REVIEW

Challenges in microbiological diagnosis of invasive *Aspergillus* infections [version 1; peer review: 2 approved]

Alexandre Alanio¹⁻³, Stéphane Bretagne¹⁻³

¹Parasitology-Myology Laboratory, Lariboisière Saint-Louis Fernand Widal hospitals, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France

²Paris-Diderot, Sorbonne Paris Cité University, Paris, France

³Institut Pasteur, CNRS, Molecular Mycology Unit, Reference National Center of Invasive Mycoses and Antifungals, Paris, France

V1 First published: 17 Feb 2017, 6(F1000 Faculty Rev):157
<https://doi.org/10.12688/f1000research.10216.1>

Latest published: 17 Feb 2017, 6(F1000 Faculty Rev):157
<https://doi.org/10.12688/f1000research.10216.1>

Abstract

Invasive aspergillosis (IA) has been increasingly reported in populations other than the historical hematology patients and there are new questions about the performance of microbiological tools. Microscopy and culture have been completed by biomarkers, either antigens or DNA, and in blood or respiratory specimens or both. First studied in hematology, the antigen galactomannan performance in serum is low in other patient populations where the pathophysiology of the infection can be different and the prevalence of IA is much lower. DNA detection with polymerase chain reaction (PCR) in blood or serum (or both) has reached a certain level of acceptance thanks to consensus methods based on real-time quantitative PCR (qPCR). When used on respiratory specimens, galactomannan and qPCR depend on standardization of the sampling and the diverse mycological procedures. Thus, culture remains the main diagnostic criterion in critically ill patients. The current trend toward more effective anti-mold prophylaxis in hematology hampers the yield of a screening strategy, as is usually performed in hematology. Therefore, circulating biomarkers as confirmatory tests should be considered and their performance should be reappraised in each new setting. The use of azole prophylaxis also raises the issue of selecting azole-resistance *Aspergillus fumigatus* isolates. Ideally, the biomarkers will be more efficient when individual genetic risks of IA are defined. Culture, though not standardized, remains a key element for the diagnosis of IA and has the advantage to easily detect molds other than *A. fumigatus*. It is still unclear whether next-generation sequencing will replace culture in the future.

Keywords

Aspergillosis, diagnosis, at-risk patients, fungal identification

Open Peer Review

Approval Status

	1	2
version 1
17 Feb 2017		

Faculty Reviews are review articles written by the prestigious Members of **Faculty Opinions**. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

1. **Stephane Ranque**, Aix Marseille University, IP-TPT, Marseille, France
Hôpital de la Timone, Assistance Publique-Hôpitaux de Marseille, Marseille, France
2. **Jacques Meis**, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

Any comments on the article can be found at the end of the article.

Corresponding author: Stéphane Bretagne (stephane.bretagne@sls.aphp.fr)

Competing interests: The authors declare that they have no competing interests.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2017 Alanio A and Bretagne S. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

How to cite this article: Alanio A and Bretagne S. **Challenges in microbiological diagnosis of invasive *Aspergillus* infections [version 1; peer review: 2 approved]** F1000Research 2017, 6(F1000 Faculty Rev):157 <https://doi.org/10.12688/f1000research.10216.1>

First published: 17 Feb 2017, 6(F1000 Faculty Rev):157 <https://doi.org/10.12688/f1000research.10216.1>

Introduction

Invasive aspergillosis (IA) is the prototype of opportunistic diseases: all of the diagnostic and therapeutics difficulties are due to the fact that only the presence of the germ cannot identify the infection. To help epidemiological studies and the evaluation of therapeutic trials, the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) proposed criteria for defining IA, incorporating clinical, imaging, and microbiological items¹. These definitions are suitable for hematological diseases—mainly, acute leukemia and hematopoietic stem cell transplant (HSCT) recipients—and, to a lesser extent, solid organ transplantations (SOTs). However, either because clinicians of other specialties are increasingly interested in IA or because of the improvement of diagnostic means, IA is now reported in other immunocompromised patient populations, such as intensive care unit (ICU) patients, patients given anti-tumor necrosis factor (anti-TNF) therapy², patients with AIDS³, as well as in hematology, in patients with chronic lymphoproliferative diseases, including multiple myeloma⁴ instead of acute leukemia⁵. Definitions based on the EORTC/MSG criteria were not well adapted to these new populations associated with a low performance of serum galactomannan (GM) testing⁶. The current challenges therefore are to adapt or develop new diagnostic microbiological criteria and to reassess the performance of biomarkers in these new populations⁷.

In parallel, new therapeutic strategies (mainly, prophylaxis in hematology) are now widely used. If the expected result is a decrease of IA prevalence, a bystander effect, though also expected, is a negative impact on the performance of biomarkers, which must be reappraised^{8,9}. Indeed, the prevalence of the disease directly impacts the performance of any test and GM in particular⁶. In parallel, this extensive use of prophylaxis has also shed light on the occurrence of azole-resistant *Aspergillus fumigatus* isolates.

New populations at risk of invasive aspergillosis

In hematology, IA is increasingly reported late after HSCT¹⁰ and in chronic lymphoproliferative disorders, mainly after several therapeutic interventions⁵. In these patients, the main risk factor of IA is not a profound and prolonged neutropenia as historically reported in acute leukemia, but instead high-dose steroid therapy^{5,10}. When IA develops during steroid therapy, the pathology of the lesions shows little fungus and chronic inflammation and a lower chance that the antigen will reach the blood stream¹¹. This probably explains why GM is released in a lower quantity in serum when patients are not neutropenic^{12,13}. The value of GM screening in chronic lymphoproliferative disorders therefore needs to be reassessed.

Another new group of patients who have been reported to be at risk of IA are the critically ill patients in an ICU without malignancies or other known risk factors for IA. High-dose corticosteroids and comorbidities such as chronic obstructive pulmonary disease, liver or renal failure, and diabetes are commonly noted as well as all causes of severe sepsis^{14–17}. The issue underlined in these patients is the value of isolation of *Aspergillus* spp. from respiratory specimens given the absence of classic imaging signs such as a

well-circumscribed nodule with halo or air crescent signs. A specific algorithm has recently been proposed to discriminate colonization from IA in critically ill patients with the introduction of “putative aspergillosis” as a new category¹⁶. Thus, putative aspergillosis represented 38% of patients with a positive *Aspergillus* culture, which could represent a high number of ICU patients. The definition of putative aspergillosis relies on four criteria: an *Aspergillus*-positive lower respiratory tract specimen (entry condition); abnormal medical imaging; and either other host risk factors or a semi-quantitative *Aspergillus*-positive culture of bronchoalveolar lavage (BAL) fluid with a positive microscopy (hyphae with morphology indicative of *Aspergillus* sp.) and without bacterial growth¹⁶. This would imply that the microbiological procedures to identify, quantify, and culture molds are similar in all microbiology laboratories, which is not the case even for hematology patients at high risk of IA¹⁸. Additionally, this definition does not make any distinction between the *Aspergillus* species, although they do not all have the same virulence and anti-fungal susceptibility profile⁵. Therefore, if the mycological criteria for defining IA in ICU patients are microscopy and isolation of *Aspergillus* spp., some consensus microbiological methods should be accepted.

In other patient populations such as patients who receive anti-TNF therapy² or patients with AIDS³, the incidence seems to remain low, although surveillance is always warranted. Similarly, a better index of suspicion is needed for the localizations that are not pulmonary, such as intestinal localizations, which are always difficult to diagnose¹⁹.

Genetic susceptibility and environmental factors

Currently, the microbiological observations for the diagnosis of IA are analyzed among patients with a similar underlying disease with the understanding that all the patients with the same factors are similarly at risk. However, one has always observed differences between patients; some develop IA and others do not in the same environment and under the same treatment. This observation has been revisited with the current availability of large-scale genetic screening means. Some donor haplotypes in Toll-like receptor 4 were shown to be associated with an increased risk of IA in hematopoietic cell transplants from unrelated donors²⁰. More recently, single-nucleotide polymorphisms in the nuclear factor kappa B (NFκB)-related genes were not found to be associated with an increased risk of developing IA²¹. Even if the current results are not very conclusive, this is an obvious field that needs to be investigated for a better assignment of anti-fungal drugs to patients at risk of IA. When this genetic susceptibility is better known, the meaning of the microbiological observation can be better interpreted.

Additionally, the environmental factors of the patients should be considered. Although IA occurs more often in outpatients, the different home or work environments are rarely investigated. There is now evidence that all patients do not share the same risk of inhaling pathogenic spores when returning home²², yet this risk is rarely taken into consideration^{23,24}.

Diagnostic means

Besides direct microscopy and culture, which remain important for identification and anti-fungal susceptibility testing of the

fungus, several biomarkers have been evaluated: GM, 1,3-B-D-glucan (BDG), and *Aspergillus* DNA. These biomarkers have been tested in blood or serum (or both) and in respiratory specimens. The main issue for the present circulating biomarkers is their weak specificity. This weak specificity is due to the analytical performance of the tests but also to the presence of the biomarkers tested in the environment, including food, inhaled air, pharmaceutical products^{25,26}, or blood tubes²⁷.

Antibody detection has been poorly studied for IA because of the underlying immunodepression and is not considered a diagnostic criterion¹. Moreover, the assays are not standardized. Therefore, they will not be commented on here despite their major interest in chronic and allergic forms of pulmonary aspergillosis²⁸.

Serum antigens: galactomannan and beta-D-glucan

GM remains a cornerstone for the diagnosis of IA, which is far ahead of BDG, whose specificity is poor because of the pan-fungal nature of this marker^{29,30}. GM is a microbiological criterion for defining IA in hematology³¹ and SOT³² patients, but there are difficulties related to studying its performance as an evaluation tool and as a diagnostic criterion³³. GM is also used as a surrogate marker to follow the efficacy of treatment³⁴. The main limitation of serum GM is the high rate of false-positive results, which fall under two categories: irreproducible results and detection not related to IA. The irreproducible positive results correspond to results that are not confirmed when retested and should be considered negative³⁵. Confirmed positive results are technically true positive, which corresponds to either a true IA or the presence of GM from environmental sources^{25,36}. There is currently no means to safely discard a GM-positive result that is not related to ongoing IA based on technical artifacts. Only the analysis of intravenous drugs or parenteral nutrition prescribed and possibly the test of batches of drugs thought to contain manufactured GM can support a false positive due to infused products, mainly antibiotics processed through mold cultures²⁵. Otherwise, it is the analysis of the evolution of the disease and medical file records that allow discrimination between true-positive or false-positive results.

Currently, the medical performance of GM is decreasing due to the widespread use of effective anti-mold prophylaxis^{8,9}. In these conditions, the screening strategy usually proposed in hematology³⁷ could become inefficient and would no longer be recommended^{8,9}. If it is used as confirmatory test, thresholds to define positivity could have to be reassessed for GM and also for other biomarkers. For instance, the GM positivity is 0.5 in two separate serum samples as used as a screening test, because the goals are to minimize the risk of IA and to limit the risk of over-treating, whereas the positivity threshold is at least 1.0 in BAL fluid sample to assess the diagnosis (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM420248.pdf>).

Recently, a lateral flow device (LFD) was compared with PCR and GM³⁸. This test is based on the detection of a new fungal antigen, an extracellular protein, using a monoclonal antibody. The best performance was achieved in combination with PCR, providing both 100% sensitivity and 100% specificity. Its main advantage should

be to provide clinicians with a rapid operational result, even if PCR can be performed later as a confirmatory test.

Polymerase chain reaction in blood/serum

The first publications on the detection of circulating *A. fumigatus* DNA appeared in the 1990s either in whole blood³⁹ or in serum⁴⁰. However, as soon as this occurred, PCR for IA was shown to be highly challenging because of the very low amount of DNA in samples. This low amount exacerbated all of the pitfalls and limits of the PCR, mainly false positives due to previously amplified products and environmental contamination and false-negative results due to residual PCR inhibitors⁴¹. In 2006, the European Aspergillus PCR Initiative (EAPCRI) was launched to seek proposals for a technical consensus. This consensus was possible thanks to the generalization of real-time quantitative PCR (qPCR), which dramatically reduces the risk of contamination from environmental amplicons and allows quantitative management of the amplification reaction to detect inhibition⁴¹. Because whole blood is technically more demanding for the extraction steps, serum appears to be a better specimen⁴². More recently, plasma has been shown to have a better sensitivity than serum and should be the preferred specimen⁴³. The performance of PCR in blood seems at least as good as GM⁴⁴, and the absence of both biomarkers (that is, GM and circulating DNA) could be sufficient to postpone anti-fungal therapy⁴⁵. As for GM, it remains unclear whether PCR should be used as a screening test⁴⁶ or as a confirmatory test in light of the widespread use of anti-mold prophylaxis⁹.

Combined use of blood biomarkers

A recent meta-analysis focused on pediatric cancer and HSCT and analyzing the biomarkers (GM, BDG, and DNA) separately concluded a poor performance of the tests, and the authors suggested combining several biomarkers for further studies⁴⁷. As soon as PCR was developed for IA diagnosis, the interest of associating GM and DNA detection was evaluated⁴⁰. Another recent meta-analysis showed that the association of both tests is highly suggestive of an active infection with a positive predictive value of 88%⁴⁵. A therapeutic strategy based on a combined surveillance of serum GM and *Aspergillus* DNA was shown to decrease the incidence of IA in high-risk hematological patients⁴⁶. The parallel use of GM and PCR was effective in reducing empirical anti-fungal treatment in hematology patients at high risk of IA³¹. The combined use of LFD, instead of GM, and qPCR seems could be a better strategy³⁸.

Biomarkers in bronchoalveolar lavage fluids

Biomarkers in respiratory specimens are more prone to subjective interpretation than the same biomarkers in blood. Indeed, a positive mold culture from a respiratory specimen can be ascribed to infection, colonization, or simple bystander observation. To switch from culture to biomarkers does not radically change the issue. Additionally, biomarker assays are more amenable to standardization compared with the same assays in respiratory specimens.

GM has also been investigated in BAL fluids and some authors advocate the value of GM testing in the ICU⁴⁸. GM has also been evaluated in association with qPCR with the delineation of quantitative thresholds both for qPCR and for GM⁴⁹. However, to be widely accepted, these thresholds should be of similar values in

different settings. Unfortunately, the BAL procedures are highly variable between centers (for example, three lavages of 50 mL versus two lavages of 20 mL); more importantly, they are highly variable between patients according to their underlying pulmonary lesions¹⁸. Therefore, there are substantial difficulties in obtaining consensus for quantitative thresholds.

The LFD described above has also been evaluated in BAL fluids. When the LFD assay was used alone in BAL of patients with hematological malignancies, the sensitivity of the LFD was moderate, around 60% to 70%, which according to the authors was possibly due to previous systemic anti-mold therapy⁵⁰. In another study on 133 ICU patients, including 16 patients with proven or probable IA, who had a positive culture or GM test, the sensitivity and specificity of the LFD was 80% and 81% respectively⁵¹. On the other hand, 18 patients without IA had a positive LFD result, although some of them grew mold in their respiratory specimens⁵¹. There is no means to decipher between LFD and other microbiological criteria which results are false-positive or false-negative. This underlines the difficulties in assessing the performance of a new assay in the absence of contributive biopsies. To overcome some limitations of using LFD alone, a different study coupled qPCR and LFD and concluded to the high performance of this strategy, as in serum⁴³, even when the patients were given systemic anti-mold therapy⁵². Therefore, as for GM or culture, an isolated positive LFD result in respiratory specimens could be limited to decipher between colonization and true infection, and the addition of several mycological tools could improve the reliability of IA diagnosis.

Exhaled volatile organic compounds—though not, strictly speaking, an antigen—have been investigated by electronic nose technology⁵³. *In vitro* investigations were conclusive to distinguish *A. fumigatus* from *Rhizopus oryzae*. The authors propose additional *in vivo* studies⁵³.

Fungal identification in biopsies

Identification of species responsible for lesions in tissue is essential to adapt anti-fungal treatments. However, tissue is frequently processed for histopathology with formalin-fixed paraffin-embedded (FFPE) tissue, which hampers the molecular identification workflow because of technical issues⁵⁴. Fresh frozen tissue gives rise to a better yield than FFPE tissue in terms of qPCR detection as demonstrated in other molecular tests^{55–57}. Once the DNA is extracted from biopsies, various strategies can be used, such as testing multiple species/genera by qPCR⁵⁸ or using sequencing of a pan-fungal barcode⁵⁹, microarrays⁶⁰, luminex-based methods⁶¹, or PCR-electrospray ionization mass spectrometry⁶².

However, pan-fungal primers can hybridize other eukaryotic DNAs. Therefore, the pan-fungal approach can be limited when tissue samples contain mainly human DNA, little fungal DNA, and potentially mixtures of fungi preventing sequencing of a single DNA barcode. The optimization of the pan-fungal primers is of utmost importance for a better representation of fungal species after PCR in complex media as in the current mycobiome studies^{63–65}.

New *Aspergillus* species and azole resistance

More and more, new mold species are reported as responsible for invasive infections. However, most of the reported cases are probable cases with isolation of these new species in respiratory specimens⁶⁶. Yet it is difficult to ascribe a new species to the pathology observed in the absence of biopsies confirming invasion. Indeed, a lot of different non-*Aspergillus* non-Mucorales species can be cultured from respiratory specimens in patients at risk of IA^{67,68}. Identification still relies on microscopy and Sanger sequencing of some barcode genes, mainly Internal Transcribed Spacer (ITS)^{69,70}, but it is expected that matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry can achieve a more rapid identification as the databases associated with the apparatus become more and more complete and reliable^{66,71}. If these species emerge after or under azole therapy, a primary resistance is feared, such as for *Mucorales*, which is now more easily diagnosed using serum qPCR^{72,73}. To know whether the new azole drug, isavuconazole, can have a specific role against these new species remains to be firmly established⁷⁴.

More challenging is the emergence of acquired azole resistance among *A. fumigatus* due to the wide use of azole drugs in agriculture and its therapeutic consequences^{75–77}. The resistance mechanism is mainly mutation in the *cyp51A* gene associated with tandem repeats⁷⁵. It is not clear whether the resistant phenotype is associated with a higher mortality because of the resistant phenotype or other factors such as delayed diagnosis⁷⁸. Indeed, in a mouse model, the virulence of isolates with *cyp51A* mutations showed reduced virulence compared with azole-susceptible isolates⁷⁹, even if this lower virulence was not confirmed in *in vitro* models⁸⁰. However, when the resistance incidence in a particular setting is over 10%, one can challenge azole therapy as the first-line therapy⁸¹. A consequence for the microbiological laboratory is the need to conduct surveillance of this resistance, at least the surveillance of the resistant phenotype¹⁸. This surveillance can be completed by searching for the mutations responsible for azole resistance, either from colonies⁸² or directly from the respiratory specimens^{83–85}. Searching for other mechanisms of azole resistance is more difficult and these other mechanisms, in contrast to mutations in the *cyp51A* gene, could be associated with higher virulence⁸⁰. When PCR is used to directly detect mutations in respiration specimens^{83,84,86}, the difficulty is to know the different percentages of wild-type and resistant *Aspergillus*⁸⁷, given that both can be simultaneously present⁸⁸.

Culture and next-generation sequencing

Given the uncertainties about biomarkers, culture remains an easy tool for the diagnosis of IA, as discussed earlier in ICU patients¹⁶, and the detection of anti-fungal-resistant phenotypes. For the direct detection of resistance without culture, next-generation sequencing (NGS) will be more widely used to describe polymorphisms between isolates and mutations occurring during infection, in particular under anti-fungal treatments⁸⁹.

The main contribution of culture is not to focus on *A. fumigatus* but also to extend the spectrum to other mold infections^{67,68}. NGS has been tested in the hope of dramatically extending this spectrum

to every species present in the sample. A first strategy is to focus on ribosomal DNA (18S, 28S, or ITS) amplification using pan-fungal primers (metataxonomics) and to obtain operational taxonomic units to describe the diversity of the fungi in a clinical specimen. This strategy is dependent on the choice of the primers described above for the identification of mold in biopsies^{63–65}. Indeed, with some primer sets, the results represent the more easily amplified species rather than the real content of the specimen.

Another strategy, called metagenomics, is to circumvent the amplification with predefined primers using whole genome sequencing. This needs extensive bioinformatic work to trim the many different sequences. This strategy is of interest if the goal is to correlate the proportion of bacterial and fungal organisms in samples⁹⁰ with a clear advantage on quantification compared with metataxonomics⁹¹. This strategy is now compared in microbiology with culturomics⁹². Culturomics consists of multiplying the culture conditions to detect low-growth microorganisms⁹³. Concordance between metagenomics and culturomics is not as good as expected⁹³, underlying the difficulties in detecting every microorganism using NGS. However, metagenomics seems more reliable than metataxonomics from the perspective of a diagnosis approach⁹¹.

Conclusions

The prognosis of IA is still dismal with an all-cause mortality around 40% at three months^{5,32}, and emerging azole resistance puts even more pressure on favorable outcome⁷⁸. Stress is often put on the development of new diagnostic tools, but given the multiplicity of risk factors and the ubiquity of molds in the environment, there is

little hope that these present microbiological difficulties will vanish in the near future. At the very least, a parallel stress should be put on the knowledge of the pathophysiology of the infection and the individual genetic susceptibility to infection. We found, for instance, evidence that the DNA detected using PCR is free circulating DNA⁹⁴. This has an immediate consequence for the pre-analytical step before nucleic acid analysis, although we cannot rule out that DNA also originates from other sources, such as fungal elements engulfed in circulating macrophages that require a different pre-analytical step to release fungal DNA from fungal elements.

For now, serum GM and DNA are the two most effective biomarkers for diagnosis, but their performance should be reappraised in new patient populations at risk of IA. Biomarkers in respiratory specimens also face difficulties in harmonizing clinical specimens. Biomarker evaluations will be hampered by the wide use of azole prophylaxis in hematology patients, which alters the kinetics of these biomarkers. This prophylaxis also exposes patients to IA due to resistant isolates and to other mold infections⁷⁶. Given the individual risk among a group of patients with similar treatments, the genetics underlying the susceptibility to IA should be further investigated to restrict the use of universal prophylaxis.

Competing interests

The authors declare that they have no competing interests.

Grant information

The author(s) declared that no grants were involved in supporting this work.

References

1. De Pauw B, Walsh TJ, Donnelly JP, *et al.*: Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008; 46(12): 1813–21. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
2. Salmon-Ceron D, Tubach F, Lortholary O, *et al.*: Drug-specific risk of non-tuberculosis opportunistic infections in patients receiving anti-TNF therapy reported to the 3-year prospective French RATIO registry. *Ann Rheum Dis*. 2011; 70(4): 616–23. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
3. Denis B, Guiguet M, de Castro N, *et al.*: Relevance of EORTC Criteria for the Diagnosis of Invasive Aspergillosis in HIV-Infected Patients, and Survival Trends Over a 20-Year Period in France. *Clin Infect Dis*. 2015; 61(8): 1273–80. [PubMed Abstract](#) | [Publisher Full Text](#)
4. Nucci M, Anaissie E: Infections in patients with multiple myeloma in the era of high-dose therapy and novel agents. *Clin Infect Dis*. 2009; 49(8): 1211–25. [PubMed Abstract](#) | [Publisher Full Text](#)
5. Lortholary O, Gangneux JP, Sitbon K, *et al.*: Epidemiological trends in invasive aspergillosis in France: the SAIF network (2005–2007). *Clin Microbiol Infect*. 2011; 17(12): 1882–9. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
6. Leeflang MM, Debets-Ossenkopp YJ, Visser CE, *et al.*: Galactomannan detection for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev*. 2008; (4): CD007394. [PubMed Abstract](#) | [Publisher Full Text](#)
7. Baddley JW: Clinical risk factors for invasive aspergillosis. *Med Mycol*. 2011; 49(Suppl 1): S7–S12. [PubMed Abstract](#) | [Publisher Full Text](#)
8. Duarte RF, Sánchez-Ortega I, Cuesta I, *et al.*: Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. *Clin Infect Dis*. 2014; 59(12): 1696–702. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
9. Patterson TF, Thompson GR 3rd, Denning DW, *et al.*: Executive Summary: Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016; 63(4): 433–42. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Kontoyiannis DP, Marr KA, Park BJ, *et al.*: Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis*. 2010; 50(8): 1091–100. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
11. Stergiopoulou T, Meletiadis J, Roilides E, *et al.*: Host-dependent patterns of tissue injury in invasive pulmonary aspergillosis. *Am J Clin Pathol*. 2007; 127(3): 349–55. [PubMed Abstract](#) | [Publisher Full Text](#)
12. Verweij PE, Weemaes CM, Curfs JH, *et al.*: Failure to detect circulating

- Aspergillus** markers in a patient with chronic granulomatous disease and invasive aspergillosis. *J Clin Microbiol.* 2000; 38(10): 3900–1.
[PubMed Abstract](#) | [Free Full Text](#)
13. Cordonnier C, Botterel F, Ben Amor R, *et al.*: Correlation between galactomannan antigen levels in serum and neutrophil counts in haematological patients with invasive aspergillosis. *Clin Microbiol Infect.* 2009; 15(1): 81–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
 14. **F** Baddley JW, Stephens JM, Ji X, *et al.*: Aspergillosis in Intensive Care Unit (ICU) patients: epidemiology and economic outcomes. *BMC Infect Dis.* 2013; 13: 29.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 15. Meersseman W, Lagrou K, Maertens J, *et al.*: Invasive aspergillosis in the intensive care unit. *Clin Infect Dis.* 2007; 45(2): 205–16.
[PubMed Abstract](#) | [Publisher Full Text](#)
 16. Blot SI, Taccone FS, van den Abeele AM, *et al.*: A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med.* 2012; 186(1): 56–64.
[PubMed Abstract](#) | [Publisher Full Text](#)
 17. **F** Delsuc C, Cottreau A, Frealle E, *et al.*: Putative invasive pulmonary aspergillosis in critically ill patients with chronic obstructive pulmonary disease: a matched cohort study. *Crit Care.* 2015; 19: 421.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 18. Alario A, Denis B, Hamane S, *et al.*: New therapeutic strategies for invasive aspergillosis in the era of azole resistance: how should the prevalence of azole resistance be defined? *J Antimicrob Chemother.* 2016; 71(8): 2075–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 19. Kazan E, Maertens J, Herbrecht R, *et al.*: A retrospective series of gut aspergillosis in haematology patients. *Clin Microbiol Infect.* 2011; 17(4): 588–94.
[PubMed Abstract](#) | [Publisher Full Text](#)
 20. **F** Bochud PY, Chien JW, Marr KA, *et al.*: Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N Engl J Med.* 2008; 359(17): 1766–77.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 21. **F** Lupiañez CB, Villaescusa MT, Carvalho A, *et al.*: Common Genetic Polymorphisms within NF- κ B-Related Genes and the Risk of Developing Invasive Aspergillosis. *Front Microbiol.* 2016; 7: 1243.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 22. **F** Barberán A, Dunn RR, Reich BJ, *et al.*: The ecology of microscopic life in household dust. *Proc Biol Sci.* 2015; 282(1814).
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 23. Rocchi S, Daguidau E, Grenouillet F, *et al.*: Azole-resistant *Aspergillus fumigatus* isolate with the TR34/L98H mutation in both a fungicide-sprayed field and the lung of a hematopoietic stem cell transplant recipient with invasive aspergillosis. *J Clin Microbiol.* 2014; 52(5): 1724–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 24. **F** Lavergne RA, Chouaki T, Hagen F, *et al.*: Home Environment as a Source of Life-Threatening Azole-Resistant *Aspergillus fumigatus* in Immunocompromised Patients. *Clin Infect Dis.* 2017; 64(1): 76–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 25. **F** Vergidis P, Razonable RR, Wheat LJ, *et al.*: Reduction in false-positive *Aspergillus* serum galactomannan enzyme immunoassay results associated with use of piperacillin-tazobactam in the United States. *J Clin Microbiol.* 2014; 52(6): 2199–201.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 26. Millon L, Grenouillet F, Crouzet J, *et al.*: False-positive *Aspergillus* real-time PCR assay due to a nutritional supplement in a bone marrow transplant recipient with GVH disease. *Med Mycol.* 2010; 48(4): 661–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
 27. Harrison E, Stahlberger T, Whelan R, *et al.*: *Aspergillus* DNA contamination in blood collection tubes. *Diagn Microbiol Infect Dis.* 2010; 67(4): 392–4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 28. Page ID, Richardson M, Denning DW: Antibody testing in aspergillosis—quo vadis? *Med Mycol.* 2015; 53(5): 417–39.
[PubMed Abstract](#) | [Publisher Full Text](#)
 29. De Vlieger G, Lagrou K, Maertens J, *et al.*: Beta-D-glucan detection as a diagnostic test for invasive aspergillosis in immunocompromised critically ill patients with symptoms of respiratory infection: an autopsy-based study. *J Clin Microbiol.* 2011; 49(11): 3783–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 30. Lamoth F, Cruciani M, Mengoli C, *et al.*: β -Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). *Clin Infect Dis.* 2012; 54(5): 633–43.
[PubMed Abstract](#) | [Publisher Full Text](#)
 31. **F** Morrissey CO, Chen SC, Sorrell TC, *et al.*: Galactomannan and PCR versus culture and histology for directing use of antifungal treatment for invasive aspergillosis in high-risk haematology patients: a randomised controlled trial. *Lancet Infect Dis.* 2013; 13(6): 519–28.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 32. **F** López-Medrano F, Fernández-Ruiz M, Silva JT, *et al.*: Clinical Presentation and Determinants of Mortality of Invasive Pulmonary Aspergillosis in Kidney Transplant Recipients: A Multinational Cohort Study. *Am J Transplant.* 2016; 16(11): 3220–34.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 33. Schwarzhager M, Sagaon-Teyssier L, Cabaret O, *et al.*: Performance of serum biomarkers for the early detection of invasive aspergillosis in febrile, neutropenic patients: a multi-state model. *PLoS One.* 2013; 8(6): e65776.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 34. Bergeron A, Porcher R, Menotti J, *et al.*: Prospective evaluation of clinical and biological markers to predict the outcome of invasive pulmonary aspergillosis in hematological patients. *J Clin Microbiol.* 2012; 50(3): 823–30.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 35. Guigue N, Lardeux S, Alario A, *et al.*: Importance of operational factors in the reproducibility of *Aspergillus* galactomannan enzyme immune assay. *PLoS One.* 2015; 10(4): e0124044.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 36. Cummings JR, Jamison GR, Boudreaux JW, *et al.*: Cross-reactivity of non-*Aspergillus* fungal species in the *Aspergillus* galactomannan enzyme immunoassay. *Diagn Microbiol Infect Dis.* 2007; 59(1): 113–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
 37. Marchetti O, Lamoth F, Mikulska M, *et al.*: ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients. *Bone Marrow Transplant.* 2012; 47(6): 846–54.
[PubMed Abstract](#) | [Publisher Full Text](#)
 38. White PL, Parr C, Thornton C, *et al.*: Evaluation of real-time PCR, galactomannan enzyme-linked immunosorbent assay (ELISA), and a novel lateral-flow device for diagnosis of invasive aspergillosis. *J Clin Microbiol.* 2013; 51(5): 1510–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 39. Loeffler J, Hebart H, Cox P, *et al.*: Nucleic acid sequence-based amplification of *Aspergillus* RNA in blood samples. *J Clin Microbiol.* 2001; 39(4): 1626–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 40. Bretagne S, Costa JM, Bart-Delabesse E, *et al.*: Comparison of serum galactomannan antigen detection and competitive polymerase chain reaction for diagnosing invasive aspergillosis. *Clin Infect Dis.* 1998; 26(6): 1407–12.
[PubMed Abstract](#) | [Publisher Full Text](#)
 41. Alario A, Bretagne S: Difficulties with molecular diagnostic tests for mould and yeast infections: where do we stand? *Clin Microbiol Infect.* 2014; 20(Suppl 6): 36–41.
[PubMed Abstract](#) | [Publisher Full Text](#)
 42. White PL, Mengoli C, Bretagne S, *et al.*: Evaluation of *Aspergillus* PCR protocols for testing serum specimens. *J Clin Microbiol.* 2011; 49(11): 3842–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 43. White PL, Barnes RA, Springer J, *et al.*: Clinical Performance of *Aspergillus* PCR for Testing Serum and Plasma: a Study by the European *Aspergillus* PCR Initiative. *J Clin Microbiol.* 2015; 53(9): 2832–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 44. White PL, Wingard JR, Bretagne S, *et al.*: *Aspergillus* Polymerase Chain Reaction: Systematic Review of Evidence for Clinical Use in Comparison With Antigen Testing. *Clin Infect Dis.* 2015; 61(8): 1293–303.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 45. **F** Arvanitis M, Anagnostou T, Mylonakis E: Galactomannan and Polymerase Chain Reaction-Based Screening for Invasive Aspergillosis Among High-Risk Hematology Patients: A Diagnostic Meta-analysis. *Clin Infect Dis.* 2015; 61(8): 1263–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 46. **F** Aguado JM, Vazquez L, Fernandez-Ruiz M, *et al.*: Serum galactomannan versus a combination of galactomannan and polymerase chain reaction-based *Aspergillus* DNA detection for early therapy of invasive aspergillosis in high-risk hematological patients: a randomized controlled trial. *Clin Infect Dis.* 2015; 60(3): 405–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 47. **F** Lehnbecher T, Robinson PD, Fisher BT, *et al.*: Galactomannan, β -D-Glucan, and Polymerase Chain Reaction-Based Assays for the Diagnosis of Invasive Fungal Disease in Pediatric Cancer and Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. *Clin Infect Dis.* 2016; 63(10): 1340–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 48. **F** Meersseman W, Lagrou K, Maertens J, *et al.*: Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. *Am J Respir Crit Care Med.* 2008; 177(1): 27–34.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 49. **F** Luong ML, Clancy CJ, Vadnaker A, *et al.*: Comparison of an *Aspergillus* real-time polymerase chain reaction assay with galactomannan testing of bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. *Clin Infect Dis.* 2011; 52(10): 1218–26.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 50. **F** Prates J, Lackner M, Eigl S, *et al.*: Diagnostic accuracy of the *Aspergillus*-specific bronchoalveolar lavage lateral-flow assay in hematological malignancy patients. *Mycoses.* 2015; 58(8): 461–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**

51. **F** Eigl S, Prattes J, Lackner M, *et al.*: Multicenter evaluation of a lateral-flow device test for diagnosing invasive pulmonary aspergillosis in ICU patients. *Crit Care*. 2015; 19: 178.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
52. **F** Johnson GL, Sarker SJ, Nannini F, *et al.*: *Aspergillus*-Specific Lateral-Flow Device and Real-Time PCR Testing of Bronchoalveolar Lavage Fluid: a Combination Biomarker Approach for Clinical Diagnosis of Invasive Pulmonary Aspergillosis. *J Clin Microbiol*. 2015; 53(7): 2103–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
53. **F** de Heer K, Vonk SI, Kok M, *et al.*: eNose technology can detect and classify human pathogenic molds *in vitro*: a proof-of-concept study of *Aspergillus fumigatus* and *Rhizopus oryzae*. *J Breath Res*. 2016; 10(3): 36008.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
54. Cabaret O, Toussain G, Abernill N, *et al.*: Degradation of fungal DNA in formalin-fixed paraffin-embedded sinus fungal balls hampers reliable sequence-based identification of fungi. *Med Mycol*. 2011; 49(3): 329–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
55. Jackson CB, Gallati S, Schaller A: qPCR-based mitochondrial DNA quantification: influence of template DNA fragmentation on accuracy. *Biochem Biophys Res Commun*. 2012; 423(3): 441–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
56. **F** Lüder Ripoli F, Mohr A, Conradine Hammer S, *et al.*: A Comparison of Fresh Frozen vs. Formalin-Fixed, Paraffin-Embedded Specimens of Canine Mammary Tumors via Branched-DNA Assay. *Int J Mol Sci*. 2016; 17(5): pii: E724.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
57. **F** Oh E, Choi YL, Kwon MJ, *et al.*: Comparison of Accuracy of Whole-Exome Sequencing with Formalin-Fixed Paraffin-Embedded and Fresh Frozen Tissue Samples. *PLoS One*. 2015; 10(12): e0144162.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
58. **F** Salehi E, Hedayati MT, Zoll J, *et al.*: Discrimination of Aspergillosis, Mucormycosis, Fusariosis, and Scedosporiosis in Formalin-Fixed Paraffin-Embedded Tissue Specimens by Use of Multiple Real-Time Quantitative PCR Assays. *J Clin Microbiol*. 2016; 54(11): 2798–803.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
59. Buitrago MJ, Aguado JM, Ballen A, *et al.*: Efficacy of DNA amplification in tissue biopsy samples to improve the detection of invasive fungal disease. *Clin Microbiol Infect*. 2013; 19(6): E271–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
60. Spiess B, Seifarth W, Hummel M, *et al.*: DNA microarray-based detection and identification of fungal pathogens in clinical samples from neutropenic patients. *J Clin Microbiol*. 2007; 45(11): 3743–53.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
61. Babady NE, Miranda E, Gilhuley KA: Evaluation of Luminex xTAG fungal analyte-specific reagents for rapid identification of clinically relevant fungi. *J Clin Microbiol*. 2011; 49(11): 3777–82.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
62. Alanio A, Garcia-Hermoso D, Mercier-Delaurie S, *et al.*: Molecular identification of *Mucorales* in human tissues: contribution of PCR electrospray-ionization mass spectrometry. *Clin Microbiol Infect*. 2015; 21(6): 594.e1–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
63. Martin KJ, Rygielwicz PT: Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BMC Microbiol*. 2005; 5: 28.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
64. Ihrmark K, Bodeker IT, Cruz-Martinez K, *et al.*: New primers to amplify the fungal ITS2 region—evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol Ecol*. 2012; 82(3): 666–77.
[PubMed Abstract](#) | [Publisher Full Text](#)
65. Bokulich NA, Mills DA: Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. *Appl Environ Microbiol*. 2013; 79(8): 2519–26.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
66. **F** Masih A, Singh PK, Kathuria S, *et al.*: Identification by Molecular Methods and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry and Antifungal Susceptibility Profiles of Clinically Significant Rare *Aspergillus* Species in a Referral Chest Hospital in Delhi, India. *J Clin Microbiol*. 2016; 54(9): 2354–64.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
67. Garcia-Hermoso D, Alanio A, Cabaret O, *et al.*: High diversity of non-sporulating moulds in respiratory specimens of immunocompromised patients: should all the species be reported when diagnosing invasive aspergillosis? *Mycoses*. 2015; 58(9): 557–64.
[PubMed Abstract](#) | [Publisher Full Text](#)
68. **F** Chowdhary A, Agarwal K, Meis JF: Filamentous Fungi in Respiratory Infections. What Lies Beyond Aspergillosis and Mucormycosis? *PLoS Pathog*. 2016; 12(4): e1005491.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
69. **F** Stielow JB, Lévesque CA, Seifert KA, *et al.*: One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia*. 2015; 35: 242–63.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
70. **F** Schoch CL, Seifert KA, Huhndorf S, *et al.*: Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proc Natl Acad Sci U S A*. 2012; 109(16): 6241–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
71. Alanio A, Beretti JL, Dauphin B, *et al.*: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for fast and accurate identification of clinically relevant *Aspergillus* species. *Clin Microbiol Infect*. 2011; 17(5): 750–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
72. Legrand M, Gits-Muselli M, Boutin L, *et al.*: Detection of Circulating *Mucorales* DNA in Critically Ill Burn Patients: Preliminary Report of a Screening Strategy for Early Diagnosis and Treatment. *Clin Infect Dis*. 2016; 63(10): 1312–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
73. **F** Millon L, Herbrecht R, Grenouillet F, *et al.*: Early diagnosis and monitoring of mucormycosis by detection of circulating DNA in serum: retrospective analysis of 44 cases collected through the French Surveillance Network of Invasive Fungal Infections (RESSIF). *Clin Microbiol Infect*. 2016; 22(9): 810.e1–810.e8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
74. **F** Marty FM, Ostrosky-Zeichner L, Cornely OA, *et al.*: Isavuconazole treatment for mucormycosis: a single-arm open-label trial and case-control analysis. *Lancet Infect Dis*. 2016; 16(7): 828–37.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
75. **F** Verweij PE, Snelders E, Kema GH, *et al.*: Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? *Lancet Infect Dis*. 2009; 9(12): 789–95.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
76. **F** Verweij PE, Zhang J, Debets AJ, *et al.*: In-host adaptation and acquired triazole resistance in *Aspergillus fumigatus*: a dilemma for clinical management. *Lancet Infect Dis*. 2016; 16(11): e251–e260.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
77. Chowdhary A, Kathuria S, Xu J, *et al.*: Emergence of azole-resistant *Aspergillus fumigatus* strains due to agricultural azole use creates an increasing threat to human health. *PLoS Pathog*. 2013; 9(10): e1003633.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
78. **F** Verweij PE, Chowdhary A, Melchers WJ, *et al.*: Azole Resistance in *Aspergillus fumigatus*: Can We Retain the Clinical Use of Mold-Active Antifungal Azoles? *Clin Infect Dis*. 2016; 62(3): 362–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
79. **F** Arendrup MC, Mavridou E, Mortensen KL, *et al.*: Development of azole resistance in *Aspergillus fumigatus* during azole therapy associated with change in virulence. *PLoS One*. 2010; 5(4): e10080.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
80. Mavridou E, Meletiadis J, Jancura P, *et al.*: Composite survival index to compare virulence changes in azole-resistant *Aspergillus fumigatus* clinical isolates. *PLoS One*. 2013; 8(8): e72280.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
81. **F** Verweij PE, Ananda-Rajah M, Andes D, *et al.*: International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*. *Drug Resist Updat*. 2015; 21–22: 30–40.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
82. Alanio A, Sitterlé E, Liance M, *et al.*: Low prevalence of resistance to azoles in *Aspergillus fumigatus* in a French cohort of patients treated for haematological malignancies. *J Antimicrob Chemother*. 2011; 66(2): 371–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
83. Zhao Y, Stensvold CR, Perlin DS, *et al.*: Azole resistance in *Aspergillus fumigatus* from bronchoalveolar lavage fluid samples of patients with chronic diseases. *J Antimicrob Chemother*. 2013; 68(7): 1497–504.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
84. Denning DW, Park S, Lass-Flörl C, *et al.*: High-frequency triazole resistance found in nonculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. *Clin Infect Dis*. 2011; 52(9): 1123–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
85. **F** Chong GM, van der Beek MT, von dem Borne PA, *et al.*: PCR-based detection of *Aspergillus fumigatus* Cyp51A mutations on bronchoalveolar lavage: a multicentre validation of the AsperGenius assay® in 201 patients with haematological disease suspected for invasive aspergillosis. *J Antimicrob Chemother*. 2016; 71(12): 3528–35.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
86. Chong GL, van de Sande WW, Dingemans GJ, *et al.*: Validation of a new *Aspergillus* real-time PCR assay for direct detection of *Aspergillus* and azole resistance of *Aspergillus fumigatus* on bronchoalveolar lavage fluid. *J Clin Microbiol*. 2015; 53(3): 868–74.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
87. Alanio A, Bretagne S: Performance evaluation of multiplex PCR including *Aspergillus*-not so simple! *Med Mycol*. 2017; 55(1): 56–62.
[PubMed Abstract](#) | [Publisher Full Text](#)

88. **F** Kolwijck E, van der Hoeven H, de Sévaux RG, *et al.*: **Voriconazole-Susceptible and Voriconazole-Resistant *Aspergillus fumigatus* Coinfection.** *Am J Respir Crit Care Med.* 2016; **193**(8): 927–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
89. **F** Hagiwara D, Takahashi H, Watanabe A, *et al.*: **Whole-genome comparison of *Aspergillus fumigatus* strains serially isolated from patients with aspergillosis.** *J Clin Microbiol.* 2014; **52**(12): 4202–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
90. **F** Lewis JD, Chen EZ, Baldassano RN, *et al.*: **Inflammation, Antibiotics, and Diet as Environmental Stressors of the Gut Microbiome in Pediatric Crohn's Disease.** *Cell Host Microbe.* 2015; **18**(4): 489–500.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
91. **F** Hilton SK, Castro-Nallar E, Pérez-Losada M, *et al.*: **Metataxonomic and Metagenomic Approaches vs. Culture-Based Techniques for Clinical Pathology.** *Front Microbiol.* 2016; **7**: 484.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
92. Hiergeist A, Gläsner J, Reischl U, *et al.*: **Analyses of Intestinal Microbiota: Culture versus Sequencing.** *ILAR J.* 2015; **56**(2): 228–40.
[PubMed Abstract](#) | [Publisher Full Text](#)
93. **F** Lagier JC, Armougom F, Million M, *et al.*: **Microbial culturomics: paradigm shift in the human gut microbiome study.** *Clin Microbiol Infect.* 2012; **18**(12): 1185–93.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
94. Alanio A, Sturny-Leclère A, Benabou M, *et al.*: **Variation in copy number of the 28S rDNA of *Aspergillus fumigatus* measured by droplet digital PCR and analog quantitative real-time PCR.** *J Microbiol Methods.* 2016; **127**: 160–3.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:

Editorial Note on the Review Process

Faculty Reviews are review articles written by the prestigious Members of **Faculty Opinions**. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

Version 1

1. Jacques Meis

¹ Centre of Expertise in Mycology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

² Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

Competing Interests: No competing interests were disclosed.

2. Stephane Ranque

¹ Aix Marseille University, IP-TPT, Marseille, France

² Parasitologie & Mycologie, Hôpital de la Timone, Assistance Publique-Hôpitaux de Marseille, Marseille, France

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research