

Lateral Flow Assays for the Diagnosis of Invasive Aspergillosis: Current Status

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Abstract

Purpose of Review Diagnosis during early stages of invasive aspergillosis (IA) and targeted antifungal treatment has the potential to improve survival significantly. Despite advances in the diagnostic arsenal, invasive mold infections remain difficult to diagnose—especially at early stages before typical radiological signs develop. Varying availability and time-to-results are important limitations of current approved biomarkers and molecular assays for diagnosis of IA. Here, we will give an update on the *Aspergillus*-specific lateral-flow device (LFD) test. We further review promising findings on feasibility of point-of-care (POC) detection of urinary excreted fungal galactomannan-like antigens.

Recent Findings POC LFD assays for detection of *Aspergillus* antigens are currently in development. The *Aspergillus*-specific LFD test, which is based on the JF5 antibody (Ab), detects an extracellular glycoprotein antigen secreted during active growth of *Aspergillus* spp. The test has shown promising results in various studies. In addition, a monoclonal Ab476-based LFD for POC detection of urinary excreted fungal galactomannan-like antigens has been developed but needs further validation.

Summary Important advances have been made in the development of LFD assays for IA. Most promising is the *Aspergillus*-specific LFD test; commercial availability is still pending, however. The search for reliable POC tests for other molds, including mucorales, continues.

Keywords *Aspergillus* lateral flow device test · Point of care · Galactomannan-like antigens · MAb476 · JF5 · Bronchoalveolar lavage · Urine · Serum · Monoclonal antibody · Invasive aspergillosis

Introduction

Invasive aspergillosis (IA) is associated with high mortality rates [1–5]. Early and reliable diagnosis and rapid initiation of appropriate antifungal therapy has been shown to improve survival significantly [6, 7]. Culture-based approaches are important for detection of fungal species and resistance testing; however, they are limited by low sensitivities—in particular during early phases of infection—and long turnaround time [8•]. Significant advances to the field were brought by the introduction of non-cultural diagnostic tests for IA in blood and bronchoalveolar lavage fluid (BALF), including galactomannan antigen (GM) testing [9•, 10–13], PCR [14••, 15, 16••, 17], and beta-D-glucan (BDG) testing [18–24] in patients at risk [25•, 26, 27, 28•, 29]. In line with the introduction of non-cultural diagnostic tests, the rate of fungal infections diagnosed pre-mortem (versus post-mortem) was shown to increase from 16 to 51% in a large autopsy study [30].

Despite these significant advancements, the availability of these non-cultural diagnostic tests and time-to-results often varies with the size, specialization, and resources of the medical institution. “Pregnancy test-like” point-of-care lateral flow

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assays for detection of *Aspergillus* antigens are currently in development and may overcome these limitations.

Aspergillus-Specific Lateral Flow Device Test

The point-of-care *Aspergillus*-specific lateral-flow device test (LFD) uses a mouse monoclonal antibody, JF5, which binds to an extracellular glycoprotein antigen secreted by *Aspergillus* spp. only during active growth [31]. The LFD can be used for testing of serum and BALF samples and shows cross-reaction with *Penicillium* spp. only [31]. While serum samples require heating, centrifugation, and addition of a buffer solution before testing, BALF samples can be tested without any pre-treatment [32]. After 15 min of incubation time, results are read by the naked eye and are interpreted depending on the intensity of the test line as negative (–) or weak (+) to strong (+++) positive and have been shown to be reproducible between laboratories and studies [33, 34].

To date, two studies evaluated the diagnostic performance of the LFD in serum samples from adult patients with hematological malignancies [14, 35•], reporting sensitivities of 40 and 82% and specificities 87 and 80% for probable/proven invasive aspergillosis (IA) according to modified EORTC/MSG criteria [36], respectively. A meta-analysis, which included also data from the LFD development study [31] (i.e., in addition to the two studies mentioned above), reported a pooled sensitivity of 68% (95% confidence interval (CI), 52–81%), specificity of 87% (95% CI, 80–92%), and diagnostic odds ratio (DOR) of 11.90 (95% CI, 3.54–39.96) for differentiating proven/probable versus no IA cases in serum samples [37•]. Another very recently published study ignored the recommendations of the manufacturer by using the LFD in serum samples without pretreatment and found poor performance confirming that pretreatment of serum samples is a necessary step and that recommendations of the manufacturer should be followed [38]. Overall, the requirement for pretreatment has been a major limitation of serum LFD testing, as has been the inconsistency of reported results. Further studies, including

multicenter studies, are needed to determine whether LFD serum testing can be recommended for clinical routine.

In contrast to serum testing, BALF LFD testing has been evaluated in a number of studies including multicenter studies and in different patient populations. Results from the first four-part, retrospective-part, prospective studies (including two multicenter studies) which evaluated the LFD in mostly patients with underlying respiratory diseases [39••] and solid organ transplant recipients [28, 40••, 41] but also a smaller proportion of patients with underlying hematological malignancies [28, 41], were summarized in the meta-analysis reporting a pooled sensitivity of 86% (95% CI, 76–93%), specificity of 93% (95% CI, 89–96%), and DOR of 65.94 (95% CI, 27.21–159.81) for IA when using BALF samples [37].

Since then, BALF LFD testing has been evaluated in multicenter studies in intensive care unit patients [42••] and patients with underlying hematological malignancies [43], as well as a number of single-center studies [16••, 44–47]. The up-to-date performance of the LFD in BALF samples for different patient groups as well as the overall performance per sample are depicted in Table 1. Published data indicates that to date, 792 BALF samples have been tested at 6 different medical universities, including 113 samples from patients with probable/proven IA and 552 samples from patients with no evidence of IA, resulting in an overall sensitivity of 73% and specificity of 90% for probable/proven IA versus no evidence for IA. While the overall positive predictive value (PPV) was 61% and the negative predictive value (NPV) 94% in samples tested to date, both PPV and NPV will depend on the prevalence of IA in tested populations as displayed in Fig. 1. For example, in a patient cohort with an IA prevalence of 1%, the PPV will be 7.6%, while the NPV will be 99.7%; the PPV will go up and the NPV down with increase of IA prevalence (e.g., 5% IA prevalence: PPV 28%, NPV 98.4%; 10% IA prevalence: PPV 45%, NPV 96.8%; 20% IA prevalence: PPV 65%, NPV 93%; always assuming 73% sensitivity and 90% specificity).

Table 1 Per BALF sample performance of the BALF *Aspergillus* LFD for probable/proven invasive pulmonary aspergillosis versus no evidence for invasive pulmonary aspergillosis in different patient cohorts (percentage and absolute numbers)^a

Patient group	Sensitivity	Specificity	PPV	NPV
Overall ^b	73% (83/113)	90% (498/552)	61% (83/137)	94% (498/528)
Solid organ transplantation	94% (15/16)	92% (89/97)	65% (15/23)	99% (89/90)
Intensive care unit	79% (26/33)	85% (176/206)	46% (26/56)	96% (176/183)
Respiratory diseases	78% (25/32)	91% (196/215)	57% (25/44)	97% (196/203)
Hematological malignancies	67% (36/54)	91% (126/139)	73% (36/49)	88% (126/144)

PPV positive predictive value, NPV negative predictive value

^a Data derived from published studies [8•, 16••, 28•, 32, 33, 39–47, 48••]:

^b Overall summarizes unique samples and is lower than the sum of subgroup samples, as some samples were classified into more than one subgroup

Fig. 1 Overall positive and negative predictive values of the bronchoalveolar lavage fluid *Aspergillus*-specific lateral flow device test in cohorts with prevalence rates of invasive aspergillosis between 1 and 30%. The overall sensitivity of 73% and specificity of 90% were used for the calculation

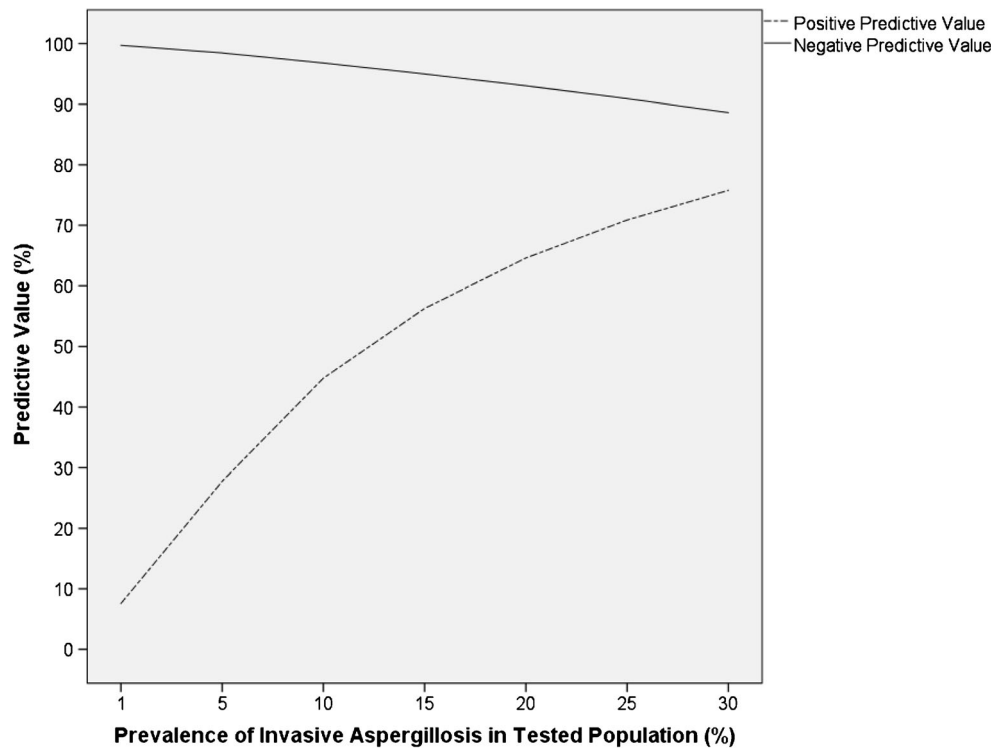


Table 2 summarizes LFD results in 127 samples from patients with possible IPA according to modified EORTC/MSG criteria [36, 49], as well as patient subgroups. The LFD resulted positive in 39% of possible IA samples versus 10% of samples from patients with no evidence of IA. When considering that diagnostic test performance calculations in the field of IA are limited by the insensitivity of all current diagnostics (including GM), these results may indicate that the LFD has additional discriminatory power in those with possible IPA, i.e., positive LFD results may provide evidence that some of those with possible IPA do in fact have (probable) IPA and a false negative GM test result. If true, positive BALF LFD test results should be considered as a future mycological factor (in addition to GM, culture, and PCR) for updated EORTC/MSG criteria.

When comparing different patient populations (Table 1), LFD sensitivity was lower in patients with underlying hematologic malignancies (67% sensitivity) compared to other patient groups. The most likely reason for lower sensitivity in patients with hematological malignancies is the frequent use of antifungal prophylaxis/empirical antifungal therapy that is beneficiary for survival in this patient population [50–56]. Similar to other fungal diagnostics, sensitivity of the LFD was reduced in the presence of antifungal prophylaxis/treatment (sensitivity 56% in those with mold active antifungals versus 86% in those without; $p = 0.0097$ Fisher’s exact test; Table 3) [8•]. The solution is combining the LFD with other biomarkers such as GM in BALF and/or serum, PCR, or novel biomarkers such as triacetylfusarinine C (TAFC) [14••,

Table 2 Performance of the BALF *Aspergillus* LFD in cases of possible invasive pulmonary aspergillosis (per BALF sample) in different patient cohorts.^a

Patient group	Positive LFD Result Percentage (absolute numbers)	Negative LFD Result Percentage (absolute numbers)
Overall ^b	39% (50/127)	61% (77/127)
<i>Solid organ transplantation</i>	33% (4/12)	66% (8/12)
<i>Intensive care unit</i>	37% (14/38)	63% (24/38)
<i>Respiratory diseases</i>	65% (20/31)	35% (11/31)
<i>Hematological malignancies</i>	32% (26/81)	68% (55/81)

^a Data derived from published studies [16••, 28•, 39•, 40, 41, 42••, 43••, 46, 47]

^b Overall summarizes unique samples and is lower than the sum of subgroup samples, as some samples were classified into more than one subgroup

Table 3 Sensitivity of BALF LFD for probable/proven IPA in patients with and without antifungal prophylaxis/therapy (information only available for a proportion of cases published).^a

	BALF LFD sensitivity for IPA overall percentage (absolute numbers)
Overall	75% (50/67)
Under mold active systemic antifungals	56% (14/25)
Without mold active antifungals	86% (36/42)

BALF bronchoalveolar lavage fluid, IPA invasive pulmonary aspergillosis, LFD lateral flow device

^a Data derived from [8•], updated with [16••, 45, 46]

[16••, 28, 43••, 45, 48••], which has been shown to increase sensitivity substantially and helps to overcome this limitation.

After issues emerged with the previous manufacturing partner, redevelopment work was undertaken by OLM diagnostics after it was given full control to develop and manufacture the assay on top of its original role as sales and marketing partner. Development work is well underway and OLM are expecting to start production over the coming months and launch the LFD by the end of 2017.

Lateral Flow Device for Galactomannan-Like Antigens in Urine

Antigen testing of urine samples may provide important advantages, including non-invasive and easy sample collection that allows for more frequent examination of large volumes, which may increase test sensitivity and also has great potential for home testing [57, 58••]. Recent studies have indicated that GM testing may be promising in urine samples [57, 59, 60], while results for urine BDG testing were less convincing [61]. Fisher and colleagues reported lower specificity of GM testing of urine specimens compared to serum (80% versus 95%) in pediatric hematologic malignancy patients; however, urine GM testing successfully identified the only case of probable IA [60]. These preliminary results were confirmed in a study conducted in adult hematologic malignancy patients by Duettmann and colleagues [62]. In that study, 242 same-day serum and urine samples were included from 75 adult patients prospectively and consecutively. Out of these 75, 10 patients met criteria for probable IA; 3 additional patients were tested positive for serum GM levels. Urine samples were not pretreated before GM testing, and urine GM levels showed a significant positive correlation with serum GM levels. Sensitivity of urine GM testing was limited and only improved when using an extremely low GM cut-off of 0.1 optical density index (ODI). With that cut-off, urine GM testing exhibited 71% sensitivity and 88% specificity for probable IA. Recently, Reischies and

colleagues showed in another prospective study in adult patients with hematological malignancies that test performance in urine samples can be improved by calculation of the urine GM/creatinine ratio, which takes urine dilution into account and may be a promising diagnostic tool for patients with hematological malignancies [57]. With a threshold of 0.26 ((urine GM [ODI] × 100)/(urine creatinine [mg/dL])), the positive predictive value of 13% was low, but the negative predictive value of >98% would qualify this diagnostic method for ruling out IA in high-risk patients [57].

Given this promising results, development of a point of care (POC) test for diagnosis of IA in urine samples has the potential of impacting patient care and associated costs significantly, as such a test may allow for home testing for IA. Dufresne and others recently reported that their new monoclonal antibody MAb476 was capable to detect GM-like antigen (Ag) in urine samples [58••]. Using in vitro and animal experiments, Dufresne and others [58••] investigated renal clearance of serum GM-like Ag in mouse models infected with *A. fumigatus* and generated MAb476. MAb476-based sandwich-ELISAs (sELISA) reliably detected GM-like Ag in bronchoalveolar lavage fluid, serum, and lung tissue of neutropenic mice and urine samples from guinea pigs after infection with air-borne IA [58••]. Experiments showed a specific affinity of MAb476 to *Aspergillus* spp. (excluding *A. terreus*) as well as *Fusarium* spp., *Paecilomyces* spp., and *Trichophyton rubrum* [58••]. MAb476 therefore differed from the EBA2 antibody used in Platelia® GM EIA, which showed also affinity to *A. terreus* and *Histoplasma capsulatum* C Ag [58••]. While these two antibodies may show affinity to different epitopes of the GM-like Ag, which would explain the differences, the detailed structure of the GM-like Ag has not yet been revealed [58••].

As a next step, Dufresne and colleagues successfully constructed a MAb476-based LFD prototype for urine POC testing. The functionality of MAb476 testing was confirmed with human urine samples, which were obtained from healthy volunteers and spiked with in vitro-produced *Aspergillus* antigen [58••]. However, when retrospectively testing stored samples which were collected from 11 patients who were categorized as probable/proven IA and showed positive serum GM results, sensitivity was imperfect, as only samples from 4 out of these 11 patients also had positive test results with the MAb476-sELISA and MAb476-LFD after pretreatment [58••]. Importantly, MAb476 testing appeared to be inhibited by an unknown substance or mix of substances in human urine, and this effect positively correlated to the specific mass of the urine samples [58••]. Boiling and centrifugation were not able to abandon the inhibition, and the relevant substances appeared to weigh less than 2 kDa [58••]. Urine sample concentration (5–10 fold),

followed by desalting/dialysis (7 kDa), resulted in a nearly complete diminution of the inhibition [58••].

While these results on development of the MAb476-LFD for urine samples were promising, it has to be kept in mind that the sample size was limited and the study settings were non-clinical and animal models in large parts. Additionally, the quality of the clinical samples and other information about the patients (like the presence of antifungal treatment) could not be satisfactorily evaluated [58••]. Referring to the planned use in clinical settings, the MAb476-LFD needs further optimization to fit requirements for a reliable POC device, which is currently ongoing.

Conclusion

Important advances have been made in the development of lateral flow assays for invasive aspergillosis. Most promising is the *Aspergillus*-specific lateral-flow device test, which is based on the JF5 antibody and has shown convincing performance in multiple clinical studies, in particular in BALF samples. Commercial availability is still pending, however. Recently, an LFD prototype for urine POC testing based on MAB476 has been constructed, which is currently undergoing further evaluation in studies with bigger sample sizes. Overall, the evaluation of the diagnostic performance of new assays in IA remains problematic, as the vast majority of IA cases are “probable” cases and there is no established diagnostic test in clinical routine that provides sensitivity high enough to qualify the test as a reliable gold standard.

The search for reliable antigen-based POC tests for other molds, including mucorales, continues [63]. Recently, an enzyme-linked immunospot assay has been developed, for detection of Mucorales-specific T cells in peripheral blood samples [63]. Mucorales-specific T cells polarized to the production of T helper type 2 cytokines were associated with proven invasive mucormycosis and may be detected by immunoenzymatic assays or immunocytofluorimetric assays [63]. Once validated, that assay may represent a breakthrough in diagnosis of invasive mucormycosis [63].

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Compliance with Ethical Standards

Conflict of Interest Sven Heldt declares that he has no conflict of interest.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ, 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.
2. Pagano L, Caira M, Candoni A, Offidani M, Martino B, Specchia G, et al. Invasive aspergillosis in patients with acute myeloid leukemia: a SEIFEM-2008 registry study. *Haematologica*. 2010;95(4):644–50.
3. Nucci M, Marr KA, Vehreschild MJ, de Souza CA, Velasco E, Cappellano P, et al. Improvement in the outcome of invasive fusariosis in the last decade. *Clin Microbiol Infect*. 2014;20(6):580–5.
4. Perkhofer S, Lass-Flörl C, Hell M, Russ G, Krause R, Honigl M, et al. The Nationwide Austrian Aspergillus registry: a prospective data collection on epidemiology, therapy and outcome of invasive mould infections in immunocompromised and/or immunosuppressed patients. *Int J Antimicrob Agents*. 2010;36(6):531–6.
5. Reischies F, Hoenigl M. The role of surgical debridement in different clinical manifestations of invasive aspergillosis. *Mycoses*. 2014;57(Suppl 2):1–14.
6. Neofytos D, Treadway S, Ostrander D, Alonso CD, Dierberg KL, Nussenblatt V, et al. Epidemiology, outcomes, and mortality predictors of invasive mold infections among transplant recipients: a 10-year, single-center experience. *Transpl Infect Dis*. 2013;15(3):233–42.
7. Ramos ER, Jiang Y, Hachem R, Kassis C, Kontoyiannis DP, Raad I. Outcome analysis of invasive aspergillosis in hematologic malignancy and hematopoietic stem cell transplant patients: the role of novel antimold azoles. *Oncologist*. 2011;16(7):1049–60.
8. Eigl S, Prattes J, Reinwald M, Thornton CR, Reischies F, Spiess B, et al. Influence of mould-active antifungal treatment on the performance of the *Aspergillus*-specific bronchoalveolar lavage fluid lateral-flow device test. *Int J Antimicrob Agents*. 2015a;46(4):401–5. **Study showing the influence of mould active antifungal prophylaxis and treatment on sensitivity of fungal diagnostics, including GM, culture and LFD.**
9. Duarte RF, Sanchez-Ortega I, Cuesta I, Arnan M, Patino B, Fernandez de Sevilla A, 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. **Groundbreaking study showing that positive predictive value of serum GM is reduced in the presence of antimould prophylaxis.**
10. Maertens J, Maertens V, Theunissen K, Meersseman W, Meersseman P, Meers S, et al. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary

- aspergillosis in patients with hematologic diseases. *Clin Infect Dis*. 2009;49(11):1688–93.
11. D'Haese J, Theunissen K, Vermeulen E, Schoemans H, De Vlieger G, Lammertijn L, et al. Detection of galactomannan in bronchoalveolar lavage fluid samples of patients at risk for invasive pulmonary aspergillosis: analytical and clinical validity. *J Clin Microbiol*. 2012;50(4):1258–63.
 12. Hoenigl M, Seeber K, Koidl C, Buzina W, Wolfner A, Duettmann W, et al. Sensitivity of galactomannan enzyme immunoassay for diagnosing breakthrough invasive aspergillosis under antifungal prophylaxis and empirical therapy. *Mycoses*. 2013;56(4):471–6.
 13. Hoenigl M, Salzer HJ, Raggam RB, Valentin T, Rohn A, Woelfler A, et al. Impact of galactomannan testing on the prevalence of invasive aspergillosis in patients with hematological malignancies. *Med Mycol*. 2012a;50(3):266–9.
 14. White PL, Parr C, Thornton C, Barnes RA. 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. **Most comprehensive study to date evaluating performance of the LFD in serum specimens.**
 15. Boch T, Reinwald M, Postina P, Cornely OA, Vehreschild JJ, Heussel CP, et al. Identification of invasive fungal diseases in immunocompromised patients by combining an *Aspergillus* specific PCR with a multifungal DNA-microarray from primary clinical samples. *Mycoses*. 2015;58(12):735–45.
 16. Eigl S, Hoenigl M, Spiess B, Heldt S, Prattes J, Neumeister P, et al. Galactomannan testing and *Aspergillus* PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. *Med Mycol*. 2016. **Important study showing that combination of serum and BALF GM with PCR improves sensitivity for diagnosing IA.**
 17. Springer J, Morton CO, Perry M, Heinz WJ, Paholcsek M, Alzheimer M, et al. Multicenter comparison of serum and whole-blood specimens for detection of *Aspergillus* DNA in high-risk hematological patients. *J Clin Microbiol*. 2013;51(5):1445–50.
 18. Reischies FM, Prattes J, Pruller F, Eigl S, List A, Wolfner A, et al. Prognostic potential of 1,3-beta-d-glucan levels in bronchoalveolar lavage fluid samples. *J Inf Secur*. 2016a;72(1):29–35.
 19. Reischies FM, Prattes J, Woelfler A, Eigl S, Hoenigl M. Diagnostic performance of 1,3-beta-d-glucan serum screening in patients receiving hematopoietic stem cell transplantation. *Transpl Infect Dis*. 2016b;18(3):466–70.
 20. Prattes J, Hoenigl M, Rabensteiner J, Raggam RB, Pruellner F, Zollner-Schwetz I, et al. Serum 1,3-beta-d-glucan for antifungal treatment stratification at the intensive care unit and the influence of surgery. *Mycoses*. 2014a;57(11):679–86.
 21. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. Beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clin Infect Dis*. 2011;52(6):750–70.
 22. Pruller F, Wagner J, Raggam RB, Hoenigl M, Kessler HH, Truschnig-Wilders M, et al. Automation of serum (1→3)-beta-D-glucan testing allows reliable and rapid discrimination of patients with and without candidemia. *Med Mycol*. 2014;52(5):455–61.
 23. Prattes J, Raggam RB, Vanstraelen K, Rabensteiner J, Hoegenauer C, Krause R, et al. Chemotherapy-induced intestinal mucosal barrier damage: a cause of falsely elevated serum 1,3-beta-d-glucan levels? *J Clin Microbiol*. 2016a;54(3):798–801.
 24. Hoenigl M, Perez-Santiago J, Nakazawa M, de Oliveira MF, Zhang Y, Finkelman MA, et al. (1→3)-beta-d-glucan: a biomarker for microbial translocation in individuals with acute or early HIV infection? *Front Immunol*. 2016;7:404.
 25. Ceesay MM, Desai SR, Berry L, Cleverley J, Kibbler CC, Pomplun S, et al. A comprehensive diagnostic approach using galactomannan, targeted beta-d-glucan, baseline computerized tomography and biopsy yields a significant burden of invasive fungal disease in at risk haematology patients. *Br J Haematol*. 2015;168(2):219–29. **Another study showing the importance of combining multiple diagnostic approaches to achieve acceptable sensitivity in patients receiving antimould prophylaxis.**
 26. Sulahian A, Porcher R, Bergeron A, Touratier S, Raffoux E, Menotti J, et al. Use and limits of (1-3)-beta-d-glucan assay (Fungitell), compared to galactomannan determination (*Platelia Aspergillus*), for diagnosis of invasive aspergillosis. *J Clin Microbiol*. 2014;52(7):2328–33.
 27. Mikulska M, Furfaro E, Viscoli C. Non-cultural methods for the diagnosis of invasive fungal disease. *Expert Rev Anti-Infect Ther*. 2015;13(1):103–17.
 28. Hoenigl M, Prattes J, Spiess B, Wagner J, Pruellner F, Raggam RB, et al. Performance of galactomannan, beta-d-glucan, *Aspergillus* lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. *J Clin Microbiol*. 2014a;52(6):2039–45. **Study showing that combining multiple diagnostic tests in BALF, including the LFD, increased sensitivity and diagnostic odds ratio.**
 29. Morrissey CO, Chen SC, Sorrell TC, Milliken S, Bardy PG, Bradstock KF, 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.
 30. Lewis RE, Cahyame-Zuniga L, Leventakos K, Chamilos G, Ben-Ami R, Tamboli P, et al. Epidemiology and sites of involvement of invasive fungal infections in patients with haematological malignancies: a 20-year autopsy study. *Mycoses*. 2013;56(6):638–45.
 31. Thornton CR. Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis. *Clin Vaccine Immunol*. 2008;15(7):1095–105.
 32. Thornton C, Johnson G, Agrawal S. Detection of invasive pulmonary aspergillosis in haematological malignancy patients by using lateral-flow technology. *J Vis Exp*. 2012;(61).
 33. Prattes J, Heldt S, Eigl S, Hoenigl M. Point of care testing for the diagnosis of fungal infections: are we there yet? *Curr Fungal Infect Rep*. 2016b;10:43–50.
 34. Wiederhold NP, Najvar LK, Bocanegra R, Kirkpatrick WR, Patterson TF, Thornton CR. Interlaboratory and interstudy reproducibility of a novel lateral-flow device and influence of antifungal therapy on detection of invasive pulmonary aspergillosis. *J Clin Microbiol*. 2013;51(2):459–65.
 35. Heldt J, Schmidt T, Thornton CR, Kotter E, Bertz H. Comparison of a novel *Aspergillus* lateral-flow device and the *Platelia*(R) galactomannan assay for the diagnosis of invasive aspergillosis following haematopoietic stem cell transplantation. *Infection*. 2013;41(6):1163–9. **Study showing that LFD results are reproducible between laboratories and studies.**
 36. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, 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.
 37. Pan Z, Fu M, Zhang J, Zhou H, Fu Y, Zhou J. Diagnostic accuracy of a novel lateral-flow device in invasive aspergillosis: a meta-analysis. *J Med Microbiol*. 2015;64(7):702–7. **Metaanalysis of the *Aspergillus* LFD performance in serum and BALF.**
 38. Metan G, Keklik M, Dinc G, Pala C, Yildirim A, Saraymen B, et al. Performance of galactomannan antigen, beta-d-glucan, and *Aspergillus*-lateral-flow device for the diagnosis of invasive aspergillosis. *Indian J Hematol Blood Transfus*. 2017;33(1):87–92.
 39. Prattes J, Flick H, Pruller F, Koidl C, Raggam RB, Palfner M, et al. Novel tests for diagnosis of invasive aspergillosis in patients with

- underlying respiratory diseases. *Am J Respir Crit Care Med*. 2014b;190(8):922–9. **Groundbreaking study of BALF LFD performance in over 200 patients with underlying pulmonary diseases.**
40. Willinger B, Lackner M, Lass-Flörl C, Prattes J, Posch V, Selitsch B, et al. Bronchoalveolar lavage lateral-flow device test for invasive pulmonary aspergillosis in solid organ transplant patients: a semiprospective multicenter study. *Transplantation*. 2014;98(8):898–902. **Multicenter study on BALF LFD performance in recipients of solid organ transplantation.**
 41. Hoenigl M, Koidl C, Duettmann W, Seeber K, Wagner J, Buzina W, et al. Bronchoalveolar lavage lateral-flow device test for invasive pulmonary aspergillosis diagnosis in haematological malignancy and solid organ transplant patients. *J Inf Secur*. 2012b;65(6):588–91.
 42. Eigl S, Prattes J, Lackner M, Willinger B, Spiess B, Reinwald M, et al. Multicenter evaluation of a lateral-flow device test for diagnosing invasive pulmonary aspergillosis in ICU patients. *Crit Care*. 2015b;19:178-015-0905-x. **Multicenter study on BALF LFD performance in recipients of solid organ transplantation.**
 43. Prattes J, Lackner M, Eigl S, Reischies F, Raggam RB, Koidl C, et al. Diagnostic accuracy of the Aspergillus-specific bronchoalveolar lavage lateral-flow assay in haematological malignancy patients. *Mycoses*. 2015a;58(8):461–9. **Multicenter study on BALF LFD performance in patients with hematologic malignancies.**
 44. Prattes J, Koidl C, Eigl S, Krause R, Hoenigl M. Bronchoalveolar lavage fluid sample pretreatment with Sputasol(R) significantly reduces galactomannan levels. *J Inf Secur*. 2015b;70(5):541–3.
 45. Prattes J, Orasch T, Eigl S, Heldt S, Duettmann W, Faserl K, et al. Diagnostic performance of bronchoalveolar lavage triacetylufusarinine C (T AFC) determination for invasive pulmonary aspergillosis in patients with hematological malignancies. *Open Forum Infect Dis*. 2016c;3(Suppl 1):1558.
 46. Heldt S, Eigl S, Prattes J, Flick H, Rabensteiner J, Neumeister P, et al. Levels of IL-6, IL-8, IL-10 and IL-17A in serum and IL-8 in bronchoalveolar lavage fluid are elevated in haematological patients with invasive pulmonary aspergillosis. *ECCMID 2017*. Poster #P0989.
 47. Miceli MH, Goggins MI, Chander P, Sekaran AK, Kizy AE, Samuel L, et al. Performance of lateral flow device and galactomannan for the detection of Aspergillus species in bronchoalveolar fluid of patients at risk for invasive pulmonary aspergillosis. *Mycoses*. 2015;58(6):368–74.
 48. Johnson GL, Sarker SJ, Nannini F, Ferrini A, Taylor E, Lass-Flörl C, 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. **Study evaluating combinations of the LFD, qPCR, and GM in immunocompromised patients.**
 49. Hoenigl M, Strenger V, Buzina W, Valentin T, Koidl C, Wolfler A, et al. European Organization for the Research and Treatment of Cancer/Mycoses study group (EORTC/MSG) host factors and invasive fungal infections in patients with haematological malignancies. *J Antimicrob Chemother*. 2012c;67(8):2029–33.
 50. Hoenigl M, Duettmann W, Raggam RB, Huber-Krassnitzer B, Theiler G, Seeber K, et al. Impact of structured personal on-site patient education on low posaconazole plasma concentrations in patients with haematological malignancies. *Int J Antimicrob Agents*. 2014b;44(2):140–4.
 51. Heimann SM, Cornely OA, Vehreschild MJ, Glossmann J, Kochanek M, Kreuzer KA, et al. Treatment cost development of patients undergoing remission induction chemotherapy: a pharmacoeconomic analysis before and after introduction of posaconazole prophylaxis. *Mycoses*. 2014;57(2):90–7.
 52. Vehreschild JJ, Ruping MJ, Wisplinghoff H, Farowski F, Steinbach A, Sims R, et al. Clinical effectiveness of posaconazole prophylaxis in patients with acute myelogenous leukaemia (AML): a 6 year experience of the Cologne AML cohort. *J Antimicrob Chemother*. 2010;65(7):1466–71.
 53. Cornely OA, Duarte RF, Haider S, Chandrasekar P, Helfgott D, Jimenez JL, et al. Phase 3 pharmacokinetics and safety study of a posaconazole tablet formulation in patients at risk for invasive fungal disease. *J Antimicrob Chemother*. 2016;71(3):718–26.
 54. Vanstraelen K, Prattes J, Maertens J, Lagrou K, Schoemans H, Peersman N, et al. Posaconazole plasma exposure correlated to intestinal mucositis in allogeneic stem cell transplant patients. *Eur J Clin Pharmacol*. 2016;72(8):953–63.
 55. Prattes J, Duettmann W, Hoenigl M. Posaconazole plasma concentrations on days three to five predict steady-state levels. *Antimicrob Agents Chemother*. 2016;60(9):5595–9.
 56. Hoenigl M, Raggam RB, Salzer HJ, Valentin T, Valentin A, Zollner-Schwetz I, et al. Posaconazole plasma concentrations and invasive mould infections in patients with haematological malignancies. *Int J Antimicrob Agents*. 2012d;39(6):510–3.
 57. Reischies FM, Raggam RB, Prattes J, Krause R, Eigl S, List A, et al. Urine galactomannan-to-creatinine ratio for detection of invasive aspergillosis in patients with hematological malignancies. *J Clin Microbiol*. 2016c;54(3):771–4. **Study that introduced urine GM testing as a useful method for clinical use, if urine concentrations are taken into account.**
 58. Dufresne SF, Datta K, Li X, Dadachova E, Staab JF, Patterson TF, et al. Detection of urinary excreted fungal galactomannan-like antigens for diagnosis of invasive aspergillosis. *PLoS One*. 2012;7(8):e42736. **Study reporting development of POC test for detection of urine GM-like antigens.**
 59. Duettmann W, Koidl C, Krause R, Lackner G, Woelfler A, Hoenigl M. Specificity of mannan antigen and anti-mannan antibody screening in patients with haematological malignancies at risk for fungal infection. *Mycoses*. 2016;59(6):374–8.
 60. Fisher BT, Zaoutis TE, Park JR, Bleakley M, Englund JA, Kane C, et al. Galactomannan antigen testing for diagnosis of invasive aspergillosis in pediatric hematology patients. *J Pediatric Infect Dis Soc*. 2012;1(2):103–11.
 61. Raggam RB, Fischbach LM, Prattes J, Duettmann W, Eigl S, Reischies F, et al. Detection of (1→3)-beta-D-glucan in same-day urine and serum samples obtained from patients with haematological malignancies. *Mycoses*. 2015;58(7):394–8.
 62. Duettmann W, Koidl C, Troppan K, Seeber K, Buzina W, Wolfler A, et al. Serum and urine galactomannan testing for screening in patients with hematological malignancies. *Med Mycol*. 2014;52(6):647–52.
 63. Potenza L, Vallerini D, Barozzi P, Riva G, Gilioli A, Forghieri F, et al. Mucorales-specific T cells in patients with hematologic malignancies. *PLoS One*. 11(2):e0149108.