
340 heating with a buffer (3 minutes) followed by centrifugation (5 minutes) is required, with a
341 total test time of approximately 25 minutes. For the GM-LFA, all samples must be pre-
342 treated with heating for 6-8 minutes and centrifugation for 5 minutes, bringing the total test
343 time to roughly 45 minutes. Notwithstanding this, Mercier and co-workers (2019a,b)
344 conclude that both tests show a good performance for diagnosis of IPA in haematology
345 patients using BALf. Both tests can be used for fast screening of patients, although
346 confirmation using an adjunct test such as GM-ELISA, PCR or culture is merited.
347
348 *Cytokines*
349
350 An important limitation of biomarker tests is that sensitivity may decrease during antifungal
351 prophylaxis or empirical therapy of patients with haematological malignancies (Eigl *et al*.,
352 2015; Prattes *et al*., 2015). To mitigate this loss of test sensitivity, alternative signature
353 molecules in BALf have recently been explored which might act as adjunct biomarkers of the
354 disease. Gonçalves *et al*. (2017) investigated the alveolar cytokine profiles of patients at high
355 risk of developing IPA. The cytokines IL-1β, IL-6, IL-8, IL-17A, IL-23, and TNF-α were
356 significantly increased among patients with the disease. However, IL-6, IL-8, and IL-23 best
357 differentiated between cases of IPA and controls, whereas the remaining cytokines
358 displayed an inconsistent contribution to discrimination. The cytokine IL-8 was the dominant
359 discriminator, with alveolar levels of $\geq$904 pg/ml predicting IPA with a sensitivity of 90%, a
360 specificity of 73%, and a negative predictive value of 88%.
361
362 *Siderophores*
363
364 Iron is essential for the growth of *Aspergillus fumigatus*, and targeting its acquisition blocks
365 infection by the pathogen (Leal *et al*., 2013). In iron-poor environments such as serum, the
366 pathogen produces low-molecular-weight siderophores to scavenge ferric iron from the
367 host (Haas, 2003; Hass *et al*., 2015). One of these, the extracellular siderophore
368 triacetylfusarinine C (TAFC), is produced following spore germination, and is detectable in
369 serum from patients with proven or probable IPA (Carrol *et al*., 2016). The siderophore is
370 also detectable in BALf, and an evaluation by Orasch *et al*. (2017) of BALf samples from
371 patients with haematological malignancies (of which 73% received antifungal prophylaxis or

empirical treatment at the time of bronchoscopy) showed that diagnostic sensitivities of the GM-ELISA and *Asp*LFD tests could be increased when used in combination with TAFC detection, in line with previous BALf GM-ELISA/*Asp*LFD combination testing (Hoenigl *et al*., 2014; Prattes *et al*., 2015).

Polymerase chain reaction

Aspergillus polymerase chain reaction (PCR) has been shown for many years to be a promising diagnostic procedure for IPA detection, especially when combined with other diagnostic biomarker tests (White *et al*., 2013; Buchheidt *et al*., 2017). Despite the widespread use of 'in-house' tests, PCR was not included in the 2008 EORTC/MSG diagnostic criteria for IPA (De Pauw *et al*., 2008) due to a lack of assay standardisation and validation. However, PCR will be included in the latest iteration of the guidelines, following systematic review of the evidence for its clinical use in comparison with antigen testing (White *et al*., 2015). The availability of commercial PCR tests has aided assay standardisation (Rath and Steinmann, 2018), with three PCR assays specifically tailored to *Aspergillus* detection, and which have been evaluated using BALf or respiratory samples. The MycAssay *Aspergillus*® assay is a real-time PCR which detects *A. fumigatus* and other *Aspergillus* spp., with a sensitivity of 80% to 94.1%, and a specificity of 87.6% to 98.6%, in non-haematology and mixed patient groups (Torelli *et al*., 2011; Guinea *et al*., 2013; Orsi *et al*., 2015). The AsperGenius® test comprises two PCR assays, one for the detection and differentiation of *Aspergillus* spp. (AsperGenius Species multiplex®), and the other (AsperGenius Resistance multiplex®) for the detection of four azole resistance markers within the Cyp51A gene of *A. fumigatus* (L98H, TR34, T289A, Y121F). The AsperGenius Species multiplex® assay contains a probe for the *A. fumigatus* complex (*A. fumigatus* and the emerging sibling pathogens *A. lentulus*, *A. udagawae*, and *A. viridinutans*), and a probe for *Aspergillus* spp. (the *A. fumigatus* complex and *A. flavus*, *A. terreus*, and *A. niger*). Using BALf, the AsperGenius® test has a sensitivity of 68.4% to 84%, and a specificity of 80% to >92% (Chong *et al*., 2016; Guegan *et al*., 2018). In a study of 91 patients with GM-ELISA positive BAlf samples, 79% were positive for *A. fumigatus* or *Aspergillus* spp. DNA, with the azole resistance mutation TR_{34}/L98H detected in 8 of the cases, and T289A/Y121F mutations in a further 3 cases (Schauwvlieghe *et al*., 2017). The MycoGenie® assay detects *A. fumigatus* and the TR34/L98H mutation, and has a test sensitivity of 71.1% and specificity of >92% when used with BALf samples (Guegan *et al*., 2018). Notwithstanding improvements in PCR standardisation, the Infectious Diseases Society of America (ISDA) currently recommends that *Aspergillus* PCR be used in individual cases if combined with other diagnostic and clinical data (Misch and Safdar, 2016; Patterson *et al*., 2016; Lass-Flörl, 2019).

Semi-invasive and non-invasive diagnostic procedures

Biomarker detection in serum

ELISA, PCR, (1→3)-β-D-glucan, and LFD tests

The literature on the relative specificities and sensititivites of fungal (1→3)-β-D-glucan tests, Bio-Rad *Platelia*® GM-ELISA, in-house PCR assays, and prototype *Asp*LFD test, for serum-based diagnosis of *Aspergillus* diseases is capacious, and has been reported extensively

419 elsewhere (Held *et al*., 2013; White *et al*., 2013; Pan *et al*., 2015; Held and Hoenigl, 2017;
420 Rath and Steinmann, 2018; Ruhnke *et al*., 2018; Patterson and Donnelly, 2019).
421 To date, there have been no studies evaluating the diagnostic performance of the CE-
422 marked *Asp*LFD when used with serum samples. However, an ELISA (GP-ELISA) which
423 employs the same mAb (JF5) used in the *Asp*LFD (and also in the experimental *Aspergillus*
424 proximity ligation assay (Mercier *et al*., 2018)) has recently been developed and CE-marked
425 by Euroimmun Medizinische Labordiagnostika AG, and its performance compared to the
426 *Platelia*® GM-ELISA for IPA diagnosis using serum samples from immunocompromised
427 patients (Dichtl *et al*., 2019). While the specificities of the GM-ELISA and GP-ELISA were 99%
428 and 96%, respectively, both assays demonstrated low sensitivities. When tests were
429 extended to all sera available in the time frame of 7 days before to 7 days after the day of
430 proven diagnosis, 47% and 56% of the cases were detected by the GM-ELISA and GP-ELISA,
431 respectively. The low sensitivities of both tests underline the need for serial tests when
432 using patient serum.
433
434 Molecular imaging using mAb JF5 and siderophores
435
436 Abnormalities in a chest-CT as diagnostic indicators of IPA (defined as dense, well-
437 delineated, nodular infiltrates with and without ground glass attenuation (the so-called
438 'halo sign'))(De Pauw *et al*., 2008) are not disease-defining, with other pathologies (non-
439 *Aspergillus* lung infections, and neoplastic and inflammatory processes) giving similar
440 anomalies. Furthermore, these radiological indicators are transient in neutropenic patients,
441 or are rare in non-neutropenic patients (Prattes *et al*., 2014). While magnetic resonance
442 imaging (MRI) provides unrivalled spatial resolution and soft tissue contrast, and can be
443 used as a detection aid for *Aspergillus* cerebral and central nervous system infections
444 (Starkey *et al*., 2014; Marzolf *et al*., 2016), its utility as an imaging modality for lung
445 infections is limited due to the lack of detectable protons in air-filled spaces and potential
446 artefacts between air-tissue interfaces.
447 For these reasons, attempts have been made to improve the accuracy of radiology for
448 diagnosis of IPA by combining positron emission tomography (PET) with CT and MRI
449 (Thornton, 2018). The *Aspergillus*-specific mAb, JF5, when conjugated to the radionuclide
450 ^{64}Cu, has been used in immunoPET/MRI as a disease-specific tracer ([^{64}Cu]NODAGA-JF5),
451 and allows accurate and sensitive non-invasive detection of *A. fumigatus* lung infections *in*
452 *vivo* (Rolle *et al*., 2016; Davies *et al*., 2017). Furthermore, humanisation of JF5 (Davies *et al*.,
453 2017) has allowed translation of the imaging technology to the clinic. A first-in-human
454 clinical trial commenced in 2018, with successful detection of IPA in a neutropenic
455 haematology patient, corroborated by BALf detection using the *Asp*LFD, GM-ELISA, and
456 culture (Thornton, pers. comm.).
457 Alternative approaches to the molecular imaging of IPA include the use of *Aspergillus*
458 siderophores in PET. When coupled to ^{68}Ga, a positron emitter with complexing properties
459 similar to those of Fe(III), the iron-chelating siderophore TAFC showed accumulation in the
460 lungs of immunosuppressed rats with IPA, with uptake correlating with severity of
461 *Aspergillus* infection (Petrik *et al*., 2010 & 2012a,b). While still at the pre-clinical stage of
462 development, a [^{68}Ga]TAFC tracer for molecular imaging of *Aspergillus* lung infections *in*
463 *vivo*, holds enormous diagnostic potential, alongside antibody-guided PET/MR imaging
464 (immunoPET/MRI), for non-invasive detection of IPA in humans.
465

466 Biomarker detection in urine
467
468 *GM antigenuria*
469
470 Evidence for the presence of *Aspergillus* carbohydrate biomarkers in urine was first shown
471 in counter-immunodiffusion assays with urine from rabbits experimentally infected with *A.*
472 *fumigatus* (Lehman and Reiss, 1978). Antiserum used in the assay was prepared from an
473 immunosuppressed and infected rabbit (rather than an animal immunised with an *A.*
474 *fumigatus* antigen preparation), and showed that a carbohydrate antigen was detected in
475 the urine of the experimentally infected rabbits. Detection of the antigen was not found in
476 uninfected animals or in animals infected with *Candida albicans*. While human urinary
477 antigen was not investigated, and the identity of the carbohydrate was not established, this
478 early study was the first to demonstrate the excretion of *A. fumigatus* carbohydrate
479 antigen(s) into the urine of animals with IPA.
480 The identity of a carbohydrate antigen in the urine of experimentally infected
481 rabbits, and in the urine of patients with IPA, was subsequently demonstrated by Dupont
482 and co-workers (Dupont *et al*., 1987). Galactomannan (GM) was detected in animal and
483 human urine samples using a sandwich ELISA employing rabbit antiserum raised against
484 purified GM. Using this assay, urinary GM was detectable throughout the course of lethal
485 aspergillosis in all 16 experimentally infected animals, in concentrations of 24 to 1,900 ng
486 GM/ml urine, with antigen excretion roughly paralleling the extent of disease.
487 Galactomannan was detected in the urine of 7 of 13 patients with IPA, with concentrations
488 of 1 to 83 ng GM/ml urine. A contemporaneous study by Bennett *et al*. (1987) showed that
489 within 24 hours after intravenous injection, 35% of galactomannan was excreted into the
490 urine of immunocompetent rabbits. Galactomannan detection in urine was also investigated
491 as a means of diagnosing experimental and spontaneous aspergillosis in cattle (Jensen *et al*.,
492 1993a). An inhibition ELISA using a GM-specific mAb EB-A1 (Stynen *et al*., 1992), reacted
493 positively with urine samples from normal cattle, and so could not be used to detect
494 systemic bovine aspergillosis. The same mAb was used by Haynes *et al*. (1990) to detect GM
495 in the urine of neutropenic patients undergoing bone marrow transplantation or remission
496 induction therapy for leukemia. In immunoblotting studies, EB-A1 showed diffuse staining
497 (indicative of carbohydrate) in urine samples from patients with IPA, but no staining with
498 urine from patients without evidence of the disease.
499 The development of a commercial latex agglutination test for *Aspergillus* GM (Pastorex
500 *Aspergillus*, Sanofi Diagnostics Pasteur, France) using another GM-reactive rat mAb, EB-A2,
501 also developed by Stynen *et al*. (1992), enabled standardised testing of GM antigenemia in
502 the serum of IPA patients as a means of diagnosing IPA. The test was also investigated as a
503 diagnostic tool for IPA using GM antigenuria in bone marrow transplant recipients (Ansorg
504 *et al*., 1994). After modification of the assay for urine testing, it had a detection limit in
505 native urine of approximately 20 ng GM/ml. Antigen was found in 79 (36.4%) of 217 serial
506 urine samples, compared to 40 (11.8%) of 340 serum samples. Overall, antigenuria
507 preceded antigenemia, and was more persistent. The sensitivity, specificity, PPV and NPV of
508 antigenuria for autopsy-proven aspergillosis and clinically-suspected *Aspergillus* infection
509 were 57%, 53%, 31% and 77%, respectively, while those of antigenemia were 43%, 53%,
510 25% and 71%, respectively. It was therefore concluded that urine testing was more reliable
511 than serum testing for the detection of *Aspergillus* GM.

512 Refinement of the latex agglutination test led to the development of a quantitative
513 sandwich ELISA for GM detection in patient serum, and which forms the basis of the
514 commercial Bio-Rad *Platelia*® ELISA for GM detection in patient serum and BALf samples.
515 The ELISA, which detects less than 1ng GM/ml sample, was also investigated as a urine test
516 for GM in patients with IPA (Stynen *et al*., 1995). When GM was detected in urine, it was
517 always lower than in matched serum samples. In addition, GM was always detected earlier
518 in serum than in urine. The maximal number of days separating patient death and the first
519 detection of GM in patient serum and urine were ≥39 and ≤12, respectively. Therefore, in
520 contrast to a previous report (Rogers *et al*., 1990), it was recommended that serum rather
521 than urine be used for the detection of GM, because of the later diagnosis obtained with
522 urine samples.
523 Since the pioneering work in the 1990s, there has been limited interest in GM
524 antigenuria as a detection method for IPA due to reports of its limited utility and high rates
525 of false positivity, which vary between 8% and 47% (Klont *et al*., 2004). However, since the
526 introduction of the *Asp*LFD POCT, which has revolutionised point-of-care testing for the
527 disease, there has been renewed interest in GM detection in urine, due to its non-invasive
528 nature and the applicability of lateral-flow technology to urine biomarker testing. Dufresne
529 *et al*. (2012) recently reported the development of a new GM-reactive mAb (mAb476), and
530 demonstrated the feasibility of using the mAb in an immunochromatographic assay for
531 detection of GM-like antigens in urine. Further development of the assay into a dip-stick
532 format has demonstrated the potential of the test to detect proven or probable IPA in
533 patients at high-risk for the disease (patients with haematological malignancy, and
534 recipients of solid organ or HSCT). Per-patient sensitivity and specificity in the overall cohort
535 was 80% and 92%, respectively, and the urine assay correlated with serum galactomannan
536 indices (Marr *et al*., 2018). While the test uses non-invasive urine samples for GM detection,
537 the requirement for sample pre-treatment means that it is unlikely to be a point-of-care test
538 for IPA. Nevertheless, it demonstrates the applicability of lateral-flow technology to urine
539 testing for *Aspergillus* diagnostic biomarkers, and for urine-based detection of other fungal
540 pathogens of humans such as *Cryptococcus* (Drain *et al*., 2019) and *Histoplasma* (Libert *et*
541 *al*., 2018).
542
543 *Urine siderophores*
544
545 In addition to cell wall carbohydrates such as GM, *A. fumigatus* and other *Aspergillus*
546 species produce proteins that are detectable in urine. These include the iron-scavenging
547 siderophores TAFC and ferricrocin. In a rat model of experimental aspergillosis, TAFC and
548 ferricrocin (FC) were quantified in urine and serum with matrix-assisted laser desorption
549 ionisation (MALDI)(Luptáková *et al*., 2017). The limits of detection of the ferri-forms of TAFC
550 and FC in rat serum were 0.28 and 0.36 ng/ml, respectively, while in urine the limits of
551 detection were 0.02 and 0.03 ng/ml, respectively. The mean concentrations of TAFC and FC
552 in urine were 0.37 and 0.63 μg/ml, respectively. In a rat model of IPA, ferricrocin and TAFC
553 detection in urine using mass spectrometry therefore appeared to provide a non-invasive
554 means of detecting *Aspergillus* lung infections.
555 The siderophore TAFC has also been evaluted as a urine biomarker of IPA in humans
556 using mass spectrometry (Hoenigl *et al*., 2019). Urine TAFC, normalised to creatinine, was
557 determined in 44 samples from 24 patients with underlying haematological malignancies
558 and probable, possible or no IPA, and compared to GM antigenuria using the Bio-Rad

559 *Platelia*® ELISA. Matched blood samples were also tested for GM, and for (1→3)-β-D-glucan
560 using the Fungitell test. The TAFC/crea index determination in urine samples showed a
561 promising performance for diagnosis of IPA, with sensitivities and specificities comparable
562 to those reported for GM in serum and BALf, and superior to GM antigenuria
563 determinations. Compared to TAFC detection in human BALf and serum samples (Carroll *et*
564 *al*., 2016; Orasch *et al*., 2017), the diagnostic performance of TAFC levels in urine seems to
565 be superior, which may be due to accumulation in the bladder as shown in recent animal
566 models (Petrik *et al*., 2014 and 2015).
567
568 **Allergic Bronchopulmonary Aspergillosis (ABPA)**
569
570 Diagnosis of ABPA is extremely difficult, reflected in a number of different diagnostic
571 definitions of the disease. To unify diagnosis and therapy of ABPA, the International Society
572 for Human and Animal Mycology (ISHAM) produced consensus-based guidelines in 2013
573 (Agarwal *et al*., 2013). Isolation of *A. fumigatus* from sputum samples can sometimes be
574 useful, but is not included in the guidelines due to its poor sensitivity and specificity. As with
575 other *Aspergillus* lung diseases, consolidation seen in a chest-CT is non-pathognomonic and
576 may indicate another pulmonary infection such as pulmonary tuberculosis. Nevertheless,
577 radiographic pulmonary opacities consistent with ABPA (Agarwal *et al*., 2013) are included
578 under 'other criteria', of which there should be at least two of three present in a patient
579 with bronchial asthma or cystic fibrosis (the other two being presence of precipitating IgG
580 antibodies against *A. fumigatus* in serum, or total eosinophil count >500 cells/μl in steroid
581 naïve patients). These additional criteria should accompany both of the 'obligatory criteria'
582 which are (1) a positive Type I skin test (immediate cutaneous hypersensitivity to *Aspergillus*
583 antigen) or elevated IgE levels against *A. fumigatus*, and (2) elevated total IgE levels (>1000
584 IU/ml).
585 The heavy reliance on serology to include or exclude ABPA has led to efforts to improve
586 test reproducibility since production of IgG and IgE antibodies to *Aspergillus* spp. is usually
587 determined using a crude antigen extract with inherent variability and cross-reactivity with
588 other fungal antigens (Reed, 1978; Crameri *et al*., 2009). The cloning of genes that encode
589 allergenic proteins of *A. fumigatus* enabled improvements in the reliability of serological
590 diagnosis of ABPA using recombinant antigens (Crameri *et al*., 1998a,b; Fricker-Hidalgo *et*
591 *al*., 2010). A recent study by Alghamdi *et al*. (2019) of CF patients with ABPA, *Aspergillus*
592 sensitisation (AS), or *Aspergillus* bronchitis (AB) showed that patients with ABPA had
593 significantly greater IgE reactivity to the allergenic proteins Asp f1, f2, f3, and f4 compared
594 to patients with AS. Patients with AB expressed higher IgG positivity to Asp f1 and Asp f2
595 compared with those with ABPA. There were very low IgE antibody levels against all
596 recombinant antigens in AS patients. Asp f1 IgG reactivity in ABPA patients correlated with
597 positive sputum culture. It was concluded that the use of multiple recombinant allergens
598 may improve the diagnostic accuracy in CF complicated with ABPA or AB. Furthermore, Asp
599 f1 reactivity might be a useful marker for guiding antifungal therapy in ABPA.
600 The role of fungi in bronchiectasis not due to CF is poorly defined. Aogáin *et al*. (2018)
601 characterised for the first time the mycobiome in bronchiectasis, assessing its clinical
602 relevance in two geographically distinct cohorts from the CAMEB study, an international
603 multicentre cross-sectional Cohort of Asian and Matched European Bronchiectasis patients.
604 The mycobiome was determined in 238 patients by targeted amplicon shotgun sequencing
605 of the 18S-28S rRNA internal transcribed spacer regions ITS1 and ITS2. The bronchiectasis

606 mycobiome profiles were dominated by *A. fumigatus* in Singapore/Kula Lumpur, and by *A.*
607 *terreus* in Dundee, the latter associated with exacerbations. High frequences of *Aspergillus*-
608 associated disease including sensitisation and ABPA were detected. This study showed that
609 the mycobiome is of clinical relevance in bronchiectasis, and screening for *Aspergillus*-
610 associated disease should be considered even in apparently stable patients.
611
612 **Chronic Pulmonary Aspergillosis (CPA)**
613
614 Symptoms of CPA (taken here as CCPA, CFPA, and SAIA/CNPA) are non-specific and, as with
615 other *Aspergillus* lung diseases, diagnosis of the disease based on culture of fungi from
616 respiratory specimens lacks sensitivity, with culture positivity as low as 11.8% (Takazono and
617 Izumikawa, 2018). Radiological findings on a chest-CT are variable and overlapping and
618 depend on the state and stage of the disease. Diagnosis of a simple pulmonary aspergilloma
619 in an immunocompetent individual is based on detection of a single lung cavity with no
620 progression over at least 3 months of observation, while an *Aspergillus* nodule is defined by
621 the presence of one or more nodules without cavitation (Denning *et al*., 2016; Muldoon *et*
622 *al*., 2016). The diseases CCPA and SAIA share the similar characteristics of one or more
623 cavities with or without fungal ball, accompanied by radiological progression such as
624 expanding thick-walled cavities and pericavitary infiltration (Denning *et al*., 2016). They
625 differ in that SAIA also involves hyphal invasion of the lung parenchyma (Hope *et al*., 2005).
626 This subtle difference creates a diagnostic dilemma, especially when histopathology is
627 lacking. The time course of radiological progression (CCPA >3 months; SAIA 1-3 months) and
628 process of cavitary formation (CCPA typically occurs in pre-existing cavities, while SAIA
629 cavities are formed during *Aspergillus* infection) are therefore needed for disease
630 differentiation, but are reliant on serial radiography. The syndrome CFPA is defined by
631 cavitation and fibrosis involving two or more lobes leading to a major loss of lung function,
632 and is generally regarded as the end result of untreated CCPA (Denning *et al*., 2003 and
633 2016).
634 Galactomannan-ELISA of serum has limited diagnostic value for CPA detection, with low
635 sensitivity and specificity even when the threshold index value for test positivity is reduced
636 from ≥1.5 (the manufacturer's recommended threshold index value for serodiagnosis of IPA
637 (Maertens *et al*., 2007)) to ≥0.5 (Kitasato *et al*., 2009). Galactomannan-ELISA of BALf showed
638 relatively higher sensitivity (77.2%) and specificity (77.0%), when the index threshold value
639 for test positivity was reduced further to ≥0.4 (Izumikawa *et al*., 2012). In a more recent
640 study (Salzer *et al*., 2018), the GM-ELISA was evaluated alongside the *Asp*LFD and levels of
641 cytokines for diagnosis of CPA (Salzer *et al*., 2018). Sensitivity and specificity of the GM-
642 ELISA with a cut-off of ≥0.5 was 41% and 100%, respectively. When the cut-off was
643 increased to ≥1.0, the sensitivity decreased to 30% while specificity remained at 100%. The
644 *Asp*LFD was positive with only 7% of patients. Therefore, based on test sensitivities, both
645 the GM-ELISA and *Asp*LFD showed insufficient performance for diagnosing CPA. However,
646 their high specificities provide high positive predictive values, which may help identify semi-
647 invasive or invasive disease. In this study, the cytokine profiles of CPA patients did not differ
648 significantly from patients with other respiratory disorders, but showed significantly higher
649 levels of IFN-γ, IL-1b, IL-6, IL-8, and TNF-α compared to healthy controls.
650 Further evaluation of the *Asp*LFD test using serum and BALf samples from CPA patients
651 has shown improved diagnostic performance (Takazono *et al*., 2019). In this study, the
652 diagnostic performance of the *Asp*LFD was compared to the GM-ELISA and (1$\rightarrow$3)-β-D-

glucan tests using samples from patients with SAIA, simple pulmonary aspergilloma, and respiratory disease controls (PTB, NTM, COPD, interstitial pneumonia, prior lung surgery, lung cancer, and bacterial infection (pneumonia, lung abscess)). The sensitivity and specificity of the *Asp*LFD using serum were 62.0% and 67.7%, respectively, while serum GM-ELISA (cut-off index value of ≥1.5) showed a sensitivity of 22% and a specificity of 92.3%, respectively. Plasma (1$\rightarrow$3)-β-D-glucan tests (cut-off value of 19.3 pg/ml), had a sensitivity of 48% and a specificity of 90.8%, respectively. Using BALf samples, the sensitivity and specificity of the *Asp*LFD were 66.7% and 69.2%, respectively, while the GM-ELISA (cut-off index value of ≥0.6) had a sensitivity of 72.7% and specificity of 83.1%. *Aspergillus* precipitating antibody had a test sensitivity of 70%. Given these results, it was concluded that the performance of the *Asp*LFD serum test was acceptable for the diagnosis of CPA as a POCT.

As with ABPA, detection of *Aspergillus*-specific IgG plays and important role in the diagnosis of CPA (Richardson and Page, 2018). There are a number of *Aspergillus*-specific IgG tests available commercially (Page *et al*., 2016; Takazona *et al*., 2019). In a study comparing six of these assays (Page *et al*., 2016), the automated ImmunoCAP fluoroenzyme immunoassay and Immunolite ELISA variant (Page *et al*., 2015) were significantly superior to the other assays (Dynamiker, Genesis, and Serion) in diagnosing CPA, with identical specificities (both 96%) and specificities (both 98%). Precipitin testing performed poorly, as did a recently introduced quantitative serum *Aspergillus fumigatus*-specific IgM assay (Yao *et al*., 2018a). Questions remain concerning the optimum cut-off values for IgG test positivities, with values provided by manufacturer's appearing to be sub-optimal for diagnosis of the disease (Page *et al*., 2016, 2019; Sehgal *et al*., 2018). Despite this, it appears that serial serum testing for *Aspergillus*-specific IgG may provide a useful means of monitoring treatment response and outcome since serum IgG levels decrease during antifungal treatment and resolution (Yao *et al*., 2018b).

The limitations of the commercial *Aspergillus*-specific IgG tests are their specificity for *A. fumigatus*, their expense, and their reliance on automation. Approximately 40% of CPA cases are caused by non-*fumigatus* spp. (e.g. *A. niger* and *A. versicolor*), which these tests would not detect (Tashiro *et al*., 2011). The cost of the tests and their reliance on automated procedures makes them unsuitable for resource-limited settings. While most CPA (and ABPA) patients are diagnosed in high-income countries, prevalence of these diseases is believed to be higher in low- to middle-income countries (Denning *et al*., 2011). Consequently, a test for CPA that requires minimal laboratory equipment, akin to the *Asp*LFD test for IPA, is needed. This need appears to have been met by a novel anti-*Aspergillus* antibody LFA manufactured by LDBIO Diagnostics, with other LFAs for *Aspergillus* immunoglobulins in development (Richardson and Page, 2018). The LDBIO Diagnostics LFA or immuno-chromatographic test (ICT) has recently been evaluated in a multicenter comparison of assays for CPA diagnosis (Piarroux *et al*., 2019). The sensitivity and specificity of the ICT were 88.9% and 96.3%, respectively, compared to 93.1% sensitivity and 94.3% specificity for an anti-*Aspergillus* IgG immunoblot assay. Since the ICT displays good diagnostic performance and complies with the ASSURED (Affordable, Sensitive, Specific, User-friendly, Equipment-free, and Delivered) criteria, it is appropriate for diagnosis of CPA in resource-limited settings. Marked improvements in IPA detection have been met through combination diagnostic testing. As advocated previously (Kobayashi and Thornton, 2014), a similar approach may prove beneficial for CPA diagnosis. A combination of serological and

biomarker tests such as the LDBIO ICT and OLM *Asp*LFD might allow rapid, simple, cheap, but accurate diagnosis of the disease.

Fusarium species pathogenic to humans

The genus *Fusarium* comprises plant, human, and animal pathogenic species with worldwide distribution (Jain *et al*., 2011; Muraosa *et al*., 2017). As a group of organisms, they are a major constraint to food security, not only as pathogen of crop plants, but also as producer of mycotoxins contaminating the food chain (Thornton and Wills, 2015). In humans, *Fusarium* spp. cause a spectrum of diseases including nail infections (onychomycosis)(Arrese *et al*., 1996; Westerberg and Voyack, 2013), bone and joint infections (Koehler *et al*., 2014), infections of the skin and of burn wounds (Nucci and Annaissie, 2002; Latenser, 2003; Gurusidappa and Mamatha, 2011; Muhammed *et al*., 2013; Nucci *et al*., 2013; van Diepeningen *et al*., 2014), mycotic eye infections (fungal keratitis)(Jurkunas *et al*., 2009; He *et al*., 2011), and deep tissue infections following combat-related blast injury (Paolino *et al*., 2012; Warkentien *et al*., 2012; Tribble and Rodriguez, 2014). Up until the 1980s, most reported *Fusarium* infections were to the nails and eyes, or were locally invasive infections. Since then, there has been an increase in the number of cases of life-threatening disseminated disease (fusariosis) in patients with haematological malignancies (García-Ruiz *et al*., 2015), in HIV patients (Esnakula *et al*., 2013), in patients with diabetes and liver cirrhosis (Chen *et al*., 2017), in solid organ transplant recipients (Muhammed *et al*., 2013; Mohanty and Sahu, 2014), and most particularly in allogeneic HSCT recipients (Fanci *et al*., 2013; Scheel *et al*., 2013; Al-Hatmi *et al*., 2016), reflecting their greater immunosuppression and profound and prolonged neutropenia. Fusariosis is now the second most common mould disease of humans after aspergillosis (Guarro, 2013), multidrug-resistant and refractory to treatment (Fanci *et al*., 2013; Al-Hatmi *et al*., 2016), with poor prognosis (Tortorano *et al*., 2014), and with mortality rates reaching 75%.

At least 70 species of *Fusarium* are able to cause infections in humans, and are grouped into species complexes (Guarro, 2013). Most infections in humans are caused by only four species; *F. petroliphilum* and *F. keratoplasticum* in the *Fusarium solani* species complex (FSSC)(Short *et al*., 2013), an unnamed species in the *Fusarium dimerum* species complex (FDSC), and a further unnamed species in the *Fusarium oxysporum* species complex (FOSC). Members of these species complexes are readily isolated from environmental samples, including soil and plant material (Thornton and Wills, 2015), air (Scheel *et al*., 2013), and water systems and plumbing fixtures (Dogget, 2000; Anaissie *et al*., 2002; Mehl and Epstein, 2008; Anaissie *et al*., 2011; Short *et al*., 2011). Indeed, contaminated water systems appear to be an important environmental source of pathogenic fusaria in community-acquired and nosocomial outbreaks of fusariosis, with a recent study showing that hospital and communal sink biofilms are heavily colonised by pathogenic species in the FSSC, FDSC, and FOSC complexes (Al-Maqtoofi and Thornton, 2016), and thus a potential source of infectious propagules.

745 **Detection of *Fusarium* diseases**
746
747 Radiology, histology and culture
748
749 In countries with high rates of fungal keratitis, confocal microscopy of the eye has proved
750 useful in the non-invasive detection of fungal structures (hyphae, pseudo-hyphae, and
751 yeast-like structures) in the infected cornea (Brasnu *et al*., 2007). However, direct
752 microscopy is non-specific, and so is unable to discriminate between the various different
753 fungi (*Aspergillus*, *Candida*, *Fusarium*) capable of causing mycotic infections of the cornea.
754 Radiological findings in CT may be useful in raising the suspicion of a *Fusarium* lung
755 infection, but are non-specific, with findings including alveolar and interstitial infiltrates,
756 nodules and cavities that are typical of other mould infections (Marom *et al*., 2008). In chest
757 CT, nodules and masses were the most common findings, with a halo sign being absent in
758 80% of cases.
759 The ESCMID and ECMM joint guidelines for diagnosis of hyalohyphomycosis strongly
760 recommend histopathology for the diagnosis of fusariosis (Tortorano *et al*., 2014). However,
761 in tissue, the hyphae of *Fusarium* typically resemble those of *Aspergillus*, with hyaline and
762 septate filaments that dichotomise at acute and right angles (Nucci and Anaissie, 2007).
763 Attempts have been made to improve the accuracy of *in situ* detection of *Fusarium* in
764 tissues, but these remain experimental, relying on immunohistochemical procedures that
765 employ cross-reactive antisera (Fukuzawa *et al*., 1995; Kaufman *et al*., 1997; Saito *et al*.,
766 1999), or on molecular detection using in-house PCR assays (Salehi *et al*., 2016). Definitive
767 diagnosis of invasive *Fusarium* infections therefore relies on culture of the fungus from skin
768 biopsies and other infected tissues (sinuses, lungs) or from the blood. Fusariosis is perhaps
769 unique amongst the invasive mould diseases, in that the pathogens' infectious propagules
770 are spread haematogenously, and can be recovered from the bloodstream in up to 82% of
771 cases (Grossman *et al*., 2012; Muhammed *et al*., 2013). In contrast, *Aspergillus* is rarely
772 detected in the blood. Haematogenous spread of *Fusarium* spores accounts for the multiple
773 necrotic skin lesions that develop in 75-90% of disseminated infections (Van Diepeningen *et*
774 *al*., 2015a), a clinical manifestation uncommon in disseminated aspergillosis. Culture from
775 tissue and blood samples enables identification of *Fusarium* based on the crescent or
776 banana-shaped macroconidia characteristic of this genus. However, identification to species
777 level, which is essential for guiding clinical management (Muhammed *et al*., 2011), is
778 difficult and relies on nucleic acid-based tests of tissue specimens or axenic cultures.
779
780 Nucleic acid tests
781
782 Ribosomal RNA gene sequences, and sequencing of the internal transcribed spacer (ITS)
783 region, a procedure widely used for the identification of other mould species, is of limited
784 value for identifying and bar-coding *Fusarium* species due to the presence of duplicated
785 divergent alleles in this region. For this reason, molecular identification of clinical isolates
786 relies on three loci; *TEF-1*α (translation elongation factor-1α)(García-Ruiz *et al*., 2015; Zarrin
787 *et al*., 2016; Muhammed *et al*., 2018), *RPB1* (the largest subunit of RNA polymerase), and
788 *RPB2* (the second largest subunit of RNA polymerase)(Al-Hatmi *et al*., 2016). Based on these
789 loci, DNA sequence databases have been constructed, and can be interrogated at
790 FUSARIUM-ID (http://isolate.fusariumdb.org/guide.php) and at CBS-KNAW
791 (http://www.westerdijkinstitute.nl/fusarium/), for the identification of environmental and

792 clinical isolates. Using a newly generated *Fusarium*-specific monoclonal antibody (ED7) that
793 detects an extracellular antigen (a 200kDa water-soluble carbohydrate), Al-Maqtoofi and
794 Thornton (2016) were able to track pathogenic fusaria in hospital and communal sink
795 biofilms, with FSSC sequence type (ST) 1-a, FOSC ST 33, and FDSC ST ET-gr isolates identified
796 using *TEF-1*α sequencing of recovered strains. This study demonstrated the specificity of
797 mAb ED7, which might find use in the detection of *Fusarium* infections in humans based on
798 detection of the 200kDa biomarker in patient samples. Multi-locus sequence typing (MLST)
799 is currently regarded as the most robust method for differentiating Fusarium species
800 complexes (Wang *et al*., 2011) or identifying isolates to species level (Tortorano *et al*.,
801 2014), with *TEF-1*α and *RPB2* employed in many MLST diagnostic regimens (Van
802 Diepeningen *et al*., 2015b).
803 There are currently no nucleic acid-based detection systems CE-marked or FDA-
804 approved for the diagnosis of human fusariosis, but a number of experimental polymerase
805 chain reaction (PCR) tests have been reported for the detection and quantification of
806 *Fusarium* DNA in murine models of fusariosis, and in human tissue specimens (Bernal-
807 Martínez *et al*., 2012; Muraosa *et al*., 2014; Salehi *et al*., 2016). A duplex RT-PCR using two
808 specific molecular beacon probes targeting a highly conserved region of the rDNA gene was
809 developed for *F. solani* and non-*Fusarium solani* DNA detection, and validated in two mouse
810 models of invasive infection (Bernal-Martínez *et al*., 2012). Specificity of the assay was
811 100%. While sensitivity of the assay in a *F. solani* infection model was 93.9% for lung tissues
812 and 86.7% for serum samples, its sensitivity in a *F. oxysporum* infection model was 87% for
813 lung tissues and 42.8% for serum samples. A *Fusarium* genus-specific and FSSC-specific RT-
814 PCR system has also been developed, which targets the 28s ribosomal RNA gene (Muraosa
815 *et al*., 2014). To apply the RT-PCR system to the molecular diagnosis of fusariosis,
816 performance was evaluated using plasma and whole blood samples from a mouse model of
817 invasive *F. solani* infection. The sensitivity of the RT-PCR system was found to be 100% in
818 plasma, but no amplification was detected in whole blood. In a retrospective multicentre
819 study, Salehi *et al*. (2016) reported the discrimination of fusariosis, aspergillosis,
820 mucormycosis, and scedosporiosis in formalin-fixed paraffin-embedded human tissue
821 specimens using multiple real-time quantitative PCR assays. The qPCR assays, targeting the
822 ITS2 region of ribosomal DNA using fluorescently labelled primers, was used to identify
823 clinically important genera and species of *Fusarium*, *Aspergillus*, *Scedosporium*, and
824 mucormycetes, and the molecular identification compared to results from histological
825 examination. *Fusarium oxysporum* and *F. solani* DNA was amplified from five specimens
826 from patients initially diagnosed by histopathology as having aspergillosis. This study
827 demonstrates that histopathological features of moulds may be easily confused in tissue
828 sections, and that qPCR can be used for the rapid and accurate identification of fungal
829 pathogens to the genus and species levels directly from FFPE tissues. Despite this, molecular
830 tests should be used only to supplement conventional laboratory tests (Tortorano *et al*.,
831 2014).
832
833 Antigen tests
834
835 At present, there are no antigen tests available commercially which are specific for *Fusarium*
836 detection. While (1$\rightarrow$3)-β-D-glucan detection can be used to diagnose fusariosis both in
837 animal models (Khan *et al*., 2008) and in humans, its pan-fungal reactivity means that it
838 lacks specificity. Disconcertingly, cross-reaction of the *Aspergillus* GM-ELISA with *Fusarium*

839 spp. has been reported, with roughly half of patients with fusariosis giving a positive test
840 result with the assay (Tortorano *et al*., 2012). The reason for this cross-reactivity is
841 uncertain, but may be due to the presence of the β-1,5-linked Gal*f* moeities recognised by
842 the GM-reactive mAb EB-A2 being present in polysaccharide antigens from fungi other than
843 *Aspergillus*, including *Fusarium* spp (Tortorano *et al*., 2012). Certainly, galactomannan has
844 been found in *Fusarium* culture supernatants (Tortorano *et al*., 2012; Wiedemann *et al*.,
845 2016). The Platelia *Candida* mannan ELISA has also been reported to cross-react with *F.*
846 *verticillioides*, *F. solani* and *F. oxysporum* (Rimek *et al*., 2003). These findings call into
847 question the validity of these tests for the specific diagnosis of aspergillosis and candidiasis,
848 respectively. Despite this, the GM-ELISA has been evaluated in humans as a means of
849 diagnosing fusariosis and as a monitoring tool for treatment response (Nucci *et al*., 2014),
850 but there is little obvious legitimacy for using this assay for fusariosis detection in the clinical
851 setting given the similar patient groups susceptible to candidiasis, aspergillosis and
852 fusariosis.
853

854 ## ***Pseudallescheria/Scedosporium*** **species complex and** ***Lomentospora***
855 ## ***prolificans***
856

857 Previously, *Pseudallescheria boydii* was considered synonymous with *Pseudallescheria*
858 *angusta*, *Pseudallescheria ellipsoidea*, and *Pseudallescheria fusoidea* (Rainer *et al*., 2000),
859 with a subsequent taxonomic study suggesting that *P. boydii* is a complex comprising six
860 species (*P. boydii*, *P. angusta*, *P. ellipsoidea*, *P. fusoidea*, *P. minutispora*, and *Scedosporium*
861 *aurantiacum*)(Gilgado *et al*., 2005). *Scedosporium apiospermum* along with *Graphium* spp.
862 (*G. capitatum*, *G. eumorphum*, and *G. penicillioides*) were considered to be synanomorphs
863 of *P. boydii* (Rainer *et al*., 2006), with *S. apiospermum* then shown to be a species distinct
864 from *P. boydii* (Gilgado *et al*., 2008), challenging previous doctrine that this fungus was the
865 asexual or anamorphic state of *P. boydii*. Currently, *Pseudallescheria* and *Scedosporium* are
866 regarded as a composite of species, the *Pseudallescheria/Scedosporium* complex (PSC)
867 (Luplertlop, 2018), with the genus *Scedosporium* now comprising *S. aurantiacum*, *S.*
868 *desertorum*, *S. minutisporum, S. cereisporum*, and *S. dehoogii*, in addition to the *S.*
869 *apiospermum* complex consisting of *S. angustum, S. apiospermum*, *S. boydii*, *S. ellipsoidea*,
870 and *S. fusoideum* (Ramirez-Garcia *et al*., 2018). Prior to the advent of molecular taxonomy,
871 the dematiaceous fungus *Scedosporium prolificans* (formerly *S. inflatum*) was believed to be
872 a member of the genus *Scedosporium*, but has since been shown to be unrelated to
873 *Scedosporium*, and has been re-classified as *Lomentospora prolificans* (Hennebert and Desai,
874 1974) with the genus *Lomentospora* being re-instated for this species (Lackner *et al*., 2014).
875 The species *S. apiospermum*, *S. aurantiacum*, and *S. boydii* are pathogenic to humans,
876 causing eumycetoma, localised cutaneous infections, muscle, joint and bone infections, and
877 fatal disseminated infections (Cortez *et al*., 2008; Taylor *et al*., 2014; Kondo *et al*., 2018;
878 Ramirez-Garcia *et al*., 2018), and are consequently the most studied species in the genus.
879 These fungi are soil saprotrophs that appear to have a proclivity for habitats impacted by
880 human activity (Kalteis *et al*., 2009; Rougeron *et al*., 2015). However, the evidence for this
881 association is questionable, since these organisms are also readily isolated from habitats
882 such as brackish waters and estuarine muds (Thornton, 2009). Notwithstanding this, the
883 numerous reports of these fungi as agents of disease in near-drowning events and following
884 natural disasters such as tsunamis due to aspiration of soil-laden water (Garzoni *et al*., 2005;

885 Leroy and Smismans, 2007; Linscott, 2007; Nakamura *et al*., 2011, 2013; Angelina *et al*.,
886 2013; Nakamura *et al*., 2013), demonstrates their abundance in the natural environment,
887 and their ability to cause life-threatening central nervous system (CNS) and cerebral
888 infections following traumatic implant of infectious propagules in the lungs (Ramirez-Garcia
889 *et al*., 2017). Less is known about the ecology of *Lomentospora prolificans*, but it is a soil-
890 and compost-borne fungus (Hennebert and Desai, 1974; Grantina-Ievina *et al*., 2013), and is
891 present, alongside members of the PSC, in estuarine muds (Thornton *et al*., 2015).
892 Pathogens in the PSC complex and *L. prolificans* are persistent colonisers of the lungs of
893 cystic fibrosis (CF) patients, and are the second most frequent moulds after *A. fumigatus* in
894 the sputum of CF patients and those with underlying respiratory diseases (Cimon *et al*.,
895 2000; Cooley *et al*., 2007; Blyth *et al*., 2010a,b; Sedlacek *et al*., 2015; Schwarz *et al*., 2015;
896 Schwarz *et al*., 2017; Tomazin and Matos, 2017; Bouchara *et al*., 2018; Schwarz *et al*., 2018).
897 While chronic airway colonisation of the lung by these fungi can lead to fatal outcomes in CF
898 patients (Borghi *et al*., 2010), the role of these fungi in CF exacerbation, other than causing
899 acute obstruction (Padoan *et al*., 2016), is not fully understood. Nothwithstanding this, a
900 trait common to all strains isolated from the CF lung is their high resistance to antifungal
901 drugs, with *L. prolificans* demonstrating multi-drug resistance (Borghi *et al*., 2010; Sedlacek
902 *et al*., 2015), and successful treatment of lung infections requiring triple antifungal drug
903 regimes in several cases (Schwarz *et al*., 2018). Important risk factors for fatal disseminated
904 infections (scedosporiosis and lomentosporiosis) include neutropenia and haematological
905 malignancy (Maertens *et al*., 2000; Rodriguez-Tudela *et al*., 2009; Song *et al*., 2009),
906 advanced AIDS (Tammer *et al*., 2011), chronic granulomatous disease (Santos *et al*., 2000),
907 allogeneic stem cell transplantation (Tamaki *et al*., 2016), and solid organ transplantation
908 (Husain *et al*., 2005). Malignancy, fungemia, central nervous system (CNS) and lung
909 involvement are prognostic factors predicting worse outcome for scedosporiosis and
910 lomentosporiosis (Seidel *et al*., 2019). Disseminated infections following lung
911 transplantation are particularly problematic (Vagefi *et al*., 2005; Morio *et al*., 2010; Rolfe *et*
912 *al*., 2013), with a *S. apiospermum* and *L. prolificans* mixed disseminated infection reported
913 recently (Balandin *et al*., 2016).
914
915 **Detection of *Scedosporium* and *Lomentospora* diseases**
916
917 Radiology, histology and culture
918
919 *Scedosporium* and *Lomentospora* infections are often clinically indistinguishable from other
920 invasive mould infections, with radiology based on chest CT providing little diagnostic worth
921 due to non-specific indicators of fungal lung infection (Dabén *et al*., 2008; Holmes *et al*.,
922 2013; Nakamura *et al*., 2013; Schwarz *et al*., 2015). In contrast, brain MRI is an important
923 diagnostic modality for detecting disseminated CNS infections in immunosuppressed stem
924 cell and solid organ transplant recipients, and in victims of near-drowning, particularly in
925 cases where antifungal treatment fails to resolve disease, or where a clear spreading focus
926 is absent (Morio *et al*., 2010; Bhuta *et al*., 2012; Holmes *et al*., 2013; Nakamura *et al*., 2013;
927 Sharma and Singh, 2015; Tamaki *et al*., 2016; Ramirez-Garcia *et al*., 2018).
928 Histopathological examination of biopsy material has limited diagnostic utility as it is
929 difficult to distinguish *Scedosporium*/*Lomentospora*-infected tissues from those infected
930 with other hyaline fungi such as *Aspergillus* and *Fusarium* species (Ramirez-Garcia *et al*.,
931 2018), although the dematiaceous (melanised) hyphae of *L. prolificans* may provide an

932 opportunity for species differentiation. Immunohistological procedures using polyclonal
933 fluorescent antibodies have attempted to improve the accuracy of *in situ* detection
934 (Kaufman *et al*., 1997), but cross-reaction with antigens from other fungi such as *Aspergillus*
935 hamper their usefulness. For these reasons, diagnosis of scedosporiosis and
936 lomentosporosis relies on the recovery of the etiological agent from clinical specimens and
937 identification using macroscopic and microscopic features (Ramsperger *et al*., 2014; Luna-
938 Rodríguez *et al*., 2019) *in vitro*. As with *Fusarium* species, accurate differentiation of species
939 is critical for guiding clinical management due to variable antifungal susceptibilities (Gilgado
940 *et al*., 2006; Cortez *et al*., 2008; Box *et al*., 2018). While *Scedosporium* and *Lomentospora*
941 species can be recovered from sterile sites such as blood, bone and tissue biopsy specimens
942 using standard mycological media, for example Saboraud's dextrose agar (SDA), their
943 frequent occurrence in sputum samples of CF patients (Sedlacek *et al*., 2015; Chen *et al*.,
944 2017) necessitated the development of selective media that prevent overgrowth by faster
945 growing *Aspergillus*, *Candida* and *Fusarium* species in polymicrobial clinical samples (Rainer
946 *et al*., 2008; Blyth *et al*., 2010; Hong *et al*., 2016). One of the most effective media for
947 selective isolation of *Scedosporium* species is *Scedosporium* Selective agar (SceSel+)(Rainer
948 *et al*., 2008; Blyth *et al*., 2010) containing the antifungal agents benomyl and dichloran that
949 limit the growth of *Aspergillus* and *Candida*. *Scedosporium* species and *L. prolificans* can
950 then be further differentiated in culture with the inclusion of cycloheximide that is
951 inhibitory to *L. prolificans* (Ramsperger *et al*., 2014).
952
953 Nucleic acid tests
954
955 Speciation of isolates within the *Pseudallescheria*/*Scedosporium* complex and their
956 discrimination from *L. prolificans* based on microscopic features requires considerable
957 expertise and can be problematic. For this reason, a number of molecular methods have
958 been developed for the detection and differentiation of these fungi in axenic culture,
959 sputum, and tissue samples including multiplex PCR (Harun *et al*., 2011); repetitive
960 sequence-based PCR (rep-PCR)(Steinmann *et al*., 2011); qPCR, loop-mediated isothermal
961 amplification (LAMP) and PCR-based reverse line blot (PCR-RLB)(Lu *et al*., 2011a,b);
962 oligonucleotide arrays (Bouchara *et al*., 2009); DNA sequencing and restriction fragment
963 length polymorphisms (RFLP)(Delhaes *et al*., 2008; Gilgado *et al*., 2005, 2008), and Rolling
964 Circle Amplification (RCA)(Lackner *et al*., 2012). These techniques and their different
965 capabilities are comprehensively reviewed elsewhere (Ramsperger *et al*., 2014; Ramirez-
966 Garcia *et al*., 2018). Despite these myriad methods for pathogen detection and speciation,
967 no single technique has been universally adopted nor are any commercial tests currently
968 recommended for this purpose. Current guidelines recommend that nucleic acid-based
969 assays be used in combination with conventional laboratory tests (Tortorano *et al*., 2014).
970
971 Proteomics and antigen tests
972
973 Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-
974 TOF/MS) has been investigated as an alternative strategy for identification of fungi within
975 the PSC (Coulibaly *et al*., 2011), and other pathogenic moulds (Santos *et al*., 2010; Cassagne
976 *et al*., 2011; Bader, 2013; Lau *et al*., 2013). This technique currently lacks the requisite
977 standardisation needed for clinical acceptability in medical mycology, but it nevertheless
978 has the potential to replace gene-sequencing methods for mould identification, as it is

979 accurate, comparatively inexpensive and relatively quick compared to sequencing methods.
980 However, it does require robust online reference databases constructed using similar fungal
981 growth and extraction procedures for interrogation of MS spectra from unknown species.
982 Commercially available MALDI-TOF/MS identification solutions are apparently inadequate
983 for *Scedosporium*/*Lomentospora* discrimination at present (Ramirez-Garcia *et al*., 2018).
984 Mass spectrometry (MS) has also recently used by Thornton *et al*. (2015) to identify the
985 antigen bound by a mAb (CA4) specific to *L. prolificans*. This IgG1 mAb is highly specific,
986 binding to an intracellular protein from *L. prolificans*, but not with proteins extracted from
987 members of the PSC or from other unrelated moulds. CA4-reactive peptides in western
988 blots of cell extracts following two-dimensional electrophoresis were identified using MS as
989 fragments of the melanin-biosynthetic enzyme tetrahydroxynaphthalene reductase (4HNR).
990 This enzyme is one of a number of enzymes involved in DHN-melanin production of
991 dematiaceous moulds such as *L. prolificans* (Al-Laaeiby *et al*., 2016). Confirmation of the CA4
992 antigen as the enzyme 4HNR was established using genome sequencing and targeted
993 disruption of the 4HNR-encoding gene. Mutants lacking the gene, and with abnormal
994 pigmentation (orange-brown spores compared to the black spores of the wild type strain),
995 were enzyme-deficient and no longer reactive with mAb CA4.
996 The study of Thornton *et al*. (2015) further demonstrates the power of hybridoma
997 technology to develop mAbs specific to pathogenic moulds, but the generation of a species-
998 specific mAb to an intracellular antigen is unusual. The majority of genus or species-specific
999 mAbs generated against cell extracts from pathogenic yeasts, yeast-like fungi, and moulds,
1000 bind to carbohydrate moieities on extracellular carbohydrate or glycoprotein antigens
1001 (Thornton *et al*., 2002; Thornton, 2009; Davies and Thornton, 2014; Al-Maqtoofi and
1002 Thornton, 2016; Davies *et al*., 2017; Morad *et al*., 2018). This is the case of mAbs specific to
1003 members of the PSC. Thornton (2009) reported the development of IgG1 (clone HG12) and
1004 IgM (clone GA3) mAbs specific to a heat-stable, water-soluble, 120kDa carbohydrate
1005 present on the surface of conidia and hyphae of *S. apiospermum*, *S. aurantiacum*, and *S.*
1006 *boydii*. The antigen is also readily detectable as an extracellular antigen that is produced in
1007 abundance during active growth of these pathogens. The mAbs do not react with the
1008 related fungus *S. dehoogii*, with *L. prolificans*, or other unrelated human pathogenic fungi.
1009 The copious production of the 120kDa antigen and the availability of highly specific mAbs
1010 for antigen detection, make this an ideal system for the development of a diagnostic test for
1011 members of the PSC. To this end, we have developed a lateral-flow assay (*Sced*-LFA) for PSC
1012 detection that comprises the IgG1 mAb HG12 (Figure 1), and which demonstrates the
1013 feasibility of developing a POCT for rapid detection of pathogens in the PSC. In addition, we
1014 have developed a quantitative sandwich ELISA that combines both mAbs HG12 and GA3.
1015 This ELISA was recently used by Box *et al*. (2018) to determine the pharmacodynamics of
1016 voriconazole in *in vitro* models of invasive pulmonary scedosporiosis. Given the recent CE-
1017 marking and commercial availability of the *Asp*LFD (OLM Diagnostics) and *Aspergillus* GP-
1018 ELISA (Euroimmun AG) which both employ the *Aspergillus*-specific mAb JF5 (Thornton,
1019 2009), the development of complementary qualitative LFA and quantitative ELISA tests for
1020 the diagnosis of scedosporiosis, based on detection of the 120kDa in human BALf and serum
1021 samples, is technically feasible.

1022

1023

1024

1025 ## Mucormycete species pathogenic to humans

1026

1027 Mucormycetes are thermotolerant, fast-growing saprotrophic moulds of the phylum
1028 Zygomycota order Mucorales*,* which are abundant in a wide range of ecological niches
1029 including soil and compost (Richardson, 2009), food (e.g. cheese where they cause spoilage
1030 (Hymery *et al*., 2014)), edible crops where they cause post-harvest storage rots (Saito *et al*.,
1031 2016), and household dust (Al-Humiany, 2010). The order Mucorales includes species in the
1032 genus *Apophysomyces*, *Cunninghamella*, *Lichtheimia*, *Mucor*, *Rhizomucor*, *Rhizopus,* and
1033 *Saksenaea*, which are separated from the entomophthoromycetes *Basiodiobolus* and
1034 *Condiobolus*, zygomycete fungi that are also capable of causing disease in humans (Binder *et*
1035 *al*., 2014; Ribes *et al*., 2000). While mucormycetes such as *Rhizopus oryzae* (syn. *Rhizopus*
1036 *arrhizus*) and *Lichtheimia corymbifera* can cause allergic reactions in humans (Sircar *et al*.,
1037 2015; Rognon *et al*., 2016), it is the ability of these fungi to cause osteoarticular mycosis
1038 (Taj-Aldeen *et al*., 2017), and rhino-orbital-cerebral (Arndt *et al*., 2009; Son *et al*., 2016), oral
1039 (Hingad *et al*., 2012), pulmonary (Hung *et al*., 2015; Wang *et al*., 2016; Iqbal *et al*., 2017),
1040 gastrointestinal (Adhikari *et al*., 2019), cutaneous (Li *et al*., 2013; Arruebarrena *et al*., 2016;
1041 Zahoor *et al*., 2016) or disseminated infections (Walsh *et al*., 2012) in pre-disposed adult
1042 and paediatric patients (Däbritz *et al*., 2011) that is of the greatest concern, with a single
1043 species, *Rhizopus oryzae* responsible for >80% of all life-threatening infections (Richardson,
1044 2009; Richardson and Page, 2018; Raghunath *et al*., 2019). This fungus and other pathogenic
1045 Mucorales (predominantly *Lichtheimia, Mucor*, and *Rhizomucor*) differ from other
1046 opportunistic moulds in their ability to infect a broader, more varied population of human
1047 hosts (Richardson, 2009; Gomes *et al*., 2011).
1048 Cutaneous and deep tissue infections typically occur by direct inoculation during
1049 trauma, such as following combat-related blast injury (Paolino *et al*., 2012; Warkentien *et*
1050 *al*., 2012; Tribble and Rodriguez, 2014; Kronen *et al*., 2017), natural disasters (Andresen *et*
1051 *al*., 2005; Fanfair *et al*., 2012), or traffic accidents (Lelievre *et al*., 2014), but are also
1052 reported in patients with diabetes or haematological malignancies (Arnáiz-García *et al*.,
1053 2009). Reports of disseminated mucormycosis, while rare, are increasing in incidence
1054 particularly in patients with uncontrolled diabetes, iron overload, and ketoacidosis (Binder
1055 *et al*., 2014; Rammaert *et al*., 2012), and also in patients with haematological malignancies
1056 and allogeneic stem cell transplants (Petrikkos *et al*., 2012; Sugui *et al*., 2011).

1057

1058 ### Detection of mucormycosis

1059

1060 Radiology, histology and culture

1061

1062 The aggressive and rapid course of mucormycete infections makes early detection critically
1063 important for the management both of localised cutaneous, deep-seated, and disseminated
1064 infections (Castrejón-Pérez *et al*., 2017). This is especially important in the context of their
1065 intrinsic resistance to short-tailed mould-active azole drugs (voriconazole (VCZ) and
1066 fluconazole) due to an evolutionary conserved amino acid substitution in the enzyme
1067 lanosterol 14α-demethylase responsible for ergosterol biosynthesis (Caramalho *et al*.,
1068 2017). This has led to an increased incidence of breakthrough infections amongst
1069 immunocompromised and high-risk patients receiving VCZ prophylaxis, with the suggestion

1070 that VCZ prophylaxis should be thought of as one of the risk factors for mucormycosis
1071 (Ustun *et al*., 2007).
1072 As with aspergillosis, fusariosis, and scedosporiosis, abnormalities observed in a chest-
1073 CT consistent with angio-invasive mould infections (nodules, halo signs, cavities, wedge-
1074 shaped infiltrates, and pleurar effusions) are not pathognomonic of pulmonary
1075 mucormycosis, and so can only raise the suspicion of mucormycosis in the context of a
1076 patient's clinical history. Despite this, the reversed halo sign in neutropenic patients with
1077 haematological malignancies appears to have high predictive value for mucormycosis
1078 (Legouge *et al*., 2014; Walsh *et al*., 2014). Computed tomography has also proven useful in
1079 the differential diagnosis of rhino-orbital-cerebral mucormycosis (ROCM) and bacterial
1080 orbital cellulitis, where the early symptoms of both diseases are similar (Son *et al*., 2016).
1081 However, it is unable to discriminate between ROCM and aspergillosis in cases of invasive
1082 fungal rhinosinusitis (Arndt *et al*., 2009). Culture of mucormycetes from biopsy samples
1083 (Hong and Park, 2017b) is problematic due to the fragile nature of mucormycete hyphae
1084 that are aseptate or pauci-septate (Skiada *et al*., 2013). Growth of mucormycetes in culture
1085 is rapid, but identification to the level of species and even genus requires considerable
1086 mycological expertise (Ziaee *et al*., 2016). As with other pathogenic moulds, MALDI-TOF/MS
1087 can be used to differentiate species once in axenic culture (Cornely *et al*., 2014; Cassagne *et*
1088 *al*., 2011; De Carolis *et al*., 2012; Schrodl *et al*., 2012), but more data are needed to
1089 authenticate this technique, and the robustness of reference databases needs to be fully
1090 evaluated.
1091 *In situ* detection of mucormycetes in tissue samples relies on the microscopic
1092 observation of characteristic broad ribbon-like hyphae that can be stained with optical
1093 brighteners such as calcofluor white or with Grocott-Gomori methamine silver (Lass-Flörl,
1094 2009; Antonov *et al*., 2015; Liao *et al*., 2015). Sputum and BALf samples can also show the
1095 characteristic broad aseptate hyphae (Glazer *et al*., 2000), which is a first indicator of
1096 mucormycosis (Al-Abbadi *et al*., 1997). However, in one case study, only 25% of sputum or
1097 BALf samples were positive pre-mortem (Kontoyiannis *et al*., 2000). Attempts have been
1098 made to improve the specificity and sensitivity of microscopy by using mucormycete-
1099 reactive antibodies in immunohistochemistry. The production of rabbit hyper-immune
1100 antisera and mAbs to immuno-dominant intracellular antigens of the Mucorales was
1101 reported by Jensen *et al*. (1994, 1996). Jensen *et al*. (1994) demonstrated that heterologous
1102 absorption rendered antisera, raised against *Rhizopus oryzae* somatic antigens,
1103 monospecific by indirect immunofluorescence, whereas heterologous absorption of
1104 *Lichtheimia corymbifera* antiserum did not abolish reactivity with *R. oryzae*. The reactivity of
1105 the heterologously absorbed antisera, and a murine IgG1 mAb (1A7B4) raised against *L.*
1106 *corymbifera*, enabled mucormycetes within bovine lesions to be identified by
1107 immunohistochemistry. In a subsequent study, Jensen *et al*. (1996) showed that a mAb
1108 (WSSA-RA-1), raised against water-soluble somatic antigens from *R. oryzae*, similarly bound
1109 to intracellular homologous antigens of between 14kDa and 110kDa. The high degree of
1110 specificity exhibited by mAb WSSA-RA-1 allowed its use in immunohistochemistry to detect
1111 the pathogen *in situ* in placenta, lymph node and gastrointestinal tract samples of cows with
1112 suspected systemic bovine zygomycosis. In a subsequent study (Jensen *et al*., 1997), the
1113 same mAb (WSSA-RA-1) was used in immunohistochemistry to impove the sensitivity and
1114 specificity of mucormycosis detection in patients with haematological malignancies.
1115
1116

1117 Nucleic acid tests
1118
1119 Identification of mucoralean fungi in culture based on morphological characteristics is
1120 notoriously difficult, particularly with strains that fail to sporulate. For this reason, nucleic
1121 acid-based procedures have been developed that enable discrimination of Mucorales at
1122 both genus- and species-level, although evidence that identification beyond genus-level aids
1123 treatment is lacking. Unlike *Fusarium*, sequencing of the internal transcribed spacer regions
1124 ITS1 and ITS2 of ribosomal RNA gene (rDNA) has been shown to be a reliable method for
1125 species differentiation in the mucormycetes (Gade *et al*., 2017), with ITS sequencing
1126 recommended by ISHAM as a first-line method of identification (Balajee *et al*., 2009).
1127 Numerous in-house molecular assays have been developed for direct detection of
1128 mucoralean DNA in clinical samples (Skiada *et al*., 2018), including BALf (Lengerova *et al*.,
1129 2014), serum (Millon *et al*., 2016), and biopsy (skin, lung, stomach mediastine, nose, parotid
1130 gland) samples (Bernal-Martínez *et al*., 2012). However, their current lack of standardisation
1131 means that they are only moderately supported in the clinical guidelines for the diagnosis of
1132 mucormycosis (Cornely *et al*., 2014; Skiada *et al*., 2018). Despite this, PCR has proved useful
1133 in the detection of mucormycete infections, especially in infections that are culture-negative
1134 (Hammond *et al*., 2011). Lengerova *et al*. (2014) used PCR followed by high-resolution melt
1135 analysis (PCR/HRMA) to detect *Rhizopus* spp., *Rhizomucor pusillus*, *Lichtheimia corymbifera*,
1136 and *Mucor* spp. in BALf samples from immunocompromised patients who were at risk of
1137 invasive fungal disease. Real-time qPCR assays specific to the species identified in the
1138 PCR/HMRA test were used to validate the test. Of the 99 BALF samples collected from 86
1139 patients with pulmonary abnormalities, 91% were negative and 9% were positive in the
1140 PCR/HMRA test, with a sensitivity and specificity 100% and 93%, respectively. By combining
1141 the postive PCR/HRMA results with positive real-time qPCR results, the specificity was
1142 increase to 98%. Due to its high NPV of 99%, the PCR/HRMA test is a fast and reliable
1143 technique for differential diagnosis of pulmonary mucormycosis in immunocompromised
1144 patients caused by the four most clinically important mucormycetes.
1145 Because of the difficulty in obtaining fungal DNA from human fluids, the possibility of
1146 detecting mucormycete DNA in paraffin-embedded and fresh tissues has been investigated
1147 in both an experimental model of disseminated mucormycosis (Dannaoui *et al*., 2010), and
1148 also in humans (Gade *et al*., 2017). The universal primers ITS1 and ITS2 were used in PCR to
1149 amplify the ITS1 region from mucormycete DNA in formalin-fixed paraffin-embedded kidney
1150 and brain tissues from mice infected with one of five species (*Rhizopus oryzae*, *Rhizopus*
1151 *microsporus*, *Lichtheimia corymbifera*, *Rhizomucor pusillus*, and *Mucor circinelloides*).
1152 Amplicons were then sequenced for species identification. Using this procedure,
1153 identification of major mucormycete species from formalin-fixed paraffin-embedded could
1154 reach 100%, provided sufficient starting material was available for DNA extraction. Gade *et*
1155 *al*. (2017) investigated the utility of a PCR targeting a 200-300 bp region of the extended 28S
1156 region of rDNA for molecular identification of DNA from mucormycetes and other fungi in
1157 formalin-fixed paraffin-embedded and fresh tissues. They demonstrated this region could
1158 be used to identify all genera and some species of clinically relevant mucormycetes. They
1159 also demonstrated that PCR amplification and direct sequencing of the extended 28S region
1160 of rDNA was more sensitive compared to targeting the ITS2 region, since they were able to
1161 detect and identify mucormycetes and other fungal pathogens in tissues from patients with
1162 histopathological and/or culture evidence of fungal infections that were negative in PCR
1163 using ITS-specific primers.

1164 Antibody and antigen tests
1165
1166 While there are no routine serological tests for diagnosis of disseminated mucormycosis,
1167 ELISA has been used to study the relationship between antibody levels and exposure to
1168 *Rhizopus microsporus* in sawmill workers with allergic alveolitis (Sandven and Eduard, 1992),
1169 while recombinant antigens have been developed for the serodiagnosis of farmer's lung
1170 caused by *Lichtheimia corymbifera* (Rognon *et al*., 2016). Detection of disseminated
1171 mucormycosis is not possible using tests for (1→3)-β-D-glucan since mucormycetes lack this
1172 carbohydrate in their cell walls (Cornely *et al*., 2014), but it can be used to rule out IPA, the
1173 most frequent differential diagnosis, or combined *Aspergillus* and Mucorales infections
1174 (Skiada *et al*., 2013). Early attempts to detect disseminated disease serologically were based
1175 on immunodiffusion (Jones and Kaufman, 1978), electrophoresis and immunoblotting
1176 (Wysong and Waldorf, 1987), and ELISA (Kaufman *et al*., 1989) of homogenate antigens
1177 challenged with sera from patients with mucormycete infections. However, non-specific
1178 reactivity with the antigen preparations was observed with sera from patients with
1179 aspergillosis and candidiasis also, which meant that the tests were unable to generically or
1180 specifically identify the etiologic agents of mucormycosis.
1181 The immunological determinant of mucoralean intracellular glycans has been shown to
1182 comprise linear (1→6)-α-linked mannopyrasonyl residues (Miyazaki *et al*., 1979), with the
1183 cell walls of yeast and hyphal phases of mucoralean fungi containing fucomannopeptide and
1184 mannoprotein (Yamada *et al*., 1982, 1983). While these antigens have provided useful
1185 targets for direct visualisation of mucoralean hyphae in tissue samples using
1186 immunohistochemistry, their intracellular nature means that they have limited applicability
1187 as circulating biomarkers of mucormycosis in BALf and serum samples. Approaches have
1188 been used to identify extracellular antigens specific to the Mucorales (Hessian & Smith
1189 1982), which might circulate in the bloodstream and therefore be suitable targets for
1190 detection by, for example, ELISA. Extracellular polysaccharides (EPS) of the Mucorales that
1191 appear, using polyclonal IgG antibodies, to be specific to this group of fungi, have been
1192 isolated and characterised (De Ruiter *et al*., 1991; 1992a,b, 1993, 1994; Notermans and
1193 Soentoro, 1986), and their immunodominant residues shown to comprise 2-*O*-methyl-D-
1194 mannose (De Ruiter *et al*., 1994).
1195 Despite these extensive early studies, and despite our improved understanding of the
1196 polysaccharide cell wall architecture of mucoralean fungi (Lecointe *et al*., 2019), no genus-
1197 or species-specific mAbs are currently available that target immunogenic EPS, and which
1198 might prove suitable for the development of immunoassays for diagnosis of mucormycosis
1199 in humans. Notwithstanding this, somatic antigens diagnostic of systemic *L. corymbifera*
1200 infections have been detected in the urine of cattle (Jensen *et al*., 1993b), indicating that
1201 biomarkers of mucormycosis circulate in the bloodstream and are excreted. This raises the
1202 possibility of developing a non-invasive test for mucormycosis based on mucormycete
1203 antigenuria, provided that suitable targets can be identified and specific mAbs can be
1204 generated.
1205
1206 # Conclusions and Future Prospects
1207
1208 One of the main priorities of the Global Action Fund for Fungal Infections (GAFFI) is
1209 availability and access of cheap and effective diagnostic tests for all, particularly those in

poorly-resourced countries. A commendable priority, but one littered with obstacles. Technically, as this review has shown, it is possible to develop highly specific and sensitive assays for the detection of some of the most problematic of human pathogens, the filamentous fungi or moulds. Furthermore, it is possible to demonstrate the clinical utility of these tests in retrospective or semi-prospectve studies. However, the vast majority of these tests remain at the experimental stage in the laboratory, with little prospect of translation to the clinic as commercially-available CE-marked or FDA-approved assays. One of the biggest obstacles to bench-to-bedside translation is the escalating costs for the regulatory approvals required at each of the different technical and commercial phases of a test's development, and during its registration as an *in vitro* medical device (IVD).

As Figure 1 and the accompanying text demonstrates, the generation of an immunoassay, a lateral-flow assay (LFA), that encompasses many of the desirable qualities of a near-patient point-of-care test for a human pathogenic fungus (short-time to result; inexpensive equipment/instrumentation; suitable for use by personnel with limited training; minimal number of steps; uncomplicated interpretation (Kozel and Wickes, 2014; Wickes and Wiederhold, 2018)) is relatively simple given the availability of a high quality monoclonal antibody (in this case a well-characterised mAb that detects a water-soluble, heat-stable, carbohydrate antigen specific to pathogens in the *Pseudallescheria*/*Scedosporium* complex (Thornton, 2009)), and the technical know-how of how to apply it to LFA technology. Production of this demonstration test took a month to complete, including antibody purification and conjugation, LFA formatting, and determination of specificity and analytical sensitivity. The limit of detection of purified antigen is 3.75ng/ml (Figure 1), which is well within the level of analytical sensitivity required for clinical detection of a circulating antigen in human samples. Despite this, the demand for a PSC-specific test is insufficient to warrant further commercial development of the *Sced*-LFA, despite the importance of this group of fungi in human disease.

To take a test such as the *Sced*-LFA from the demonstration phase through to prototyping and then into test validation and verification with an FDA-approved manufacturer, requires investment beyond the capabilities of many diagnostic companies, certainly beyond a small to mid-size enterprise (SME), without a cast-iron guarantee of financial return and profit within a short period of time post CE-marking or FDA approval. This guarantee is unlikely to be met with tests for emerging or rare pathogens such as *Fusarium*, *Lomentospora*, *Scedosporium*, or the mucormycetes, especially given the investment and time required for CE-marking and FDA approval of an IVD, and subsequent integration of a novel test into hospital diagnostic workstreams. With every hospital or regional healthcare authority demanding in-house validation of a test's performance compared to its own established diagnostic procedures, it can typically take several years before a test is widely accepted, and is purchased in sufficient numbers so as to justify the developer's costs for R&D, manufacture, marketing, sales and distribution of the test. These same principles apply to any diagnostic test, whether it be an antigen test such as the *Sced*-LFA, or a molecular diagnostic test for fungal DNA (Wickes and Wiederhold, 2018).

If GAFFI's desire for cheap and effective diagnostic tests for all is to be met, there needs to be a major re-think in the way that we, as a community of medical mycologists, diagnosticians, and medical practitioners, work together to better support the diagnostics sector to facilitate the development and translation of novel fungal diagnostics to the clinical setting. Without this community support, and without much-needed financial assistance from government funding agencies who have traditionally paid insufficient

attention to human mycoses and their detection, the availability of cheap, easy-to-use, and effective diagnostic tests will be limited to those pathogens which infect sufficiently large number of individuals, such as *Aspergillus*, *Cryptococcus*, and *Pneumocystis*, to warrant diagnostic test development. An exemplar of where community and funding agency support worked to the patient's advantage was the Aspergillus Technology Consortium (AsTeC) for aspergillosis clinical laboratory diagnostics. A similar approach needs to be adopted for other invasive mould diseases.

Acknowledgments

This work was carried out, in part, during tenure of a grant from Innovate UK (reference number 105440). The author would like to thank Richard Campbell of LateralDx for supply of the image in Figure 1B.

References

Abdolrasouli, A., Scourfield, A., Rhodes, J., Shah, A., Elborn, J. S., Fisher, M. C., Schelenz, S., and Armstrong-James, D. (2018). High prevalence of triazole resistance in *Aspergillus fumigatus* isolates in a specialist cardiothoracic centre. *Int. J. Antimicrob. Agents* **52,** 637-642.

Adhikari, S., Gautam, A. R., Paudyal, B., Sigdel, K. R., and Basnyat, B. (2019). Case report: gastric mucormycosis - a rare but important differential diagnosis of upper gastrointestinal bleeding in an area of *Helicobacter pylori* endemicity. *Wellcome Open Research* **4,** 5.

Agarwal, R., Chakrabati, A., Shah, A., Gupta, D., Meis, J. F., Guleria, R., Moss, R., Denning, D. W., and For the ABPA complicating asthma ISHAM working group. (2013). Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin. Exp. Allergy* **43,** 850-873.

Alghamdi, N.S., Barton, R., Wilcox, M., and Peckham, D. (2019). Serum IgE and IgG reactivity to *Aspergillus* recombinant antigens in patients with cystic fibrosis. *J. Med. Microbiol*. **68,** 924-929.

Al-Abbadi, M. A., Russo, K., and Wilkinson, E. J. (1997). Pulmonary mucormycosis diagnosed by bronchoalveolar lavage: a case report and review of the literature. *Pediatr. Pulmonol*. **23,** 222-225.

Al-Hatmi, A. M. S., Hagen, F., Menken, S. B. J., Meis, J. F., and de Hoog, G. S. (2016). Global molecular epidemiology and genetic diversity of *Fusarium*, a significant emerging group of human opportunists from 1958 to 2015. *Emerg. Microbes. Infect*. **5,** e124.

Al-Humiany, A. A. (2010). Opportunistic pathogenic fungi of the house dust in Turubah, Kingdom of Saudia Arabia. *Aust. J. Basic and Appl. Sci*. **4,** 122-126.

Al-Laaeiby, A., Kershaw, M. J., Penn, T. J., and Thornton, C. R. (2016). Targeted disruption of melanin biosynthesis genes in the human pathogenic fungus *Lomentospora prolificans* and its consequences for pathogen survival. *Int. J. Mol. Sci*. **17,** 444.

Al-Maqtoofi, M., and Thornton, C. R. (2016). Detection of human pathogenic *Fusarium* species in hospital and communal sink biofilms by using a highly specific monoclonal antibody. *Environ. Microbiol*. **18,** 3620-3634.

1302 Anaissie, E. J., Penzak, S. R., and Dignani, M. C. (2002). The hospital water supply as a source
1303 of nosocomial infections: a plea for action. *Arch. Intern. Med.* **162,** 1483-1492.
1304 Anaissie, E. J., Kuchar, R. T., Rex, J. H., Francesconi, A., Kasai, M., Müller, F-M. C., Mario, L-C.,
1305 Summerbell, R. C., Dignani, M. C., Chanock, S. J., and Walsh, T. J. (2001). Fusariosis
1306 associated with pathogenic *Fusarium* species colonisation of a hospital water system: a
1307 new paradigm for the epidemiology of opportunistic mold infections. *Clin. Infect. Dis*.
1308 **33,** 1871-1878.
1309 Andresen, D., Donaldson, A., Choo, L., Knox, A., Klaasen, M., Ursic, C., Vonthethoff, L., Krilis,
1310 S., and Konecny, P. (2005). Multifocal cutaneous mucormycosis complicating
1311 polymicrobial wound infection in a tsunami survivor from Sri Lanka. *Lancet* **365,** 876-
1312 878.
1313 Angelini, A., Drago, G., and Ruggieri, P. (2013). Post-tsunami primary *Scedosporium*
1314 *apiospermum* osteomyelitis of the knee in an immunocompetent patient. *Int. J. Infect.*
1315 *Dis*. **17,** e646-e649.
1316 Ansorg, R., von Heinegg, E. H., and Rath, P. M. (1994). *Aspergillus* antigenuria compared to
1317 antigenemia in bome marrow transplant recipients. *Eur. J. Clin. Microbiol. Infect. Dis*.
1318 **13,** 582-589.
1319 Antonov, N. K., Tang, R., and Grossman, M. E. (2015). Utility of touch preparation for rapid
1320 diagnosis of cutaneous mucormycosis. *JAAD Case Reports* **1,** 175-177.
1321 Aogáin, M. M., Chandrasekaran, R., Lim, A. Y. H., Low, T. B., Tan, G. L., Hassan, T., Ong, T. H.,
1322 Ng, A. H. Q., Bertrand, D., Koh, J. Y., Pang, S. L., Lee, Z. Y., *et al*. (2018). Immunological
1323 corollary of the pulmonary microbiome in bronchiectasis: the CAMEB study. *Eur. Respir.*
1324 *J.* **52,** 1800766.
1325 Arrese, J. E., Piérard-Franchimont, C., and Piérard, G. E. (1996). Fatal hyalohyphomycosis
1326 following *Fusarium* onychomycosis in an immunocompromised patient. *Am. J.*
1327 *Dermatopathol*. **18,** 196-198.
1328 Arnáiz-García, M. E., Alonso-Peña, D., del Carmen González-Vela, M., García-Palomo, J. D.,
1329 Sanz-Giménez-Rico, J. R., and Arnáiz-García, A. M. (2009). Cutaneous mucormycosis:
1330 report of five cases and review of the literature. *J. Plast. Reconstr. Aesthet. Surg*. **62,**
1331 e434-e441.
1332 Arndt, S., Aschendorff, A., Echternach, M., Daemmrich, T. D., and Maier, W. (2009). Rhino-
1333 orbital-cerebral mucormycosis and aspergillosis: differential diagnosis and treatment.
1334 *Eur. Arch. Otorhinolaryngol*. **266,** 71-76.
1335 Arruebarrena, G. A., Romano, A. E., Sciarretta, J., Hopkins, M. A., and Davis, J. M. (2016).
1336 Cutaneous mucormycosis in a trauma patient with a new diagnosis of diabetes mellitus.
1337 *Surgical Infection Case Reports* **1,** 4-7.
1338 Baddley, J. W., Marr, K. A., Andes, D. R., Walsh, T. J., Kauffman, C. A., Kontoyiannis, D. P., Ito,
1339 J. I., Balajee, S. A., Pappas, P. G., and Moser, S. A. (2009). Patterns of susceptibility of
1340 *Aspergillus* isolates recovered from patients enrolled in the Transplant-Associated
1341 Infection Surveillance Network. *J. Clin. Microbiol*. **47,** 3271-3275.
1342 Bader, O. (2013). MALDI-TOF-MS-based species identification and typing approaches in
1343 medical mycology. *Proteomics* **13,** 788-799.
1344 Balajee, S. A., Borman, A. M., Brandt, M. E., Cano, J., Cuenca-Estrella, M., Dannaoui, E.,
1345 Guarro, J., Haase, G., Kibbler, C. C., Meyer, W., O'Donnell, K., Petti, C. A., *et al*. (2009).
1346 Sequence-based identification of *Aspergillus*, *Fusarium*, and mucorales species in the
1347 clinical mycology laboratory: where are we and where should we go from here? *J. Clin.*
1348 *Microbiol*. **47,** 877-884.

1349 Balandin, B., Aguilar, M., Sánchez, I., Monzón, A., Rivera, I., Salas, C., Valdivia, M., Alcántara,
1350 S., Pérez, A., and Ussetti, P. (2016). *Scedosporium apiospermum* and *S. prolificans* mixed
1351 disseminated infection in a lung transplant recipient: an unusual case of long-term
1352 survival with combined systemic and local antifungal therapy in intensive care unit.
1353 *Med. Mycol. Case Rep*. **11,** 53-56.
1354 Barnes, R. A. (2013). Directed therapy for fungal infections: focus on aspergillosis. *J.*
1355 *Antimicrob. Chemother.* **68,** 2431-2434.
1356 Bennett, J. E., Friedman, M. M., and Dupont, B. (1987). Receptor-mediated clearance of
1357 *Aspergillus* galactomannan. *J. Infect. Dis*. **155,** 1005-1010.
1358 Bernal-Martínez, L., Buitrago, M. J., Castelli, M. V., Rodríguez-Tudela, J. L., and Cuenca-
1359 Estrella, M. (2012). Detection of invasive infection caused by *Fusarium solani* and non-
1360 *Fusarium solani* species using a duplex quantitative PCR-based assay in a murine model
1361 of fusariosis. *Med. Mycol*. **50,** 270-275.
1362 Bernal-Martínez, L., Buitrago, M. J., Castelli, M. V., Rodríguez-Tudela, J. L., and Cuenca-
1363 Estrella, M. (2013). Development of a single tube multiplex real-time PCR to detect the
1364 most clinically relevant Mucormycete species. *Clin. Microbiol. Infect*. **19,** E1-E7.
1365 Bhuta, S., Hsu, C. C.-T., and Kwan, G. N. C. (2012). *Scedosporium apiospermum*
1366 endophthalmitis: diffusion-weighted imaging in detecting subchoroidal abscess. *Clin.*
1367 *Ophthalmol*. **6,** 1921-1924.
1368 Binder, U., Maurer, E., and Lass-Flörl. (2014). Mucormycosis - from the pathogens to the
1369 disease. *Clin. Microbiol. Infect*. **20,** 60-66.
1370 Blackwell, M. (2011). The fungi: 1, 2, 3 … 5.1 million species? *Am. J. Bot*. **98,** 426-438.
1371 Blyth, C. C., Harun, A., Middleton, P. G., Sleiman, S., Lee, O., Sorrell, T. C., Meyer, W., and
1372 Chen, S. C.-A. (2010a). Detection of occult *Scedosporium* species in respiratory tract
1373 specimens from patients with cystic fibrosis by use of selective media. *J. Clin. Microbiol*.
1374 **48,** 314-316.
1375 Blyth, C. C., Middleton, P. G., Harun, A., Sorrel, T. C., Meyer, W., and Chen, S. C.-A. (2010b).
1376 Clinical associations and prevalence of *Scedosporium* spp. in Australian cystic fibrosis
1377 patients: identification of novel risk factors? *Med. Mycol*. **48,** S37-S44.
1378 Bongomin, F., Gago, S., Oladele, R. O., and Denning, D. W. (2017). Global and multi-national
1379 prevalence of fungal diseases - estimate precision. *J. Fungi* **3,** 57.
1380 Borghi, E., Iatta, R., Manca, A., Montagna, M. T., and Morace, G. (2010). Chronic airway
1381 colonisation by *Scedosporium apiospermum* with a fatal outcome in a patient with
1382 cystic fibrosis. *Med. Mycol*. **48,** S108-S113.
1383 Bouchara, J.-P., Hsieh, H. Y., Croquefer, S., Barton, R., Marchais, V., Pihet, M., and Chang, T.
1384 C. (2009). Development of an oligonucleotide array for direct detection of fungi in
1385 sputum samples from patients with cystic fibrosis. *J. Clin. Microbiol*. **47,** 142-152.
1386 Bouchara, J.-P., Symoens, F., Schwartz, C., and Chaturvedi, V. (2018). Fungal respiratory
1387 infections in cystic fibrosis (CF): recent progress and future research agenda.
1388 *Mycopathologia* **183,** 1-5.
1389 Box, H., Negri, C., Livermore, J., Whalley, S., Johnson, A., McEntee, L., Alastruey-Izquidero,
1390 A., Meis, J. F., Thornton, C. R., and Hope, W. (2018). Pharmacodynamics of voriconazole
1391 for invasive pulmonary scedosporiosis. *Antimicrob. Agents Chemotherap*. **62,** e02516-
1392 17.
1393 Brasnu, E., Bourcier, T., Dupas, B., Degorge, S., Rodallec, T., Laroche, L., Borderie, V., and
1394 Baudouin, C. (2007). *In vivo* confocal microscopy in fungal keratitis. *Br. J. Ophthalmol*.
1395 **91,** 588-591.

1396 Brown, G. D., Denning, D. W., and Levitz, S. M. (2012a). Tackling human fungal infections.
1397 *Science* **336,** 647.
1398 Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G., and White, T. C.
1399 (2012b). Hidden killers: human fungal infections. *Sci. Transl. Med*. **4,** 165rv13.
1400 Buchheidt, D., Reinwald, M., Hofmann, W.-K., Bloch, T., and Seiss, B. (2017). Evaluating the
1401 use of PCR for diagnosing invasive aspergillosis. *Exp. Rev. Mol. Diagn*. **17,** 603-610.
1402 Caramalho, R., Tyndall, J. D. A., Monk, B. C., Larentis, T., Lass-Flörl, C., and Lackner, M.
1403 (2017). Intrinsic short-tailed azole resistance in mucormycetes is due to an evolutionary
1404 conserved amino acid substitution of the lanosterol 14α-demethylase. *Sci. Rep*. **7,**
1405 15898.
1406 Carroll, C. S., Amankwa, K. N., Pinto, L. J., Fuller, J. D., and Moore, M. M. (2016). Detection of
1407 serum siderophores by LC-MS/MS as a potential biomarker of invasive aspergillosis.
1408 *PLoS ONE* **11,** e0151260.
1409 Carsin, A., Romain, T., Ranque, S., Reynaud-Gaubert, M., Dubus, J.-C., Mège, J.-L., and Vitte,
1410 J. (2017). *Aspergillus fumigatus* in cystic fibrosis: an update on immune interactions and
1411 molecular diagnostics in allergic bronchopulmonary aspergillosis. *Allergy* **72,** 1632-1642.
1412 Cassagne, C., Ranque, S., Normand, A. C., Forquet, P., Thiebault, S., Planard, C., Hendrickx,
1413 M., and Piarroux, R. (2011). Mould routine identification in the clinical laboratory by
1414 matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. *PLoS ONE*
1415 **6,** e28425.
1416 Castrejón-Pérez, A. D., Miranda, I., Welsh, O., Welsh, E. C., and Ocampo-Candiani, J. (2017).
1417 Cutaneous mucormycosis. *An. Bras. Dermatol*. **92,** 304-311.
1418 Chan, J. F.-W., Lau, S. K.-P., Wong, S. C.-Y., To, K. K.-W., So, S. Y.-C., Leung, S. S.-M., Chan, S.-
1419 M., Pang, C.-M., Xiao, C., Hung, I. F.-N., Cheng, V. C.-C., Yuen, K.-Y., *et al*. (2016). A 10-
1420 year study reveals clinical and laboratory evidence for the 'semi-invasive' properties of
1421 chronic pulmonary aspergillosis. *Emerg. Microb. Infect*. **5,** e37.
1422 Chen, M., Kondori, N., Deng, S., Gerrits van den Ende, A. H. G., Lackner, M., Liao, W., de
1423 Hoog, G. S. (2017). Direct detection of *Exophiala* and *Scedosporium* species in sputa of
1424 patients with cystic fibrosis. *Med. Mycol*. **56,** 695-702.
1425 Chen, Y.-J., Chou, C.-L., Lai, K.-J., and Lin, Y.-J. (2017). *Fusarium* brain abscess in a patient
1426 with diabetes mellitus and liver cirrhosis. *Acta Neurol. Taiwan*. **26,** 128-132.
1427 Chong, G. M., van der Beek, M. T., von dem Borne, A., Boelens, J., Steel, E., Kampinga, G. A.,
1428 Span, L. F., Lagrou, K., Maertens, J. A., Dingemans, G. J., Gaajetaan, G. R., van Tegelen,
1429 D. W., *et al*. (2016). PCR-based detection of *Aspergillus fumigatus* Cyp51A mutations on
1430 bronchoalveolar lavage: a multicentre validation of the AsperGenius® assay in 201
1431 patients with haematological disease suspected for invasive aspergillosis. *J. Antimicrob.*
1432 *Chemother*. **71,** 3528-3535.
1433 Chotirmall, S. H., and McElvaney, N. G. (2014). Fungi in the cystic fibrosis lung: bystanders or
1434 pathogens? *Int. J. Biochem. Cell Biol*. **52,** 161-173.
1435 Chotirmall, S. H., and Martin-Gomez, M. T. (2018). *Aspergillus* species in bronchiectasis:
1436 challenges in the cystic fibrosis and non-cystic fibrosis airways. *Mycopathologia* **183,** 45-
1437 59.
1438 Chowdhary, A., Agarwal, K., Kathuria, S., Gaur, S. N., Randhawa, H. S., and Meis, J. F. (2014).
1439 Allergic bronchopulmonary mycosis due to fungi other than *Aspergillus*: a global
1440 overview. *Crit. Rev. Microbiol*. **40,** 30-48.

1441 Cimon, B., Carrere, J., Vinatier, J. F., Chazalette, J. P., Chabasse, D., and Bouchara, J. P.
1442 (2000). Clinical significance of *Scedosporium apiospermum* in patients with cystic
1443 fibrosis. *Eur. J. Clin. Microbiol. Infect. Dis*. **19,** 53-56.
1444 Cooley, L., Spelman, D., Thursky, K., and Slavin. M. (2007). Infection with *Scedosporium*
1445 *apiospermum* and *S. prolificans*, Australia. *Emerg. Infect. Dis*. **13,** 1170-1177.
1446 Cornely, O. A., Arikan-Akdagli, S., Dannaoui, E., Groll, A. H., Lagrou, K., Chakrabarti, A.,
1447 Lanternier, F., Pagano, L., Skiada, A., Akova, M., Arendrup, M. C., Boekhout, T., *et al*.
1448 (2014). ESCMID and ECMM joint clinical guidelines for the diagnosis and management
1449 of mucormycosis 2013. *Clin. Microbiol. Infect*. **20,** 5-26.
1450 Cortez, K. J., Roilides, E., Quiroz-Telles, F., Meletiadis, J., Antachopoulos, C., Knudsen, T.,
1451 Buchanan, W., Milanovich, J., Sutton, D. A., Fothergill, A., Rinaldi, M. G., Shea, Y. R., *et*
1452 *al.* (2008). Infections caused by *Scedosporium* spp. *Clin. Microbiol. Rev*. **21,** 157-197.
1453 Coulibaly, O., Marinach-Patrice, C., Cassagne, C., Piarroux, R., Mazier, D., and Ranque, S.
1454 (2011). *Pseudallescheria*/*Scedosporium* complex species identification by matrix-
1455 assisted laser desorption ionisation-time of flight mass spectrometry. *Med. Mycol*. **49,**
1456 621-626.
1457 Cramer, R. (1998a). Recombinant *Aspergillus fumigatus* allergens: from the nucleotide
1458 sequences to clinical applications. *Int. Arch. Allergy Immunol*. **115,** 99-114.
1459 Crameri, R., Hemmann, S., Ismail, C., Menz, G., and Blaser, K. (1998b). Disease-specific
1460 recombinant antigens for the diagnosis of allergic bronchopulmonary aspergillosis. *Int.*
1461 *Immunol*. **10,** 1211-1216.
1462 Crameri, R., Zeller, S., Glaser, A. G., Vilhelmsson, M., and Rhyner, C. (2009). Cross-reactivity
1463 among fungal antigens: a clinically relevant phenomenon? *Mycoses* **52,** 99-106.
1464 Dabén, R. P., de Lucas, E. M., Cuesta, L. M., Velasco, T. P., Garica, J. A., Landeras, R., Duarte,
1465 M. L., and Bermúdez, A. (2008). Imaging findings of pulmonary infection casued by
1466 *Scedosporium prolificans* in a deep immunocompromised patient. *Emerg. Radiol*. **15,**
1467 47-49.
1468 Däbritz J., Attarbaschi, A., Tintelnot, K., Kollmar, N., Kremens, B., v. Loewenich, F. D., Schrod,
1469 L., Schuster, F., Wintergerst, U., Weig, M., Lehrnbecher, T., and Groll, A. H. (2011).
1470 Mucormycosis in paediatric patients: demographics, risk factors and outcome of 12
1471 contemporary cases. *Mycoses* **54,** e785-e788.
1472 Dannaoui, E., Schwartz, P., Slany, M., Loeffler, J., Jorde, A. T., Cuenca-Estrella, M., Hauser, P.
1473 M., Shrief, R., Huerre, M. Freiberger, T., Gaustad, P., Rodriguez-Tudela, J. L., *et al*.
1474 (2010). Molecular detection and identification of zygomycete species from paraffin-
1475 embedded tissues in a murine model of disseminated zygomycosis: a collaborative
1476 European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Fungal
1477 Infection Group (EFISG) evaluation. *J. Clin.Microbiol*. **48,** 2043-2046.
1478 Davies, G., and Thornton, C. R. (2014). Differentiation of the emerging human pathogens
1479 *Trichosporon asahii* and *Trichosporon asteroides* from other pathogenic yeasts and
1480 moulds by using species-specific monoclonal antibodies. *PLoS ONE* **9,** e84789.
1481 Davies, G., Rolle, A-M., Maurer, A., Spycher, P. R., Schillinger, C., Solouk-Saran, D.,
1482 Hasenberg, M., Weski, J., Fonslet, J., Dubois, A., Boschetti, F., Denat, F., *et al*. (2017).
1483 Towards translational immunoPET/MR imaging of invasive pulmonary aspergillosis: the
1484 humanised monoclonal antibody JF5 detects *Aspergillus* lung infections *in vivo*.
1485 *Theranostics* **7,** 3398-3414.
1486 De Carolis, E., Posteraro, B., Lass-Flörl, C., Vella, A., Florio, A. R., Torelli, R., Girmenia, C.,
1487 Colozza, C., Tortorano, A. M., Sanguinetti, M., and Fadda, G. (2012). Species

1488 identification of *Aspergillus*, *Fusarium*, and Mucorales with direct surface analysis by
1489 matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. *Clin.*
1490 *Microbiol. Infect*. **18,** 475-484.
1491 Deja, M., Wolf, S., Weber-Carstens, S., Lehmann, T-N., Adler, A., Ruhnke, M., Tintelnot, K.
1492 (2006). Gastrointestinal zygomycosis caused by *Mucor indicus* in a patient with acute
1493 traumatic brain injury. *Med. Mycol*. **44,** 683-687.
1494 Delhaes, L., Harun, A., Chen, S. C., Nguyen, Q., Slavin, M., Heath, C. H., Maszewska, K.,
1495 Halliday, C., Robert, V., Sorrel, T. C., Australian *Scedosporium* (AUSCEDO) Study Group,
1496 and Meyer, W. (2008). Molecular typing of Australian *Scedosporium* isolates showing
1497 genetic variability and numerous *S. aurantiacum*. *Emerg. Infect. Dis*. **14,** 282-290.
1498 Denning, D. W., Riniotis, K., Dobrashian, R., and Sambatakou, H. (2003). Chronic cavitary and
1499 fibrosing pulmonary and pleural aspergillosis: case series, proposed nomenclature
1500 change, and review. *Clin. Infect. Dis*. **37,** S265-S280.
1501 Denning, D. W., Pleuvry, A., and Cole, D. C. (2011). Global burden of chronic pulmonary
1502 aspergillosis as a sequel to pulmonary tuberculosis. *Bull. World Health Org*. **89,** 864-872.
1503 Denning, D. W., Pleuvry, A., and Cole, D. C. (2013). Global burden of allergic
1504 bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary
1505 aspergillosis in adults. *Med. Mycol*. **51,** 361-370.
1506 Denning, D. W., Pashley, C., Hartl, D., Wardlaw, A., Godet, C., Del Gaccio, S., Delhaes, L., and
1507 Sergejeva, S. (2014). Fungal allergy in asthma - state of the art and research needs. *Clin.*
1508 *Transl. Allergy* **4,** 14.
1509 Denning, D. W., Cadranel, J., Beigelman-Aubry, C., Ader, F., Chakrabarti, A., Blot, S., Ullmann,
1510 A. J., Dimopoulos, G., and Lange, C. (2016). Chronic pulmonary aspergillosis: rationale
1511 and clinical guidelines for diagnosis and management. *Eur. Respir. J.* **47,** 45-68.
1512 De Pauw, B., Walsh, T. J., Donnelly, J. P., Stevens, D. A., Edwards, J. E., Calandra, T., Pappas,
1513 P. G., Maertens, J., Lortholary, O., Kauffman, C. A., Denning, D. W., Patterson, T. F., *et al*.
1514 (2008). Revised definitions of invasive fungal disease from the European Organisation
1515 for Research and Treatment of Cancer/Invasive Fungal Infections Co-operative Group
1516 and the National Institute of Allergy and Infectious Diseases Mycoses Study Group
1517 (EORTC/MSG) Consensus Group. *Clin. Infect. Dis*. **46,** 1813-1821.
1518 De Ruiter, G. A., van der Lugt, A. W., Voragen, A. G. J., and Rombouts, F. M. (1991). High-
1519 performance size-exclusion chromatography and ELISA detection of extracellular
1520 polysaccharides from Mucorales. *Carbohydr. Res*. **215,** 47-57.
1521 De Ruiter, G. A., van Bruggen-van der Lugt, A. W., Nout, M. J. R., Middelhoven, W. J.,
1522 Soentoro, P. S. S., Notermans, S. H. W., and Rombouts, F. M. (1992a). Formation of
1523 antigenic extracellular polysaccharides by selected strains of *Mucor* spp., *Rhizopus* spp.,
1524 *Rhizomucor* spp., *Absidia corymbifera* and *Syncephalastrum racemosum*. *Antonie*
1525 *Leeuwenkoek* **62,** 189-199.
1526 De Ruiter, G. A., Josso, S. L., Colquhoun, I. J., Voragen, A. G. J., and Rombouts, F. M. (1992b).
1527 Isolation and characterisation of β(1-4)-D-glucuronans from extracellular
1528 polysaccharides of moulds belonging to Mucorales. *Carbohydr. Polym.* **18,** 1-7.
1529 De Ruiter, G. A., van Bruggen-van der Lugt, A. W., Bos, W., Notermans, S. H. W., Rombouts,
1530 F. M., and Hofstra, H. (1993). The production and partial characterisation of a
1531 monoclonal IgG antibody specific for the moulds belonging to the order Mucorales. *J.*
1532 *Gen. Microbiol*. **139,** 1557-1564.
1533 De Ruiter, G. A., van Bruggen-van der Lugt, A. W., Mischnick, P., Smid, P., van Boom, J. H.,
1534 Notermans, S. H., and Rombouts, F. M. (1994). 2-*O*-Methyl-D-mannose residues are

1535 immunodominant in extracellular polysaccharides of *Mucor racemosus* and related
1536 molds. *J. Biol. Chem*. **269,** 4299-4306.
1537 Dichtl, K., Seybold, U., Ormanns, S., Horns, H., and Wagener, J. (2019). Evaluation of a novel
1538 *Aspergillus* antigen ELISA. *J. Clin. Microbiol*. **57,** e00136-19.
1539 Dogget, M. S. (2000). Characterisation of fungal biofilms within a municipal water
1540 distribution system. *Appl. Env. Microbiol*. **66,** 1249-1251.
1541 Drain, P. K., Hong, T., Krows, M., Govere, S., Thulare, H., Wallis, C. L., Gosnell, B. I., Moosa,
1542 M-Y., Bassett, I. V., and Celum, C. (2019). Validation of clinic-based cryptococcal antigen
1543 lateral-flow assay screening in HIV-infected adults in South Africa. *Sci. Rep*. **9,** 2687.
1544 Dufresene, S. F., Datta, K., Li, X., Dadachova, E., Staab, J. F., Patterson, T. F., Feldmesser, M.,
1545 and Marr, K. A. (2012). Detection of urinary excreted fungal galactomannan-like
1546 antigens for diagnosis of invasive aspergillosis. *PLoS ONE* **7,** e42736.
1547 Dupont, B., Huber, M., Kim, S. J., and Bennett, J. E. (1987). Galactomannan antigenemia and
1548 antigenuria in aspergillosis: studies in patients and experimentally infected rabbits. *J.*
1549 *Infect. Dis*. **155,** 1-11.
1550 Dutkiewicz, R., and Hage, C. A. (2010). *Aspergillus* infections in the critically ill. *Proc. Am.*
1551 *Thorac. Soc*. **7,** 204-209.
1552 Eigl, S., Prattes, J., Reinwald, M., Thornton, C. R., Reischies, F., Spiess, B., Neumeister, P.,
1553 Zollner-Schwetz, I., Raggam, R. B., Flik, H., Buchheidt, D., Krause, R., *et al*. (2015).
1554 Influence of mould-active antifungal treatment on the performance of the *Aspergillus*-
1555 specific bronchoalveolar lavage fluid lateral-flow device test. *Int. J. Antimicrob. Agents*.
1556 **46,** 401-405.
1557 Eigl, S., Hoenigl, M., Spiess, B., Heldt, S., Prattes, J., Neumeister, P., Wölfler, A.,
1558 Rabensteiner, J., Prueller, F., Krause, R., Reinwald, M., Flick, H., *et al*. (2017).
1559 Galactomannan testing and *Aspergillus* PCR in same-day bronchoalveolar lavage and
1560 blood samples for diagnosis of invasive aspergillosis. *Med. Mycol*. **55,** 528-534.
1561 Esnakula, A. K., Summers, I., and Naab, T. J. (2013). Fatal disseminated *Fusarium* infection in
1562 a human immunodeficiency virus positive patient. *Case Rep. Infect. Dis*. Article ID
1563 379320.
1564 Fanci, R., Pini, G., Bartolesi, A. M., and Pecile, P. (2012). Refactory disseminated fusariosis by
1565 *Fusarium verticillioides* in a patient with acute myeloid leukaemia relapsed after
1566 allogeneic hematopoietic stem cell transplantation: a case report and literature review.
1567 *Rev. Iberoam. Micol*. **30,** 51-53.
1568 Fanfair, R. N., Benedict, K., Bos, J., Bennett, S. D., Lo, Y-C., Adebanjo, T., Etienne, K., Deak, E.,
1569 Derado, G., Shieh, W-J., Drew, C., Zaki, S., *et al*. (2012). Necrotizing cutaneous
1570 mucormycosis after a tornado in Joplin, Missouri, in 2011. *New Engl. J. Med*. **367,** 2214-
1571 2225.
1572 Fisher, M. C., Hawkins, N. J., Sanglard, D., and Gurr, S. J. (2018). Worldwide emergence of
1573 resistance to antifungal drugs challenges human health and food security. *Science* **360,**
1574 739-742.
1575 Fricker-Hidalgo, H., Coltey, B., Llerena, C., Renversez, J-C., Grillot, R., Pin, I., Pelloux, H., and
1576 Pinel, C. (2010). Recombinant allergens combined with biological markers in the
1577 diagnosis of allergic bronchopulmonary aspergillosis in cystic fibrosis patients. *Clin.*
1578 *Vacc. Immunol.* **17,** 1330-1336.
1579 Fukuzawa, M., Inaba, H., Hayama, M., Sakaguchi, N., Sano, K., Ito, M., and Hotchi, M. (1995).
1580 Improved detection of medically important fungi by immunoperoxidase staining with
1581 polyclonal antibodies. *Virchows Arch*. **427,** 407-414.

1582 Gade, L., Hurst, S., Balajee, S. A., Lockhart, S. R., and Litvintseva, A. P. (2017). Detection of
1583 mucormycetes and other pathogenic fungi in formalin fixed paraffin embedded and
1584 fresh tissues using the extended region of 28S rDNA. *Med. Mycol*. **55,** 385-395.
1585 García-Ruiz, J. C., Olazábal, I., Pedroso, R. M. A., López-Soria, L., Velaso-Benito, V., Sanchez-
1586 Aparicio, J. A., Navajas, A., Montejo, M., and Moragues, M.-D. Disseminated fusariosis
1587 and hematologic malignancies, a still devastating association. Report of three new
1588 cases. *Rev. Iberoam. Micol*. **32,** 190-196.
1589 Garzoni, C., Emonet, S., Legout, L., Benedict, R., Hoffmeyer, P., Bernard, L., and Garbino, J.
1590 (2005). Atypical infections in tsunami survivors. *Emerg. Infect. Dis*. **11,** 1591-1593.
1591 Gilgado, F., Cano, J., Gené, J., and Guarro, J. (2005). Molecular phylogeny of the
1592 *Pseudallescheria boydii* complex: proposal of two new species. *J. Clin. Microbiol*. **43,**
1593 4930-4942.
1594 Gilgado, F., Serena, C., Cano, J., Gené, J., and Guarro, J. (2006). Antifungal susceptibilities of
1595 of the species of the *Pseudallescheria boydii* complex. *Antimicrob. Agents Chemotherap*.
1596 **50,** 4211-4213.
1597 Gilgado, F., Cano, J., Gené, J., Sutton, D. A., and Guarro, J. (2008). Molecular and phenotypic
1598 data supporting distinct species statuses for *Scedosporium apiospermum* and
1599 *Pseudallescheria boydii* and the proposed new species *Scedosporium dehoogii*. *J. Clin.*
1600 *Microbiol*. **46,** 766-771.
1601 Glazer, M., Nusair, S., Breuer, R., Lafair, J., Sherman, Y., and Berkman, N. (2000). The role of
1602 BAL in the diagnosis of pulmonary mucormycosis. *Chest J*. **117,** 279-282.
1603 Gomes, M. Z. R., Lewis, R. E., and Kontoyiannis, D. P. (2011). Mucormycosis caused by
1604 unusual mucormycetes, non-*Rhizopus*, -*Mucor*, and -*Lichtheimia* species. *Clin. Microbiol.*
1605 *Rev*. **24,** 411-445.
1606 Gonçalves, S. M., Lagrou, K., Rodrigues, C. S., Campos, C. F., Bernal-Martínez, L., Rodrigues,
1607 F., Silvestre, R., Alcazar-Fuoli, L., Maertens, J. A., Cunha, C., and Carvalho, A. (2017).
1608 Evaluation of bronchoalveolar lavage fluid cytokines as biomarkers for invasive
1609 pulmonary aspergillosis in at-risk patients*. Front. Microbiol*. **8,** 2362.
1610 Grantina-Ievina, L., Andersone, U., Berkolde-Pīre, D., Nikolajeva, V., and Ievinsh, G. (2013).
1611 Critical tests for determination of microbiological quality and biological activity in
1612 commercial vermicompost samples of different origins. *Appl. Microbiol. Biotechnol*. **97,**
1613 10541-10554.
1614 Grossman, M. E., Fox, L. P., Kovarik, C., and Rosenbach, M. (2012). Hyalohyphomycosis. In
1615 *Cutaneous manifestations of infection in the immunocompromised host* (pp. 65-88).
1616 New York: Springer.
1617 Guarro, J. (2013). Fusariosis, a complex infection caused by a high diversity of fungal species
1618 refractory to treatment. *Eur. J. Clin. Microbiol. Infect. Dis*. **32,** 1491-1500.
1619 Guegan, H., Robert-Gangneux, F., Camus, C., Belaz, S., Marchand, T., Baldeyrou, M., and
1620 Gangneux, J. P. (2018). Improving the diagnosis of invasive aspergillosis by the detection
1621 of *Aspergillus* in bronchoalveolar lavage fluid: comparison of non-culture-based assays.
1622 *J. Infect.* **76,** 196-205.
1623 Guinea, J., Padilla, C., Escribano, P., Muñoz, P., Padilla, B., Gijón, P., and Bouza, E. (2013).
1624 Evaluation of MycAssay™ *Aspergillus* for diagnosis of invasive pulmonary aspergillosis in
1625 patients without haematological cancer. *PLoS One* **8,** e61545.
1626 Gurusidappa, S. B., and Mamatha, H. S. (2011). Fusarial skin lesion in immunocompromised.
1627 *Ind. J. Cancer* **48,** 116-117.

1628 Gundacker, N. D., and Baddley, J. W. (2015). Fungal infections in the era of biologic
1629 therapies. *Curr. Clin. Micro. Rpt*. **2,** 76-83.
1630 Haas, H. (2003). Molecular genetics of fungal siderophore biosynthesis and update: the role
1631 of siderophores in iron uptake and storage. *Appl. Microbiol. Biotechnol*. **62,** 316-33.
1632 Haas, H., Petrik, M., and Descristoforo, C. (2015). An iron-mimicking, Trojan horse-entering
1633 fungi - has the time come for molecular imaging of fungal infections? *PLoS Pathog*. **11,**
1634 e1004568.
1635 Hammond, S. P., Bialek, R., Milner, D. A., Petschnigg, E. V., Baden, L. R., and Marty, F. M.
1636 (2011). Molecular methods to improve diagnosis and identification of mucormycosis. *J.*
1637 *Clin. Microbiol.* **49,** 2151-2153.
1638 Harun, A., Blyth, C. C., Gilgado, F., Middleton, P., Chen, S. C.-A., and Meyer, W. (2011).
1639 Development and validation of a multiplex PCR for detection of *Scedosporium* spp. in
1640 respiratory tract specimens from patients with cystic fibrosis. *J. Clin. Microbiol*. **49,**
1641 1508-1512.
1642 Hawksworth, D. L. (1991). The fungal dimension of biodiversity: magnitude, significance, and
1643 conservation. *Mycol. Res*. **95,** 641-655.
1644 Hawksworth, D. L. (2011). Naming *Aspergillus* species: progress towards one name for each
1645 species. *Med. Mycol*. **49,** S70-S76.
1646 Haynes, K. A., Latgé, J. P., and Rogers, T. R. Detection of *Aspergillus* antigens associated with
1647 invasive aspergillosis. *J. Clin. Microbiol*. **28,** 2040-2044.
1648 He, D., Hao, J., Zhang, B., Yang, Y., Song, W., Zhang, Y., Yokoyama, K., and Wang, L. (2011).
1649 Pathogenic spectrum of fungal keratitis and specific identification of *Fusarium solani*.
1650 *Invest. Ophthalmol. Vis. Sci*. **52,** 2804-2808.
1651 Held, J., Schmidt, Thornton, C. R., Kotter, E., and Bertz, H. (2012). Comparison of a novel
1652 *Aspergillus* lateral-flow device and the Platelia galactomannan assay for the diagnosis of
1653 invasive aspergillosis following haematopoietic stem cell transplantation. *Infection* **41,**
1654 1163-1169.
1655 Held, S., and Hoenigl, M. (2017). Lateral fow assays for the diagnosis of invasive
1656 aspergillosis: current status. *Curr. Fungal Infect. Rep*. **11,** 45-51.
1657 Hennebert, G. L., and Desai, B. G. (1974). *Lomentospora prolificans*, a new hyphomycete
1658 from greenhouse soil. *Mycotaxon* **1,** 45-50.
1659 Hessian, P., and Smith, J. M. B. (1982). Antigenic characterisation of some potentially
1660 pathogenic mucoraceous fungi. *Sabouraudia* **20,** 209-216.
1661 Hingad, N., Kumar, G., and Deshmukh, R. (2012). Oral mucormycosis causing necrotizing
1662 lesion in a diabetic patient: a case report. *Int. J. Oral Maxillofacial Pathol*. **3,** 8-12.
1663 Hoenigl, M., Prattes, J., Spiess, B., Wagner, J., Prueller, F., Raggam, R. B., Posch, V.,
1664 Duettmann, W., Hoenigl, K., Wölfler, A., Koidl, C., Buzina, W., *et al*. (2014). Performance
1665 of galactomannan, beta-d-glucan, *Aspergillus* lateral-flow device, conventional culture,
1666 and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary
1667 aspergillosis. *J. Clin. Microbiol*. **52,** 2039-2045.
1668 Hoenigl, M., Orasch, T., Faserl, K., Prattes, J., Loeffler, J., Springer, J., Gsaller, F., Reischies, F.,
1669 Duettmann, W., Raggam, R. B., Linder, H., and Haas, H. (2019). Triacetylfusarinine C: a
1670 urine biomarker for diagnosis of invasive aspergillosis. *J. Infect.* **78,** 150-157.
1671 Holmes, N. E., Trevillyan, J., Kidd, S. E., and Leong, T. Y.-M. (2013). Locally extensive angio-
1672 invasive *Scedosporium prolificans* infection following resection for squamous cell lung
1673 carcinoma. *Med. Mycol. Case Rep*. **2,** 98-102.

1674 Hong, G., Miller, H. B., Allgood, S., Lee, R., Lechtzin, N., and Zhang, S. X. (2017a). Use of
1675 selective fungal culture media increases rates of detection of fungi in the respiratory
1676 tract of cystic fibrosis patients. *J. Clin. Microbiol*. **55,** 1122-1130.
1677 Hong, Y., and Park, J. (2017b). The role of transbronchial lung biopsy in diagnosing
1678 pulmonary mucormycosis in a critical care unit. *Korean J. Crit. Care Med*. **32,** 205-210.
1679 Hope, W. W., Walsh, T. J., and Denning, D. W. (2005). The invasive and saprophytic
1680 syndromes due to *Aspergillus* spp. *Med. Mycol*. **43,** S207-S238.
1681 Horré, R., Marklein, G., Siekmeier, R., Nidermajer, S., Reiffert, S. M. (2009). Selective
1682 isolation of *Pseudallescheria* and *Scedosporium* species from respiratory tract
1683 specimens of cystic fibrosis patients. *Respiration* **77,** 320-324.
1684 Howard, S. J., Cerar, D., Anderson, M. J., Albarrag, A., Fisher, M. C., Pasqualotto, A. C.,
1685 Laverdiere, M, Arendrup, M. C., Perlin, D. S., and Denning, D. W. (2009). Frequency and
1686 evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure.
1687 *Emerg. Infect. Dis.* **15,** 1068-1076.
1688 Hung, H-C., Shen, G-Y., Chen, S-C., Yeo, K-J., Tsao, S-M., Lee, M-C., and Lee, Y-T. (2015).
1689 Pulmonary mucormycosis in a patient with systemic lupus erythematosus: a diagnostic
1690 and treatment challenge. *Case Rep. Infect. Dis.* **2015,** 478789.
1691 Husain, S., Munoz, P., Forrest, G., Alexander, B. D., Somani, J., Brennan, K., Wagener, M. M.,
1692 and Singh, N. (2005). Infections due to *Scedosporium apiospermum* and *Scedosporium*
1693 *prolificans* in transplant recipients: clinical characteristics and impact of antifungal
1694 agent therapy on outcome. *Clin. Infect. Dis*. **40,** 89-99.
1695 Hymery, N., Vasseur, V., Coton, M., Mounier, J., Jany, J-L., Barbier, G., and Coton, E. (2014).
1696 Filamentous fungi and mycotoxins in cheese: a review. *Compr. Rev. Food Sci. Food Saf.*
1697 **13,** 437-456.
1698 Iqbal, N., Irfan, M., Jabeen, K., Kazmi, M. M., Tariq, M. U. (2017). Chronic pulmonary
1699 mucormycosis: an emerging fungal infection in diabetes mellitus. *J. Thorac. Dis*. **9,** E121-
1700 E125.
1701 Izumikawa, K., Yamamoto, Y., Mihara, T., Takazono, T., Morinaga, Y., Kurihara, S., Nakamura,
1702 S., Imamura, Y., Miyazaki, T., Nishino, T., Tsukamoto, M., Kakeya, H., *et al*. (2012).
1703 Bronchoalveolar lavage galactomannan for the diagnosis of chronic pulmonary
1704 aspergillosis. *Med. Mycol*. **50,** 811-817.
1705 Jain, P. K., Gupta, V. K., Misra, A. K., Gaur, R., Bajpai, V., and Issar, S. (2011). Current status
1706 of *Fusarium* infection in human and animal. *Asian J. Anim. Vet. Adv.* **6,** 201-227.
1707 Jenks, J. D., Mehta, S. R., Taplitz, R., Aslam, S., Reed, S. L., and Hoenigl, M. (2018). Point-of-
1708 care diagnosis of invasive aspergillosis in non-neutropenic patients: *Aspergillus*
1709 galactomannan lateral flow assay versus *Aspergillus*-specific lateral flow device test in
1710 bronchoalveolar lavage. *Mycoses* **62,** 230-236.
1711 Jenks, J. D., Mehta, S. R., Taplitz, R., Law, N., Reed, S. L., and Hoenigl, M. (2019).
1712 Bronchoalveolar lavage *Aspergillus* galactomannan lateral flow assay versus *Aspergillus*-
1713 specific lateral flow device test for diagnosis of invasive pulmonary aspergillosis in
1714 patients with hematological malignancies. *J. Infect.* **78,** 249-259.
1715 Jensen, H. E., Stynen, D., Sarfati, J., and Latge, J.-P. (1993a). Detection of galactomannan and
1716 the 18kDa antigen from *Aspergillus fumigatus* in serum and urine from cattle with
1717 systemic aspergillosis. *J. Vet. Med. B*. **40,** 397-408.
1718 Jensen, H. E., Frandsen, P. L., Schønheyder, H. (1993b). Experimental systemic bovine
1719 zygomycosis with reference to pathology and secretion of antigen into urine. *J. Vet.*
1720 *Med. B*. **40,** 55-65.

1721 Jensen, H. E., Aalbæk, B., and Schønheyder, H. (1994). Immunohistochemical identification
1722 of aetiological agents of systemic bovine zygomycosis. *J. Comp. Path*. **110,** 65-77.
1723 Jensen, H. E., Aalbæk, B., Lind, P., and Krogh, H. V. (1996). Immunohistochemical diagnosis
1724 of systemic bovine zygomycosis by murine monoclonal antibodies. *Vet. Pathol*. **33,** 176-
1725 183.
1726 Jensen, H. E., Salonen, J., and Ekfors, T. O. (1997). The use of immunohistochemistry to
1727 improve sensitivity and specificity in the diagnosis of systemic mycoses in patients with
1728 haematological malignancies. *J. Pathol*. **181,** 100-105.
1729 Johnson, G. L., Sarker, S. J., Nannini, F., Ferrini, A., Taylor, E., Lass-Flörl, C., Mutschlechner,
1730 W., Bustin, S. A., and Agrawal, S. G. (2015). *Aspergillus*-specific lateral-flow device and
1731 real-time PCR testing of bronchoalveolar lavage fluid: a combination biomarker
1732 approach for clinical diagnosis of invasive pulmonary aspergillosis. *J. Clin. Microbiol*. **53,**
1733 2103-2108.
1734 Jones, K. D., and Kaufman, L. (1978). Development and evaluation of an immunodiffusion
1735 test for diagnosis of systemic zygomycosis (mucormycosis): preliminary report. *J. Clin.*
1736 *Microbiol*. **7,** 97-103.
1737 Jurkunas, U., Behlau, I., and Colby, K. (2009). Fungal keratitis: changing pathogens and risk
1738 factors. *Cornea* **28,** 638-643.
1739 Kalteis, J., Rainer, J., and de Hoog, G. S. (2009). Ecology of *Pseudallescheria* and
1740 *Scedosporium* species in human-dominated and natural environments and their
1741 distribution in clinical samples. *Med. Mycol*. **47,** 398-405.
1742 Kaufman, L., Turner, L. F., and McLaughlin, D. W. (1989). Indirect Enzyme-Linked
1743 Immunosorbent Assay for zygomycosis. *J. Clin. Microbiol*. **27,** 1979-1982.
1744 Kaufman, L., Standard, P. G., Jalbert, M., and Kraft, D. E. (1997). Immunohistologic
1745 identification of *Aspergillus* spp. and other hyaline fungi by using polyclonal fluorescent
1746 antibodies. *J. Clin. Microbiol*. **35,** 2206-2209.
1747 Kaymaz, D., Ergün, P., Candemir, I., and Çicek, T. (2016). Chronic necrotising pulmonary
1748 aspergillosis presenting as transient thoracic mass: a diagnostic dilemma. *Respir. Med.*
1749 *Case Rep*. **19,** 140-142.
1750 Khan, Z. U., Ahmad, S., and Theyyathel, A. M. (2008). Diagnostic value of DNA and (1→3)-β-
1751 D-glucan detection in serum and bronchoalveolar lavage of mice experimentally
1752 infected with *Fusarium oxysporum*. *J. Med. Microbiol*. **57,** 36-42.
1753 Kitasato, Y., Tao, Y., Hoshino, T., Tachibana, K., Inoshima, N., Yoshida, M., Takata, S.,
1754 Okabayashi, K., Kawasaki, M., Iwanaga, T., and Aizawa, H. (2009). Comparison of
1755 *Aspergillus* galactomannan antigen testing with a new cut-off index and *Aspergillus*
1756 precipitating antibody testing for the diagnosis of chronic pulmonary aspergillosis.
1757 *Respirology* **14,** 701-708.
1758 Klont, R. R., Mennink-Kersten, M. A., and Verweij, P. E. (2004). Utility of *Aspergillus* antigen
1759 detection in specimens other than serum specimens. *Clin. Infect. Dis*. **39,** 1467-1474.
1760 Kobayashi, M., and Thornton, C. R. (2014). Serological diagnosis of chronic pulmonary
1761 aspergillosis (CPA) and *Aspergillus* lateral-flow device (LFD). *Eur. Resp. J*. **44,** P2501.
1762 Koehler, P., Tacke, D., and Cornely, O. A. (2014). Bone and joint infections by *Mucorales*,
1763 *Scedosporium*, *Fusarium*, and even rarer fungi. *Crit. Rev. Microbiol*. **9,** 1-14.
1764 Kondo, M., Goto, H., and Yamanaka, K. (2018). Case of *Scedosporium aurantiacum* infection
1765 in a subcutaneous abscess. *Med. Mycol. Case Rep*. **20,** 26-27.
1766 Kontoyiannis, D. P., Wessel, V. C., Bodey, G. P., and Rolston, K. V. (2000). Zygomycosis in the
1767 1990s in tertiary care cancer center. *Clin. Infect. Dis*. **30,** 851-856.

1768 Kontoyiannis, D. P., Marr, K. A., Park, B. J., Alexander, B. D., Anaissie, E. J., Walsh, T. J., Ito, J.,
1769 Andes, D. R., Baddley, J. W., Brown, J. M., Brumble, L. M., Freifeld, A. G., *et al*. (2010).
1770 Prospective surveillance for invasive fungal infections in hematopoietic stem cell
1771 transplant recipients, 2001-2006: overview of the Transplant-Associated Infection
1772 Surveillance Network (TRANSNET) database. *Clin. Infect. Dis*. **50,** 1091-1100.
1773 Kousha, M., Tadi, R., and Soubani, A. O. (2011). Pulmonary aspergillosis: a clinical review.
1774 *Eur. Respir. Rev.* **20,** 156-174.
1775 Kozel, T. R., and Wickes, B. (2014). Fungal diagnostics. *Cold Spring Harb. Perspect. Med.* **4,**
1776 a019299.
1777 Kronen, R., Liang, S. Y., Bochicchio, G., Bochicchio, K., Powderly, W. G., and Spec, A. (2017).
1778 Invasive fungal infections secondary to traumatic injury. *Int. J. Infect. Dis*. **62,** 102-111.
1779 Kyi, C., Hellmann, M. D., Wolchok, J. D., Chapman, P. B., and Postow, M. A. (2014).
1780 Opportunistic infections in patients treated with immunotherapy for cancer. *J.*
1781 *Immunother. Cancer* **2,** 19.
1782 Lackner, M., Najafzadeh, M. J., Sun, J., Lu, Q., and de Hoog, G. S. (2012). Rapid identification
1783 of *Pseudallescheria* and *Scedosporium* strains by using rolling circle amplification. *Appl.*
1784 *Environ. Microbiol*. **78,** 126-133.
1785 Lackner, M., de Hoog, G. S., Yang, L., Moreno, L. F., Ahmed, S. A., Andreas, F., Kalteis, J.,
1786 Nagl, M., Lass-Flörl, C., Risslegger, B., Rambach, G., Speth, C., *et al*. (2014). Proposed
1787 nomenclature for *Pseudallescheria*, *Scedosporium* and related genera. *Fungal Divers*.
1788 **67,** 1-10.
1789 Lamoth, F., and Calandra, T. (2017). Early diagnosis of invasive mould infections and disease.
1790 *J. Antimicrob. Chemotherap.* **72,** i19-i28.
1791 Lanternier, F., Cypowyj, S., Picard, C., Bustamante, J., Lortholary, O., Casanova, J-L., and
1792 Puel, A. (2013). Primary immunodeficiencies underlying fungal infections. *Curr. Opin.*
1793 *Pediatr*. **25,** 736-747.
1794 Lass-Flörl, C. (2009). Zygomycosis: conventional laboratory diagnosis. *Clin. Microbiol. Infect*.
1795 **15,** 60-65.
1796 Lass-Flörl, C. (2019). How to make a fast diagnosis in invasive aspergillosis. *Med. Mycol*. **57**,
1797 S155-S160.
1798 Latenser, B. A. (2003). *Fusarium* infections in burn patients: a case report and review of the
1799 literature. *J. Burn Care Rehabil*. **24,** 285-288.
1800 Latgé, J-P. (1999). *Aspergillus fumigatus* and Aspergillosis. *Clin. Microbiol. Rev*. **12,** 310-350.
1801 Lau, A. F., Drake, S. F., Calhoun, L. B., Henderson, C. M., and Zelany, A. M. (2013).
1802 Development of a clinically comprehensive database and a simple procedure for
1803 identification of moulds from solid media by matrix-assisted laser desorption ionisation-
1804 time of flight mass spectrometry. *J. Clin. Microbiol*. **51,** 1828-1834.
1805 Leal, S. M., Roy, S., Vareechon, C., deJesus Carion, S., Clark, H., Lopez-Berges, M. S., diPietro,
1806 A., Schrettl, M., Beckmann, N., Redl, B., Haas, H., and Pearlman, E. (2013). Targeting
1807 iron acquisition blocks infection with the fungal pathogens *Aspergillus fumigatus* and
1808 *Fusarium oxysporum*. *PLoS Pathog*. **9,** e1003436.
1809 Lecointe, K., Cornu, M., Leroy, J., Coulon, P., and Sendid, B. (2019). Polysaccharides cell wall
1810 architecture of Mucorales. *Front. Microbiol*. **10,** 469.
1811 Legouge, C., Caillot, D, Chrétien, M. L., Lafon, I., Ferrant, E., Audia, S., Pages, P. B., Roques,
1812 M., Estivalet, L., Martin, L., Maitre, T., Bastie, J. N., *et al*. (2014). The reversed halo sign:
1813 pathognomonic pattern of pulmonary mucormycosis in leukemic patients with
1814 neutropenia? *Clin. Infect. Dis*. **58,** 672-678.

1815 Lehmann, P. F., and Reiss, E. (1978). Invasive aspergillosis: antiserum for circulating antigen
1816 produced after immunisation with serum from infected rabbits. *Infect. Immun*. **20,** 570-
1817 572.
1818 Lelievre, L., Garcia-Hermoso, D., Abdoul, H., Hivelin, M., Chouaki, T., Toubas, D., Mamez, A-
1819 C., Lantieri, L., Lotholary, O., and Lanternier, F. (2014). Posttraumatic mucormycosis.
1820 *Medicine* **93,** 395-404.
1821 Lengerova, M., Racil, Z., Hrncirova, I., Kocmanova, I., Volfova, P., Ricna, D., Bejdak, P.,
1822 Moulis, M., Pavlovsky, Z., Weinbergerova, B., Toskova, M., and Mayer, J. (2014). Rapid
1823 detection and identification of mucormycetes in bronchoalveolar lavage samples from
1824 immunocompromised patients with pulmonary infiltrates by use of high-resolution melt
1825 analysis. *J. Clin. Microbiol*. **52,** 2824-2828.
1826 Leroy, P., and Smismans, A. (2007). Long-term risk of atypical fungal infection after near-
1827 drowning episodes: in reply. *Paediatrics* **119,** 417-418.
1828 Li, H., Hwang, S. K., Zhou, C., Du, J., and Zhang, J. (2013). Grangrenous cutaneous
1829 mucormycosis caused by *Rhizopus oryzae*: a case report and review of primary
1830 cutaneous mucormycosis in China over past 20 years. *Mycopathologia* **176,** 123-128.
1831 Liao, G. P., Karanjawala, B. E., Baba, S., Khalil, K. G., and Irani, A. D. (2015). A 23-year-old
1832 diabetic female with persistent pneumonia and chronic lung abscess. *Ann. Am. Thorac.*
1833 *Soc.* **12,** 1231-1234.
1834 Libert, D., Procop, G. W., and Ansari, M. Q. (2018). *Histoplasma* urinary antigen testing
1835 obviates the need for coincident serum antigen testing. *Am. J. Clin. Pathol*. **149,** 362-
1836 368.
1837 Limper, A. H., Adenis, A., Le, T., and Harrsion, T. S. (2017). Fungal infections in HIV/AIDS.
1838 *Lancet Infect. Dis*. **17,** e334-e443.
1839 Linscott, A. J. (2007). Natural disasters - a microbe's paradise. *Clin. Microbiol. Newslett*. **29,**
1840 57-62.
1841 Lu, Q., Gerrits van den Ende, A. H. G., Bakkers, J. M. J. E., Sun, J., Lackner, M., Najafzadeh, M.
1842 J., Melchers, W. J. G., Li, R., and de Hoog, G. S. (2011a). Identification of
1843 *Pseudallescheria* and *Scedosporium* species by three molecular methods. *J. Clin.*
1844 *Microbiol*. **49,** 960-967.
1845 Lu, Q., Gerrits van den Ende, A. H. G., de Hoog, G. S., Li, R., Accoceberry, I., Durand-Joly, I.,
1846 Bouchara, J.-P., Hernandez, F., and Delhaes, L. (2011b). Reverse line blot hybridisation
1847 screening of *Pseudallescheria*/*Scedosporium* species in patients with cystic fibrosis.
1848 *Mycoses* **54,** 5-11.
1849 Luna-Rodríguez, C. E., de Treviño-Rangel, R., Montoyas, A. M., Becerril-García, M. A.,
1850 Andrade, A., and González, G. M. (2019). *Scedosporium* spp.: chronicle of an emerging
1851 pathogen. *Medicina Universitaria* **21,** 4-13.
1852 Luplertlop, N. (2018). *Pseudallescheria*/*Scedosporium* complex species: from saprobic to
1853 pathogenic fungus. *J. Mycol. Med*. **28,** 249-256.
1854 Luptáková, D., Pluháček, T., Petřik, M., Novák, J., Palyzová, A., Sokolová, L., Škríba, A.,
1855 Šedivá, B., Lemr, K., and Havlíček, V. (2017). Non-invasive and invasive diagnoses of
1856 aspergillosis in a rat model by mass spectrometry. *Sci. Rep*. **7,** 16523.
1857 Maertens, J., Lagrou, K., Deweerdt, H., Surmont, I., Verhoef, G. E. G., Verhaegen, J., and
1858 Boogaerts, M. A. (2000). Disseminated infection by *Scedosporium prolificans*: an
1859 emerging fatality among haematology patients. Case report and review. *Ann. Hematol*.
1860 **79,** 340-344.

1861 Maertens, J. A., Klont, R., Masson, C., Theunissen, K., Meersseman, W., Lagrou, K., Heinen,
1862 C., Crépin, B., Van Eldere, J., Tabouret, M., Donnelly, J. P., and Verweij, P. E. (2007).
1863 Optimisation of the cutoff value for the *Aspergillus* double-sandwich enzyme
1864 immunoassay. *Clin. Infect. Dis*. **44,** 1329-1336.
1865 Máiz, L., Girón, R., Olveira, C., Vendrell, M., Nieto, R., and Martinez-García. (2015).
1866 Prevalence and factors associated with isolation of *Aspergillus* and *Candida* from
1867 sputum in patients with non-cystic fibrosis bronchiectasis. *Respiration* **89,** 396-403.
1868 Máiz, L., Nieto, R., Cantón, R., de la Pedrosa, E. G. G., and Martinez-García. (2018). Fungi in
1869 bronchiectasis: a concise review. *Int. J. Mol. Sci.* **19,** 142.
1870 Marom, E. M., Holmes, A. M., Bruzzi, J. F., Truong, M. T., and O'Sullivan, P. J., and
1871 Kontoyiannis, D. P. (2008). Imaging of pulmonary fusariosis in patients with hematologic
1872 malignancies. *Am. J. Roentgenol*. **190,** 1605-1609.
1873 Marr, K. A., Datta, K., Mehta, S., Ostrander, D. B., Rock, M., Francis, J., and Feldmesser, M.
1874 (2018). Urine antigen detection as an aid to diagnose invasive aspergillosis. *Clin. Infect.*
1875 *Dis*. **67,** 1705-1711.
1876 Marzolf, G., Sabou, M., Lannes, B., Cotton, F., Meyronet, D., Galanaud, D., Cottier, J-P.,
1877 Grand, S., Desal, H., Kreutz, J., Schenck, M., Meyer, N., *et al*. (2016). Magnetic
1878 resonance imaging of cerebral aspergillosis: imaging and pathological correlations. *PLoS*
1879 *ONE* **11,** e0152475.
1880 Mehl, H. L., and Epstein, L. (2008). Sewage and community shower drains are environmental
1881 reservoirs of *Fusarium* species complex group 1, a human and plant pathogen. *Env.*
1882 *Microbiol*. **10,** 219-227.
1883 Mercier, T., Guldentops, E., van Daele, R., and Maertens, J. (2018). Diagnosing invasive mold
1884 infections: what is next. *Curr. Fungal Infect. Rep*. **12,** 161-169.
1885 Mercier, T., Schauwvlieghe, A., de Kort, E., Dunbar, A., Reynders, M., Guldentops, E.,
1886 Rijnders, B., Verweij, P. E., Lagrou, K., and Maertens, J. (2019a). Diagnosing invasive
1887 pulmonary aspergillosis in hematology patients: a retrospective multicentre evaluation
1888 of a novel lateral flow device. *J. Clin. Microbiol*. **57,** e01913-18.
1889 Mercier, T., Dunbar, A., de Kort, E., Schauwvlieghe, A., Reynders, M., Guldentops, E.,
1890 Blijlevens, N. M. A., Vonk, A. G., Rijnders, B., Verweij, P. E., Lagrou, K., and Maertens, J.,
1891 on behalf of the Dutch-Belgian Mycosis Study Group. (2019). Lateral flow assays for
1892 diagnosing invasive pulmonary aspergillosis in adult hematology patients: A
1893 comparative multicenter study. *Med. Mycol.* **0,** 1-9.
1894 Millon, L., Herbrecht, R., Grenouillet, F., Morio, F., Alanio, A., Letscher-Bru, V., Cassaing, S.,
1895 Chouaki, T., Kaufmann-Lacroix, C., Poirier, P., Toubas, D., Augereau, O., *et al*. (2016).
1896 Early diagnosis and monitoring of mucormycosis by detection of circulating DNA in
1897 serum: retrospective analysis of 44 cases collected through the French Surveillance
1898 Network of Invasive Fungal Infections (RESSIF). *Clin. Microbiol. Infect*. **22,** 810.e1-
1899 810.e8.
1900 Misch, E. A., and Safdar, N. (2016). Updated guidelines for the diagnosis and management of
1901 aspergillosis. *J. Thorac. Dis*. **8,** E1771-E1776.
1902 Miyazaki, T., Hayashi, O., Oshima, Y., and Yadomae, T. (1979). Studies on fungal
1903 polysaccharides: The immunological determinant of the serologically active substances
1904 from *Absidia cylindrospora*, *Mucor hiemalis* and *Rhizopus nigricans*. *J. Gen. Microbiol*.
1905 **111,** 417-422.
1906 Mohanty, N. K., and Sahu, S. (2014). *Fusarium solani* infection in a kidney transplant
1907 recipient. *Indian J. Nephrol.* **24,** 312-314.

1908 Morad, H. O. J., Wild, A-M., Wiehr, S., Davies, G., Maurer, A., Pichler, B. J., and Thornton, C.
1909 R. (2018). Pre-clinical imaging of invasive candidiasis using immunoPET/MR. *Front.*
1910 *Microbiol*. **9,** 1996.
1911 Morio, F., Horeau-Langlard, D., Gay-Andrieu, F., Talarmin, J.-P., Haloun, A., Treilhaud, M.,
1912 Despins, P., Jossic, F., Nourry, L., Danner-Boucher, I., Pattier, S., Bouchara, J.-P., *et al*.
1913 (2010). Disseminated *Scedosporium*/*Pseudallescheria* infection after double-lung
1914 transplantation in patients with cystic fibrosis. *J. Clin. Microbiol*. **48,** 1978-1982.
1915 Mortensen, K. L., Mellado, E., Lass-Florl, C., Rodriguez-Tudela, J. L., Johansen, H.K., and
1916 Arendrup, M. C. (2010). Environmental study of azole-resistant *Aspergillus fumigatus*
1917 and other aspergilli in Austria, Denmark, and Spain. *Antimicrob. Agents Chemother*. **54,**
1918 4545-4549.
1919 Muhammed, M., Coleman, J. J., Carneiro, H. A., and Mylonakis, E. (2011). The challenge of
1920 managing fusariosis. *Virulence* **2,** 91-96.
1921 Muhammed, M., Anagnostou, T., Desalermos, A., Kourkoumpetis, T. K., Carneiro, H. A.,
1922 Glavis-Bloom, J., Coleman, J. J., and Mylonakis, E. (2013). *Fusarium* infection - Report of
1923 26 cases and review of 97 cases from the literature. *Medicine* **92,** 305-316.
1924 Muldoon, E. G., Sharman, A., Page, I., Bishop, P., and Denning, D. W. (2016). *Aspergillus*
1925 nodules; another presentation of chronic pulmonary aspergillosis. *BMC Pulm. Med*. **16,**
1926 123.
1927 Muraosa, Y., Schreiber, A. Z., Trabasso, P., Matsuzawa, T., Taguchi, H., Moretti, M. L.,
1928 Mikami, Y., and Kamei, K. (2014). Development of cycling probe-based real-time PCR
1929 system to detect *Fusarium* species and *Fusarium solani* species complex (FSSC). *Int. J.*
1930 *Med. Microbiol*. **304,** 505-511.
1931 Muraosa, Y., Oguchi, M., Yahiro, M., Watanabe, A., Yaguchi, T., and Kamei, K. (2017).
1932 Epidemiological study of *Fusarium* species causing invasive and superficial fusariosis in
1933 Japan. *Med. Mycol. J.* **58E,** E5-E13.
1934 Nakamura, Y., Utsumi, Y., Suzuki, N., Nakajima, Y., Murata, O., Sasaki, N., Nitanai, H.,
1935 Nagashima, H., Miyamoto, S., Yaegashi, J., Hatakeyama, T., Shibano, Y., *et al*. (2011).
1936 Multiple *Scedosporium apiospermum* abscesses in a woman survivor of a tsunami in
1937 northeastern Japan: a case report. *J. Med. Case Rep.* **25,** 526.
1938 Nakamura, Y., Suzuki, N., Nakajima, Y., Utsumi, Y., Murata, O., Nagashima, H., Saito, H.,
1939 Sasaki, N., Fujimura, I., Ogino, Y., Kato, K., Terayama, Y., *et al*. (2013). *Scedosporium*
1940 *aurantiacum* brain abscess after near-drowning in a survivor of a tsunami in Japan.
1941 *Respir. Investig*. **51,** 207-211.
1942 Notermans, S., and Soentoro, P. S. S. (1986). Immunological relationship of extra-cellular
1943 polysaccharide antigens produced by different mould species. *Antonie Leeuwenhoek* **52,**
1944 393-401.
1945 Nucci, M., and Anaissie, E. (2002). Cutaneous infection by *Fusarium* species in healthy and
1946 immunocompromised hosts: implications for diagnosis and management. *Clin. Infect.*
1947 *Dis*. **35,** 909-920.
1948 Nucci, M., and Anaissie, E. (2007). *Fusarium* infections in immunocompromised patients.
1949 *Clin. Microbiol. Rev.* **20,** 695-704.
1950 Nucci, M., Varon, A. G., Garnica, M., Akiti, T., Barreiros, G., Trope, B. M., and Nouér, S. A.
1951 (2013). Increased incidence of invasive fusariosis with cutaneous portal of entry, Brazil.
1952 *Emerg. Infect. Dis.* **19,** 1567-1572.

1953 Nucci, M, Carlesse, F., Cappellano, P., Varon, A. G., Seber, A., Garnica, M., Nouér, S. A., and
1954 Colombo, A. L. (2014). Earlier diagnosis of invasive fusariosis with *Aspergillus* serum
1955 galactomannan testing. *PLoS ONE* **9,** e87784.
1956 Nwankwo, L., Periselneris, J., Cheong, J., Thompson, K., Darby, P., Leaver, N., Schelenz, S.,
1957 and Armstrong-James, D. (2018). A prospective real-world study of the impact of
1958 antifungal stewardship program in a tertiary respiratory-medicine setting. *Antimicrob.*
1959 *Agents Chemother*. **62,** e00402-18.
1960 Ohba, H., Miwa, S., Shirai, M., Kanai, M., Eifuku, T., Suda, T., Hayakawa, H., and Chida, K.
1961 (2012). Clinical characteristics and prognosis of chronic pulmonary aspergillosis. *Resp.*
1962 *Med*. **106,** 724-729.
1963 Orasch, T., Prattes, J., Faserl, K., Eigl, S., Düttmann, W., Lindner, H., Haas, H., and Hoenigl, M.
1964 (2018). Bronchoalveolar lavage triacetylfusarinine C (TAFC) determination for diagnosis
1965 of invasive pulmonary aspergillosis in patients with hematological malignancies. *J.*
1966 *Infect*. **75,** 370-373.
1967 Orsi, C. F., Gennari, W., Venturelli, C., La Regina, A., Pecorari, M., Righi, E., Machetti, M., and
1968 Blasi, E. (2012). Performance of 2 commercial real-time polymerase chain reaction
1969 assays for the detection of *Aspergillus* and *Pneumocystis* DNA in bronchoalveolar lavage
1970 fluid samples from critical care patients. *Diagn. Microbiol. Infect. Dis.* **73,** 138-143.
1971 Padoan, R., Poli, P., Colombrita, D., Borghi, E., Timpano, S., and Berlucchi, M. (2016). Acute
1972 *Scedosporium apiospermum* endobronchial infection in cystic fibrosis*. Ped. Infect. Dis. J.*
1973 **35,** 701-702.
1974 Page, I. D., Richardson, M. D., and Denning, D. (2015). Antibody testing in aspergillosis - quo
1975 vadis? *Med. Mycol*. **53,** 417-439.
1976 Page, I. D., Richardson, M. D., and Denning, D. (2016). Comparison of six *Aspergillus*-specific
1977 IgG assays for the diagnosis of chronic pulmonary aspergillosis. *J. Infect*. **72,** 240-249.
1978 Page, I. D., Richardson, M. D., and Denning, D. (2019). Siemens Immulite *Aspergillus*-specific
1979 IgG assay for chronic pulmonary aspergillosis diagnosis. *Med. Mycol*. **57,** 300-307.
1980 Pan, Z., Fu, M., Zhang, J., Zhou, H., Fu, Y., and Zhou, J. (2015). Diagnostic accuracy of a novel
1981 lateral-flow device in invasive aspergillosis: a meta-analysis. *J. Med. Microbiol*. **64,** 702-
1982 707.
1983 Paolino, K. M., Henry, J. A., Hospenthal, D. R., Wortmann, G. W., and Hartzell, J. D. (2012).
1984 Invasive fungal infections following combat-related injury. *Mil. Med*. **177,** 681-685.
1985 Pappas, P. G., Alexander, B. D., Andes, D. R., Hadley, S., Kauffman, C. A., Freifeld, A.,
1986 Anaissie, E. J., Brumble, L. M., Herwaldt, L., Ito, J., Kontoyiannis, D. P., Lyon, G. M., *et al*.
1987 (2010). Invasive fungal infections among organ transplant recipients: results of the
1988 Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin. Infect. Dis*. **50,**
1989 1101-1111.
1990 Patterson, T. F., Thompson, G. R., Denning, D. W., Fishman, J. A., Hadley, S., Herbrecht, R.,
1991 Kontoyiannis, D. P., Marr, K. A., Morrison, V. A., Nguyen, M. H., Segal., B. H., Steinbach,
1992 W. J., *et al*. (2016). Practice guidelines for the diagnosis and management of
1993 aspergillosis: 2016 update by the Infectious Diseases Society of America. *Clin. Infect.*
1994 *Dis*. **15,** e1-e60.
1995 Patterson, T. F., and Donnelly, J. P. (2019). New concepts in diagnostics for invasive
1996 mycoses: non-culture-based methodologies. *J. Fungi* **5,** 9.
1997 Pegorie, M., Denning, D. W., and Welfare, W. (2017). Estimating the burden of invasive and
1998 serious fungal disease in the United Kingdom. *J. Infect*. **74,** 60-71.

1999 Pergam, S. A. (2017). Fungal pneumonia in patients with hematologic malignancies and
2000 hematopoietic cell transplantation. *Clin. Chest Med*. **38,** 279-294.
2001 Petrik, M., Haas, H., Dobrozemsky, G., Lass-Flörl, C., Helbok, A., Blatzer, M., Dietrich, H., and
2002 Decristiforo, C. (2010). ^{68}Ga-siderophores for PET imaging of invasive pulmonary
2003 aspergillosis: proof of principle. *J. Nucl. Med.* **51,** 639-645.
2004 Petrik, M., Fransenn, G. M., Haas, H., Laverman, P., Hörtnagl, C., Schrettl, M., Helbok, A.,
2005 Lass-Flörl, C., and Decristiforo, C. (2012a). Preclinical evaluation of two ^{68}Ga-
2006 siderophores as potential radiopharmaceuticals for *Aspergillus fumigatus* infection
2007 imaging. *Eur. J. Nucl. Med. Mol. Imaging* **39,** 1175-1183.
2008 Petrik, M., Haas, H., Schrettl, M., Helbok, A., Blatzer, M., and Decristiforo, C. (2012b). *In vitro*
2009 and *in vivo* evaluation of selected (68)Ga-siderophores for infection imaging. *Nucl. Med.*
2010 *Biol*. **39,** 361-369.
2011 Petrik, M., Haas, H., Laverman, P., Schrettl, M., Franssen, G. M., Blatzer, M., and
2012 Decristiforo, C. (2014). ^{68}Ga-triacetylfusarinine C and ^{68}Ga-ferrioxamine E for *Aspergillus*
2013 infection imaging: uptake specificity in various microrganisms. *Mol. Imaging Biol*. **16,**
2014 102-108.
2015 Petrik, M., Vlckova, A., Novy, Z., Urbanek, L., Haas, H., and Decristoforo, C. (2015). Selected
2016 ^{68}Ga-siderophores versus ^{68}Ga-colloid and ^{68}Ga-citrate: biodistribution and small animal
2017 imaging in mice. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub*. **159,** 60-
2018 66.
2019 Petrikkos, G., Skiada, A., Lortholary, O., Roilides, E., Walsh, T., and Kontoyiannis, D. P.
2020 (2012). Epidemiology and clinical maifestations of mucormycosis. *Clin. Infect. Dis*. **54,**
2021 S23-S34.
2022 Piarroux, R. P., Romain, T., Martin, A., Vainqueur, D., Vitte, J., Lachaud, L., Gangneux, J-P.,
2023 Gabriel, F., Fillaux, J., and Ranque, S. (2019). Multicenter evaluation of a novel
2024 immunochromatographic test for anti-aspergillus IgG detection. *Front. Cell. Infect.*
2025 *Microbiol*. **9,** 12.
2026 Postina, P., Skladny, J., Boch, T., Cornely, O. A., Hamprecht, A., Rath, P-M., Steinmann, J.,
2027 Bader, O., Miethke, T., Dietz, A., Merker, N., Hofmann, W-K., *et al*. (2018). Comparison
2028 of two molecular assays for detection and characterisation of *Aspergillus fumigatus*
2029 triazole resistance and Cyp51A mutations in clinical isolates and primary clinical
2030 samples of immunocompromised patients. *Front. Microbiol*. **9,** 555.
2031 Prattes, J., Flick, H., Prüller, F., Koidl, C., Raggam, R. B., Palfner, M., Eigl, S., Buzina, W.,
2032 Zollner-Schwetz, I., Thornton, C. R., Krause, R., and Hoenigl, M. (2014). Novel tests for
2033 diagnosis of invasive aspergillosis in patients with underlying respiratory diseases. *Am.*
2034 *J. Resp. Crit. Care. Med.* **190,** 922-929.
2035 Raghunath, V., Priyanka, K. H. R., Kiran, C. G., and Deepthi, R. M. (2018). Mucormycosis - can
2036 the diagnosis be challenging at times? *SRM J. Res. Dent. Sci*. **9,** 191-196.
2037 Rainer, J., de Hoog, G. S., Wedde, M., Graser, Y., and Gilges, S. (2000). Molecular variability
2038 of *Pseudallescheria boydii*, a neurotropic opportunist. *J. Clin. Microbiol*. **38,** 3267-3273.
2039 Rainer, J., and de Hoog, G. S. (2006). Molecular taxonomy and ecology of *Pseudallescheria*,
2040 *Petriella* and *Scedosporium prolificans* (Microascaceae) containing opportunist agents
2041 on humans. *Mycol. Res*. **110,** 151-160.
2042 Rainer, J., Kalteis, J, de Hoog, S. G., and Summerbell, R. C. (2008). Efficacy of a selective
2043 isolation procedure for members of the *Pseudallescheria boydii* complex. *Antonie*
2044 *Leeuwenkoek* **93,** 315-322.

2045 Ramirez-Garcia, A., Pellon, A., Rementeria, A., Buldain, I., Barreto-Bertger, E., Rollin-
2046 Pinheiro, R., de Meirelles, J. V., Xisto, M. I. D. S., Ranque, S., Havlicek, V., Vandeputte, P.,
2047 Le Govic, Y., *et al*. (2018). *Scedosporium* and *Lomentospora*: an updated overview of
2048 underrated opportunists. *Med. Mycol*. **56,** S102-S125.
2049 Rammaert, B., Lanternier, F., Poirée, S., Kania, R., and Lortholary, O. (2012). Diabetes and
2050 mucormycosis: a complex interplay. *Diabetes and Metabolism* **38,** 193-204.
2051 Ramsperger, M., Duan, S., Sorrell, T. C., Meyer, W., and Chen, S. C.-A. (2014). The genus
2052 *Scedosporium* and *Pseudallescheria*: current challenges in laboratory diagnosis. *Curr.*
2053 *Clin. Micro. Rpt*. **1,** 27-36.
2054 Rankin, N. E. (1953). Disseminated aspergillosis and moniliasis associated with
2055 agranulocytosis and antibiotic therapy. *Br. Med. J.* **1,** 918-919.
2056 Rath, P-M., and Steinmann, J. (2018). Overview of commercially available PCR assays for the
2057 detection of *Aspergillus* spp. DNA in patient samples. *Front. Microbiol*. **9,** 740.
2058 Reed, C. (1978). Variability in antigenicity of *Aspergillus fumigatus*. *J. Allergy Clin. Immunol.*
2059 **61,** 227-229.
2060 Reinwald, M., Boch, T., Hofmann, W. K., and Buchheidt, D. (2016). Risk of infectious
2061 complications in hemato-oncological patients treated with kinase inhibitors. *Biomark*
2062 *Insights* **10,** 55-68.
2063 Ribes, J. A., Vanover-Sams, C. L., and Baker, D. J. (2000). Zygomycetes in human disease.
2064 *Clin. Microbiol. Rev.* **13,** 236-301.
2065 Richardson, M. (2009). The ecology of the Zygomycetes and its impact on environmental
2066 exposure. *Clin. Microbiol. Infect*. **15,** 2-9.
2067 Richardson, M., and Page, I. (2018). Role of serological tests in the diagnosis of mold
2068 infections. *Curr. Fungal Infect. Rep*. **12,** 127-136.
2069 Rimek, D., Singh, J., and Kappe, R. (2003). Cross-reactivity of the PLATELIA *CANDIDA* antigen
2070 detection enzyme immunoassay with fungal extracts. *J. Clin. Microbiol*. **41,** 3395-3398.
2071 Rivero-Menendez, O., Alastruey-Izquidero, A., Mellado, E., and Cuenca-Estrella, M. Triazole
2072 resistance in *Aspergillus* spp.: a worldwide problem? *J. Fungi* **2,** 21.
2073 Rodriguez-Tudela, J. L., Berenguer, J., Guarro, J., Kantarcioglu, A. S., Horre, R., de Hoog, G. S.,
2074 and Cuenca-Estrella, M. (2009). Epidemiology and outcome of *Scedosporium prolificans*
2075 infection, a review of 162 cases. *Med. Mycol*. **47,** 359-370.
2076 Rogers, T. R., Haynes, K. A., and Barnes, R. A. (1990). Value of antigen detection in predicting
2077 invasive pulmonary aspergillosis. *Lancet* **336,** 1210-1213.
2078 Rognon, B., Barrera, C., Monod, M., Valot, B., Roussel, S., Quadroni, M., Jouneau, S., Court-
2079 Fortune, I., Caillaud, D., Fellrath, J-M., Dalphin, J-C., Reboux, G., *et al*. (2016).
2080 Identification of antigenic proteins from *Lichtheimia corymbifera* for farmer's lung
2081 disease diagnosis. *PLoS ONE* **11,** e0160888.
2082 Rolfe, N. E., Haddad, T. J., and Wills, T. S. (2013). Management of *Scedosporium*
2083 *apiospermum* in a pre- and post-lung transplant patient with cystic fibrosis. *Med. Mycol.*
2084 *Case Rep*. **2,** 37-39.
2085 Rolle, A-M., Hasenberg, M., Thornton, C. R., Solouk-Saran, D., Männ, L., Weski, J., Maurer,
2086 A., Fischer, E., Spycher, P. R., Boschetti, F., Stegemann-Koniszewski, S., Bruder, D., *et al*.
2087 (2016). ImmunoPET/MR imaging allows specific detection of *Aspergillus* lung infection
2088 *in vivo*. *Proc. Natl. Acad. Sci. U.S.A*. **113,** E1026-E1033.
2089 Rougeron, A., Schullar, G., Leto, J., Sitterié, E., Landry, D., Bougnoux, M-E., Kobi, A.,
2090 Bouchara, J-P., and Giraud, S. (2015). Human-impacted areas of France are

2091 environmental reservoirs of the *Pseudallescheria boydii*/*Scedosporium apiospermum*
2092 species complex. *Env. Microbiol*. **17,** 1039-1048.
2093 Ruhnke, M., Behre, G., Buchheidt, D., Christopeit, M., Hamprecht, A., Heinz, W., Heussel, C-
2094 P., Horger, M., Kurzai, O., Karthaus, M., Löffler, J., Maschmeyer, G., *et al*. (2018).
2095 Diagnosis of invasive fungal diseases in haematology and oncology: 2018 update of the
2096 recommendations of the infectious diseases working party of the German society for
2097 hematology and medical oncology (AGIHO). *Mycoses* **61,** 796-813.
2098 Saito, T., Imaizumi, M., Kudo, K., Hotchi, M., Chikaoka, S., Yoshinari, M., Suwabe, N., Sato,
2099 A., Suzuki, H., and Iinuma, K. (1999). Disseminated *Fusarium* infection identified by the
2100 immunohistochemical staining in a patient with a refractory leukemia. *Tohoku J. Exp.*
2101 *Med*. **187,** 71-77.
2102 Saito, S., Michailides, T. J., and Xiao, C. L. (2016). Mucor rot - an emerging postharvest
2103 disease of mandarin fruit caused by *Mucor piriformis* and other *Mucor* spp. in California.
2104 *Plant Dis*. **100,** 1054-1063.
2105 Salehi, E., Hedayati, M. T., Zoll, J., Rafati, H., Ghasemi, M., Doroudinia, A., Abastabar, M.,
2106 Tolooe, A., Snelders, E., van der Lee, H. A., Rijs, A. J. M. M., Verweij, P. E., *et al*.
2107 Discrimination of aspergillosis, mucormycosis, fusariosis, and scedosporiosis in
2108 formalin-fixed paraffin-embedded tissue specimens by use of multiple real-time
2109 quantitative PCR assays. *J. Clin. Microbiol*. **54,** 2798-2803.
2110 Salonen, J., Lehtonen, O-P., Teräsjärvi, M-R., and Nikoskelainen, J. (2000). *Aspergillus*
2111 antigen in serum, urine and bronchoalveolar lavage specimens of neutropenic patients
2112 in relation to clinical outcome. *Scand. J. Infect. Dis*. **32,** 485-490.
2113 Salzer, H. J. F., Prattes, J., Flick, H., Reimann, M., Heyckendorf, J., Kalsdorf, B., Obersteiner,
2114 S., Gaede, K. I., Herzmann, C., Johnson, G. L., Lange, C., and Hoenigl, M. (2018).
2115 Evaluation of galactomannan testing, the *Aspergillus*-specific lateral-flow device test
2116 and levels of cytokines in bronchoalveolar lavage fluid for diagnosis of chronic
2117 pulmonary aspergillosis. *Front. Microbiol*. **9,** 2223.
2118 Sandven, P. E. R., and Eduard, W. (1992). Detection and quantification of antibodies against
2119 *Rhizopus* by enzyme-linked immunosorbent assay. *APMIS* **100,** 981-987.
2120 Santos, P. E., Oleastra, M., Galicchio, M., and Zelazko, M. (2000). Fungal infections in
2121 paediatric patients with chronic granulomatous disease. *Rev. Iberoam. Micol*. **17,** 6-9.
2122 Santos, C., Paterson, R. R. M., Venancio, A., and Lima, N. (2010). Filamentous fungal
2123 characterisation by matrix-assisted laser desorption/ionisation time of flight
2124 spectrometry. *J. Appl. Microbiol*. **108,** 378-385.
2125 Schauwvlieghe, A. F. A. D., Vank, A. G., Buddingh, E. P., Hoe, R. A. S., Dalm, V. A., Klaassen, C.
2126 H. W., and Rijnders, B. J. A. (2017). Detection of azole-susceptible and azole-resistant
2127 *Aspergillus* coinfection by cyp51A PCR amplicon melting curve analysis. *J. Antimicrob.*
2128 *Chemotherap*. **72,** 3047-3050.
2129 Scheel, C. M., Hurst, S. F., Barreiros, G., Akiti, T., Nucci, M., and Balajee, S. A. (2013).
2130 Molecular analyses of *Fusarium* isolates recovered from a cluster of invasive mold
2131 infections in a Brazilian hospital. *BMC Infect. Dis*. **13,** 49.
2132 Schrödl, W., Heydel, T., Schwartze, V. U., Hoffmann, K., Grosse-Herrenthey, A., Walther, G.,
2133 Alastruey-Izquierdo, A., Rodriguez-Tudela, J. L., Olias, P., Jacobsen, I. D., de Hoog, G. S.,
2134 and Voigt, K. (2012). Direct analysis and identification of pathogenic *Lichtheimia* species
2135 by matrix-assisted laser desorption ionisation-time of flight analyser-mediated mass
2136 spectrometry. *J. Clin. Microbiol*. **50,** 419-427.

2137 Schumock, G. T., Li, E. C., Suda, K. J., Wiest, M.D., Stubbings, J., Mutusiak, L. M., Hunkler, R.
2138 J., and Vermeulen, L. C. (2016). National trends in prospective drug expenditures and
2139 projections for 2016. *Am. J. Health Sys. Pharm.* **73,** 1058-1075.
2140 Schwarz, C., Thronicke, A., Staab, D., and Tintelnot, K. (2015). *Scedosporium apiospermum*: a
2141 fungal pathogen in a patient with cystic fibrosis. *JMM Case Rep.* **2,** 1-5.
2142 Schwarz, C., Brandt, C., Antweiler, E., Krannich, A., Staab, D., Schmitt-Grohé, S., Fischer, R.,
2143 Hartl, D., Thronicke, A., and Tintelnot, K. (2017). Prospective multicentre German study
2144 on pulmonary colonisation with *Scedosporium*/*Lomentospora* species in cystic fibrosis:
2145 epidemiology and new association factors. *PLoS ONE* **12,** e0171485.
2146 Schwarz, C., Brandt, C., Whitaker, P., Sutharsan, S., Skopnik, H., Gartner, S., Smazny, C., and
2147 Röhmel, J. F. (2018). Invasive pulmonary fungal infections in cystic fibrosis.
2148 *Mycopathologia* **183,** 33-43.
2149 Schwartz, R. A. (2004). Superficial fungal infections. *Lancet* **364,** 1173-1182.
2150 Sedlacek, L., Graf, B., Schwarz, C., Albert, F., Peter, S., Würstl, B., Wagner, S., Klotz, M.,
2151 Becker, A., Haase, G., Laniado, G., Kahl, B., *et al*. (2015). Prevalence of *Scedosporium*
2152 species and *Lomentospora prolificans* in patients with cystic fibrosis in a multicentre
2153 trial by use of a selective medium. *J. Cystic Fibrosis* **14,** 237-241.
2154 Segal, B. H. (2009). Aspergillosis. *N. Engl. J. Med*. **360,** 1870-1884.
2155 Sehgal, I. S., Choudhary, H., Dhooria, S., Aggrawal, A. N., Garg, M., Chakrabarti, A., and
2156 Agarwal, R. (2018). Diagnostic cut-off of *Aspergillus fumigatus*-specific IgG in the
2157 diagnosis of chronic pulmonary aspergillosis. *Mycoses* **61,** 770-776.
2158 Seidel, D., Meißner, A., Lackner, M., Piepenbrock, E., Salmanton-García, J., Stecher, M.,
2159 Mellinghoff, S., Hamprecht, A., Graeff, L. D., Köhler, P., Cheng, M. P., Denis, J., *et al*.
2160 (2019). Prognostic factors in 264 adults with invasive *Scedosporium* spp. and
2161 *Lomentospora prolificans* infection reported in the literature and FungiScope. *Crit. Rev.*
2162 *Microbiol*. **45,** 1-21.
2163 Sharma, A., and Singh, D. (2015). *Scedosporium apiospermum* causing brain abscess in a
2164 renal allograft recipient. *Saudi J. Kidney Dis. Transpl*. **26,** 1253-1256.
2165 Short, D. P. G., O'Donnell, K., Zhang, N., Juba, J. H., and Geiser, D. M. (2011). Widespread
2166 occurrence of diverse human pathogenic types of the fungus *Fusarium* detected in
2167 plumbing drains. *J. Clin. Microbiol*. **49,** 4264-4272.
2168 Short, D. P. G., O'Donnell, K., Thrane, U., Nielsen, K. F., Zhang, N., Juba, J. H., and Geiser, D.
2169 M. (2013). Phylogenetic relationships among members of the *Fusarium solani* species
2170 complex in human infections and the descriptions of *F. keratoplasticum* sp. nov. and *F.*
2171 *petroliphilum* stat. nov. *Fungal Genet. Biol.* **53,** 59-70.
2172 Sircar, G., Saha, B., Mandal, R. S., Pandey, N., Saha, S., and Bhattacharya, S. G. (2015).
2173 Purification, cloning and immuno-biochemical characterisation of a fungal aspartic
2174 protease allergen Rhi o 1 from the airborne mold *Rhizopus oryzae*. *PLoS ONE* **10,**
2175 e0144547.
2176 Skiada, A., Lanternier, F., Groll, A. H., Pagano, L., Zimmerli, S., Herbrecht, R., Lortholary, O.,
2177 and Petrikkos, G. L. (2013). Diagnosis and treatment of mucormycosis in patients with
2178 hematological malignancies: guidelines from the 3rd European Conference on Infections
2179 in Leukemia (ECIL 3). *Haematologica* **98,** 492-504.
2180 Skiada, A., Lass-Floerl, C., Klimko, N., Ibrahim, A., Roilides, E., and Petrikkos, G. (2017).
2181 Challenges in the diagnosis and treatment of mucormycosis. *Med. Mycol*. **56,** S93-S101.
2182 Smith, N. L., and Denning, D. W. (2011). Underlying conditions in chronic pulmonary
2183 aspergillosis including simple aspergilloma. *Eur. Resp. J.* **37,** 865-872.

2184 Son, J. H., Lim, H. B., Lee, S. H., Yang, J. W., and Lee, S. B. (2016). Early differential diagnosis
2185 of rhino-orbito-cerebral mucormycosis and bacterial orbital cellulitis: based on
2186 computed tomography findings. *PLoS ONE* **11,** e0160897.
2187 Song, M. J., Lee, J. H., and Lee, N. Y. (2009). Fatal disseminated *Scedosporium prolificans*
2188 infection in a paediatric patient with acute lymphoblastic leukemia. *Mycoses* **54,** 81-83.
2189 Starkey, J., Moritani, T., and Kirby, P. (2014). MRI and CNS fungal infections: review of
2190 aspergillosis to histoplasmosis and everything else. *Clin. Neuroradiol*. **24,** 217-230.
2191 Steinmann, J., Schmidt, D., Buer, J., and Rath, P.-M. (2011). Discrimination of *Scedosporium*
2192 *prolificans* against *Pseudallescheria boydii* and *Scedosporium apiospermum* by semi-
2193 automated repetitive sequence-based PCR. *Med. Mycol*. **49,** 475-483.
2194 Stynen, D., Sarfati, J., Goris, A., Prévost, M.-C., Lesourd, M., Kamphuis, H., Darras, V., and
2195 Latgé, J.-P. (1992). Rat monoclonal antibodies against *Aspergillus* galactomannan.
2196 *Infect. Immun*. **60,** 2237-2245.
2197 Stynen, D., Goris, A., Sarfati, J., and Latgé, J.-P. (1995). A new sensitive sandwich enzyme-
2198 linked immunosorbent assay to detect galactofuran in patients with invasive
2199 aspergillosis. *J. Clin. Microbiol*. **33,** 497-500.
2200 Sugui, J. A., Christensen, J. A., Bennett, J. E., Zelazny, A. M., and Kwon-Chung, K. J. (2011).
2201 Hematogenously disseminated skin disease caused by *Mucor velutinosus* in a patient
2202 with acute myeloid leukemia. *J. Clin. Microbiol*. **49,** 2728-2732.
2203 Sugui, J. A., Kwon-Chung, K. J., Juvvadi, P. R., Latge, J-P., and Steinbach, W. J. (2015).
2204 *Aspergillus fumigatus* and related species. *Cold Spring Harb. Perpect. Med*. **5,** a019786.
2205 Taj-Aldeen, S. J., Rammaert, B., Gamaletsou, M. N., Sipsas, N. V., Zeller, V., Roilides, E.,
2206 Kontoyiannis, D. P., Miller, A. O., Petraitis, V., Walsh, T. J., and Lortholary, O. (2015).
2207 Osteoarticular infections caused by non-*Aspergillus* filamentous fungi in adult and
2208 pediatric patients. *Medicine* **94,** 1-13.
2209 Taj-Aldeen, S. J., Gamaletsou, M. N., Rammaert, B., Sipsas, N. V., Zeller, V., Roilides, E.,
2210 Kontoyiannis, D. P., Henry, M., Petraitis, V., Moriyama, B., Denning, D. W., Lortholary,
2211 O., *et al*. (2017). Bone and joint infections caused by mucormycetes: a challenging
2212 osteoarticular mycosis of the twenty-first century. *Med. Mycol*. **55,** 691-704.
2213 Takazono, T., and Izumikawa, K. (2018). Recent advances in diagnosing chronic pulmonary
2214 aspergillosis. *Front. Microbiol*. **9,** 1810.
2215 Takazono, T., Ito, Y., Tashiro, M., Nishimura, K., Saijo, T., Yamamoto, K., Imamura, Y.,
2216 Miyazaki, T., Yanagihara, K., Mukae, H., and Izumikawa, K. (2019). Evaluation of
2217 *Aspergillus*-specific Lateral-flow device test using serum and bronchoalveolar lavage
2218 fluid for diagnosis of chronic pulmonary aspergillosis. *J. Clin. Microbiol*. **57,** e00095-19.
2219 Tamaki, M., Nozaki, K., Onishi, M., Yamamoto, K., Ujiie, H., and Sugahara, H. (2016). Fungal
2220 meningitis caused by *Lomentospora prolificans* after allogeneic hematopoietic stem cell
2221 transplantation. *Transpl. Infect. Dis.* **18,** 601-605.
2222 Tammer, I., Tintelnot, K., Braun-Dullaeus, R. C., Mawrin, C., Scherlach, C., Schlüter, D., and
2223 König, W. (2011). Infections due to *Pseudallescheria*/*Scedosporium* species in patients
2224 with advanced HIV disease - a diagnostic and therapeutic challenge. *Int. J. Infect. Dis*.
2225 **15,** e422-e429.
2226 Tashiro, T., Izumikawa, K., Tashiro, M., Takazono, T., Morinaga, Y., Yamamoto, K., Imamura,
2227 Y., Miyazaki, T., Seki, M., Kakeya, H., Yamamoto, Y., Yanagihara, K., *et al*. (2011).
2228 Diagnostic significance of *Aspergillus* species isolated from respiratory samples in an
2229 adult pneumology ward. *Med. Mycol*. **49,** 581-587.

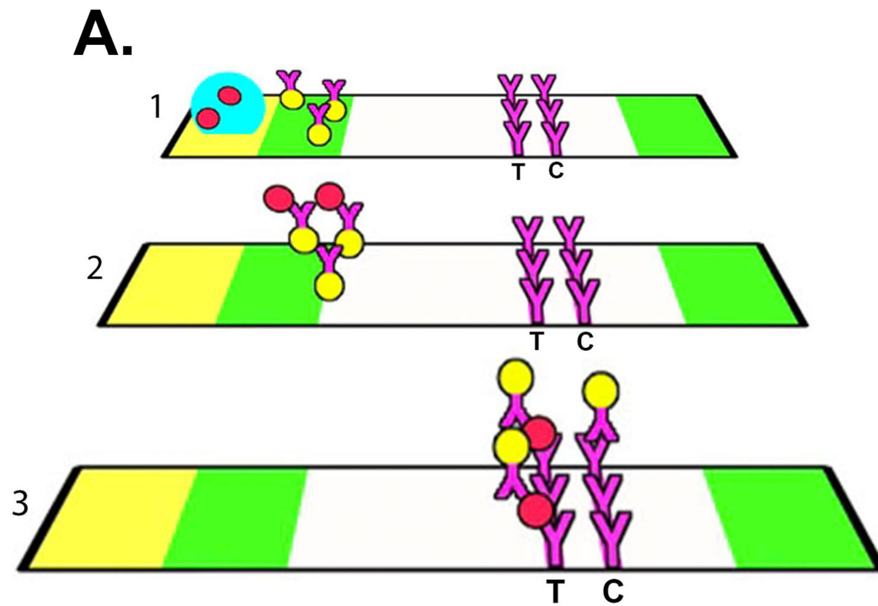
2230 Taylor, A., Talbot, J., Bennett, P., Martin, P., Makara, M., and Barrs, V. R. (2014).
2231 Disseminated *Scedosporium prolificans* infection in a Labrador retriever with immune
2232 mediated haemolytic anaemia. *Med. Mycol. Case Rep.* **6,** 66-69.
2233 Thornton, C. R. (2008). Development of an immunochromatographic lateral-flow device for
2234 rapid serodiagnosis of invasive aspergillosis. *Clin. Vaccine Immunol*. **15,** 1095-1105.
2235 Thornton, C. R. (2009). Tracking the emerging human pathogen *Pseudallescheria boydii* by
2236 using highly specific monoclonal antibodies. *Clin. Vaccine Immunol*. **16,** 756-764.
2237 Thornton, C. R. (2010). Detection of invasive aspergillosis. *Adv. Appl. Microbiol*. **70,** 187-216.
2238 Thornton, C. R. (2014). Breaking the mould - novel diagnostic and therapeutic strategies for
2239 invasive pulmonary aspergillosis in the immune deficient patient. *Exp. Rev. Clin.*
2240 *Immunol*. **6,** 771-780.
2241 Thornton, C. R., Ryder, L. S., Le Cocq, K., and Soanes, D. M. (2015). Identifying the emerging
2242 human pathogen *Scedosporium prolificans* by using a species-specific monoclonal
2243 antibody that binds to the melanin biosynthetic enzyme tetrahydroxynaphthalene
2244 reductase. *Environ. Microbiol*. **17,** 1023-1038.
2245 Thornton, C. R., and Wills, O. E. (2015). Immunodetection of fungal and oomycete
2246 pathogens: established and emerging threats to human health, animal welfare and
2247 global food security. *Crit. Rev. Microbiol*. **41,** 27-51.
2248 Thornton, C. R. (2018). Molecular imaging of invasive pulmonary aspergillosis using
2249 immunoPET/MRI: the future looks bright. *Front. Microbiol*. **9,** 691.
2250 Tomazin, G., and Matos, T. (2017). Fungal infections in patients with cystic fibrosis. *Zdrav*
2251 *Vestn*. **86,** 42-52.
2252 Torelli, R., Sanguinetti, M., Moody, A., Pagano, L., Caira, M., De Carolis, E., Fuso, L., De
2253 Pascale, G., Bello, G., Antonelli, M., Fadda, G., and Posteraro, B. (2011). Diagnosis of
2254 invasive aspergillosis by a commercial real-time PCR assay for *Aspergillus* DNA in
2255 bronchoalveolar lavage fluid samples from high-risk patients compared to a
2256 galactomannan enzyme immunoassay. *J. Clin. Microbiol*. **49,** 4273-4278.
2257 Torres, H. J., and Kontoyiannis, D. (2011). Hyalohyphomycosis (hyaline molds). In C. A.
2258 Kauffman, P. G. Pappas, J. D. Sobel, & W. E. Dismukes (Eds.), *Essentials of clinical*
2259 *mycology* (pp. 281-304). New York: Springer Science and Business Media.
2260 Tortorano, A. M., Esposto, M. C., Prigitano, A., Grancini, A., Ossi, C., Cavanna, C., and Cascio,
2261 G. L. (2012). Cross-reactivity of *Fusarium* spp. in the *Aspergillus* galactomannan enzyme-
2262 linked immunosorbent assay. *J. Clin. Microbiol*. **50,** 1051-1053.
2263 Tortorano, A. M., Richardson, M., Roilides, E., van Diepeningen, A., Caira, M., Munoz, P.,
2264 Johnson, E., Meletiadis, J., Pana, Z.-D., Lackner, M., Verweij, P., Freiberger, T., *et al*.
2265 (2014). ESCMID and ECMM joint guidelines on diagnosis and management of
2266 hyalohyphomycosis: *Fusarium* spp., *Scedosporium* spp. and others. *Clin. Microbiol.*
2267 *Infect*. **20,** 27-46.
2268 Tribble, D. R., and Rodriguez, C. J. (2014). Combat-related invasive fungal wound infections.
2269 *Curr. Fungal Infect. Rep*. **8,** 277-286.
2270 Ullmann, A. J., Aguado, J. M., Arikan-Akdagli, S., Denning, D. W., Groll, A. H., Lagrou, K., Lass-
2271 Flörl, C., Lewis, R. E., Munoz, P., Verweij, P. E., Warris, A., Ader, F., *et al*. (2017).
2272 Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017
2273 ESCMID-ECMM-ERS guideline. *Clin. Microbiol. Infect*. **24,** e1-e38.
2274 Ustun, C., Farrow, S., DeRemer, D., Fain, H., and Jillela, A. P. (2007). Early fatal *Rhizopus*
2275 infection on voriconazole prophylaxis following allogeneic stem cell transplantation.
2276 *Bone Marrow Transpl*. **39,** 807-808.

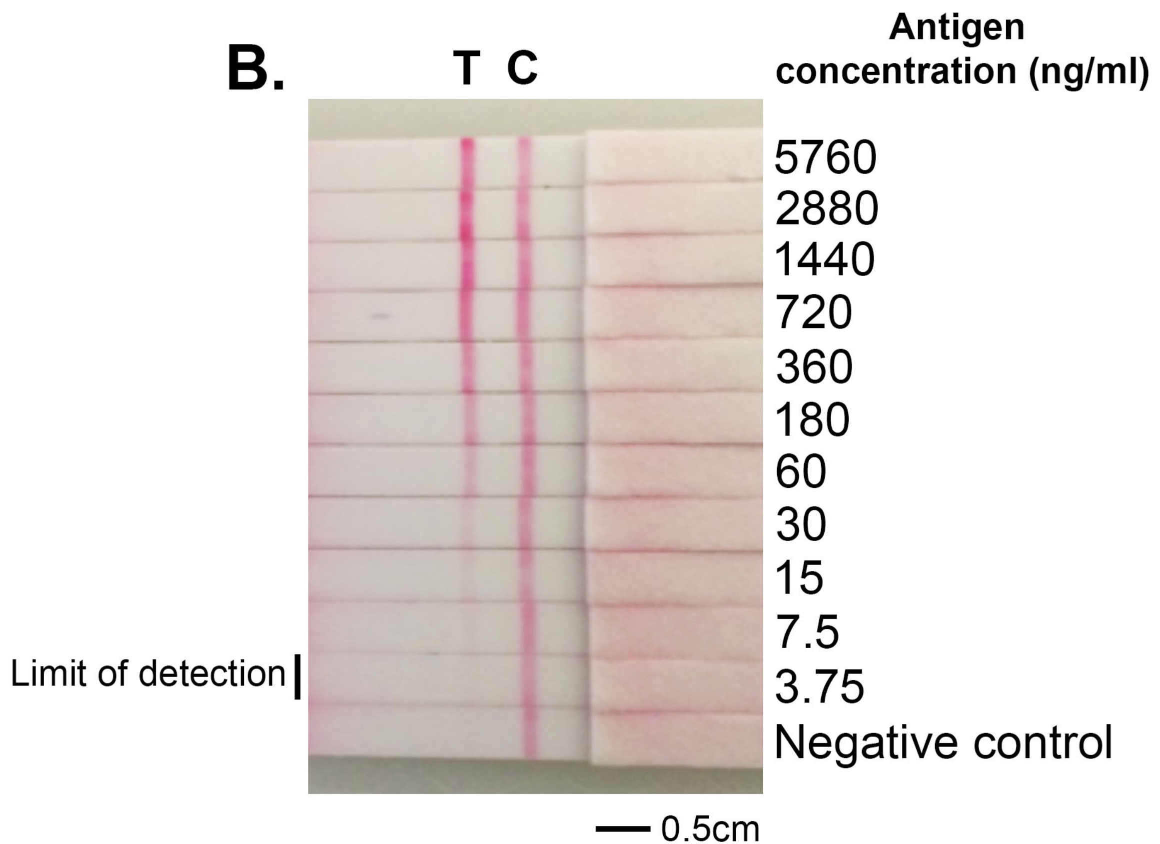
2277 Vagefi, M. R., Kim, E. T., Alvarado, R. G., Duncan, J. L., Howes, E. L., and Crawford, J. B.
2278 (2005). Bilateral endogenous *Scedosporium prolificans* endophthalmitis after lung
2279 transplantation. *Am. J. Ophthalmol*. **139,** 370-373.
2280 Van der Elst, K. C., Brouwers, C. H., van den Heuvel, E. R., van Wanrooy, M. J., Uges, D. R.,
2281 van der Werf, T. S., Kosterink, J. G., Span, L. F., and Alffenaar, J. W. (2015).
2282 Subtherapeutic posaconazole exposure and treatment outcome in patients with
2283 invasive fungal disease. *Ther. Drug. Monit.* **37,** 766-771.
2284 Van der Linden, J. W. M., Warris, A., and Verweij, P. E. (2011). *Aspergillus* species
2285 intrinsically resistant to antifungal agents. *Med. Mycol*. **49,** S82-S89.
2286 Van Diepeningen, A. D., Brankovics, B., Iltes, J., van der Lee, T. A. J., and Waalwijk, C.
2287 (2015a). Diagnosis of *Fusarium* infections: approaches to identification by the clinical
2288 mycology laboratory. *Curr. Fungal Infect. Rep*. **9,** 135-143.
2289 Van Diepeningen, A. D., Feng, P., Ahmed, S., Sudhadham, M., Bunyaratavej, S., and de Hoog,
2290 G. S. (2015b). Spectrum of *Fusarium* infections in tropical dermatology evidenced by
2291 multilocus sequencing typing diagnostics. *Mycoses* **58,** 48-57.
2292 Verweij, P.E., Snelders, E., Kema, G. H., Mellado, E., and Melchers, W. J. (2009). Azole
2293 resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? *Lancet*
2294 *Infect. Dis*. **9,** 789-795.
2295 Verweij, P.E., Zhang, J., Debets, A. J. M., Meis, J. F., van de Veerdonk, F., Schoustra, S. E.,
2296 Zwaan, B. J., and Melchers, W. J. G. (2016). In-host adaptation and acquired triazole
2297 resistance in *Aspergillus fumigatus*: a dilemma for clinical management. *Lancet Infect.*
2298 *Dis.* **16,** e251-260.
2299 Walsh, T. J., Gamaletsou, M. N., McGinnis, M. R., Hayden, R. T., and Kontoyiannis, D. P.
2300 (2012). Early clinical and laboratory diagnosis of invasive pulmonary, extrapulmonary,
2301 and disseminated mucormycosis (zygomycosis). *Clin. Infect. Dis*. **54**, S55-60.
2302 Walsh, T. J., Skiada, A., Cornely, O. A., Roilides, E., Ibrahim, A., Zaoutis, T., Groll, A.,
2303 Lortholary, O., Kontoyiannis, D. P., and Petrikkos, G. (2014). Development of new
2304 strategies for early diagnosis of mucormycosis from bench to bedside. *Mycoses* **57,** 2-7.
2305 Wang, H., Xiao, M., Kong, F., Chen, S., Dou, H.-T., Sorrell, T., Li, R.-Y., and Xu, Y.-C. (2011).
2306 Accurate and practical identification of 20 *Fusarium* species by seven-locus sequence
2307 analysis and reverse line blot hybridization, and an *in vitro* antifungal susceptibility
2308 study. *J. Clin. Microbiol*. **49,** 1890-1898.
2309 Wang, X-M, Guo, L-C., Xue, S-L., and Chen, Y-B. (2016). Pulmonary mucormycosis: a case
2310 report and review of the literature. *Oncology Lett.* **11,** 3049-3053.
2311 Warkentien, T., Rodriguez, C., Lloyd, B., Wells, J., Weintrob, A., Dunne, J. R., Ganesan, A., Li,
2312 P., Bradley, W., Gaskins, L. J., Seillier-Moiseiwitsch, F., Murray, C. K., *et al*. (2012).
2313 Invasive mold infections following combat-related injuries. *Clin. Infect. Dis*. **55,** 1441-
2314 1449.
2315 Westerberg, D. P., and Voyack, M. J. (2013). Onychomycosis: current trends in diagnosis and
2316 treatment. *Am. Fam. Physician* **88,** 762-770.
2317 White, P. L., Parr, C., Thornton, C. R., and Barnes, R. A. (2013). An evaluation of real-time
2318 PCR, galactomannan ELISA and a novel lateral-flow device for the diagnosis of invasive
2319 aspergillosis. *J. Clin. Microbiol*. **51,** 1510-1516.
2320 White, P. L., Wingard, J. R., Bretagne, S., Löffler, J., Patterson, T. F., Slavin, M. A., Barnes, R.
2321 A., Pappas, P. G., and Donnelly, J. P. (2015). *Aspergillus* polymerase chain reaction:
2322 systematic review of evidence for clinical use in comparison with antigen testing. *Clin.*
2323 *Infect. Dis*. **61,** 1293-1303.

2324 Wickes, B. L., and Wiederhold, N. P. (2018). Molecular diagnostics in medical mycology. *Nat.*
2325 *Comm*. **9,** 5135.
2326 Wiedemann, A., Kakoschke, T. K., Speth, C., Rambach, G., Ensinger, C., Jensen, H. E., and
2327 Ebel, F. (2016). Distinct galactofuranose antigens in the cell wall and culture
2328 supernatants as a means to differentiate *Fusarium* from *Aspergillus* species. *Int. J. Med.*
2329 *Microbiol*. **306,** 381-390.
2330 Wysong, D. R., and Waldorf, A. R. (1987). Electrophoretic and immunoblot analyses of
2331 *Rhizopus arrhizus* antigens. *J. Clin. Microbiol*. **25,** 358-363.
2332 Yamada, H., Ohshima, Y., and Miyazaki, T. (1982). Characteristion of fucomannopeptide and
2333 mannoprotein from *Absidia cylindrospora*. *Carbohydr. Res*. **110,** 113-126.
2334 Yamada, H., Hiraiwa, M., and Miyazaki, T. (1983). Characterisation of mannoproteins from
2335 yeast and mycelial forms of *Mucor rouxii*. *Carbohydr. Res*. **119,** 129-140.
2336 Yao, Y., Zhou, H., Shen, Y., Yang, Q., Ye, J., Fu, Y., Lu, G., Lou, H., Yu, Y., and Zhou, J. (2018a).
2337 Evaluation of a quantitative serum *Aspergillus fumigatus*-specific IgM assay for
2338 diagnosis of chronic pulmonary aspergillosis. *Clin. Respir. J.* **12,** 2566-2572.
2339 Yao, Y., Zhou, H., Yang, Q., Lu, G., Yu, Y., Shen, Y., and Zhou, J. (2018b). Serum *Aspergillus*
2340 *fumigatus*-specific IgG antibody decreases after antifungal treatment in chronic
2341 pulmonary aspergillosis patients. *Clin. Respir. J.* **12,** 1772-1774.
2342 Zahoor, B., Kent, S., and Wall, D. (2016). Cutaneous mucormycosis secondary to penetrative
2343 trauma. *Int. J. Care Injured* **47,** 1383-1387.
2344 Zarrin, M., Ganj, F., and Faramarzi, S. (2016). Development of a polymerase chain reaction-
2345 restriction fragment length polymorphism method for identification of the *Fusarium*
2346 genus using the transcription elongation factor-1α gene. *Biomed. Rep*. **5,** 705-708.
2347 Zhang, L., Guo, Z., Xie, S., Zhou, J., Chen, G., Feng, J., and Huang, Y. (2019). The performance
2348 of galactomannan in combination with 1,3-β-D-glucan or aspergillus-lateral flow device
2349 for the diagnosis of invasive aspergillosis: evidences from 13 studies. *Diag. Microbiol.*
2350 *Infect. Dis.* **93,** 44-53.
2351 Ziaee, A., Zia, M., Bayat, M., and Hashemi, J. (2016). Molecular identification of *Mucor* and
2352 *Lichtheimia* species in pure cultures of zygomycetes. *Jundishapur J. Microbiol*. **9,**
2353 e35237.
2354 Zoran, T., Sartori, B., Sappl, L., Aigner, M., Sánchez-Reus, F., Rezusta, A., Chowdhary, A., Taj-
2355 Aldeen, S. J., Arendrup, M. C., Oliveri, S., Kontoyiannis, D. P., Alastruey-Izquidero, A., *et*
2356 *al*. (2018). Azole-resistance in *Aspergillus terreus* and related species: an emerging
2357 problem or rare phenomenon? *Front. Microbiol*. **9,** 516.
2358
2359
2360
2361
2362
2363
2364
2365
2366
2367
2368
2369
2370

2371 **Figure and Legend**

Thornton Figure 1.





2372

2373

2374

2375 **Figure 1.** Demonstration lateral-flow assay (*Sced*-LFA) for the detection of pathogens within
2376 the *Pseudallescheria*/*Scedosporium* species complex. **A.** Schematic diagram showing the
2377 internal workings of the *Sced*-LFA test. The monoclonal antibody HG12 (Thornton, 2009) is
2378 immobilized in the test zone (T) on a porous nitrocellulose membrane. Anti-mouse
2379 immunoglobulin immobilised to the membrane in a separate zone serves as an internal
2380 control (C). On addition of 100μl of analyte (for example bronchoalveolar lavage) to the
2381 release pad (1), mAb HG12 which is conjugated to NanoAct cellulose nanobeads (CNB) in
2382 the release pad (Y-shaped structures with yellow spheres), binds to the diagnostic 120kDa
2383 antigen (red spheres) and the antibody-CNB/antigen complex then moves along the porous
2384 membrane by capillary action (2). The same mAb immobilized in the test zone (T) binds to
2385 the complex (3), resulting in a positive test line. Any mAb-CNB conjugate that migrates
2386 unbound to the target antigen, binds to the internal control (C) indicating that the assay has
2387 run correctly. Where the 120kDa diagnostic antigen is present in the analyte, 2 lines appear
2388 (T and C) indicating infection, but samples that are antigen negative (no infection) result in a
2389 single control line (C) only. **B.** Test results using filtrates from an actively growing culture of
2390 *S. apisopermum*, and with mAb HG12 conjugated to red CNB particles. Positive tests results
2391 (T and C both present) are visible for antigen concentrations ≥3.75ng/ml filtrate (the limit of
2392 detection). The negative control (antigen-negative culture medium) produces a single
2393 internal control line only. The single-step *Sced*-LFA test is highly specific, quick (taking only
2394 10 minutes to perform from addition of sample to interpretation of result), and is stable for
2395 18 months at room temperature, making it ideal for use in resource-limited settings. The
2396 image shown in B is courtesy of the *Sced*-LFA contract manufacturer LateralDx. Scale bar =
2397 0.5cm.

2398
2399
2400
2401
2402
2403
2404
2405
2406
2407
2408
2409
2410
2411
2412
2413
2414
2415
2416
2417
2418