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Point-of-care diagnostics for invasive aspergillosis: nearing the finish line

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Abstract:

Introduction: The spectrum of disease caused by *Aspergillus* spp. is dependent on the immune system of the host, with invasive aspergillosis (IA) its most severe manifestation. Early and reliable diagnosis of *Aspergillus* disease is important to decrease associated morbidity and mortality from IA.

Areas covered: The following review searched Pub Med for literature published since 2007 and will give an update on the current point-of-care diagnostic strategies for the diagnosis of IA, discuss needed areas of improvement for these tests, and future directions.

Expert opinion: Several new diagnostic tests for IA – including point-of-care tests - are now available to complement conventional galactomannan (GM) testing. In particular, the *Aspergillus*-specific Lateral Flow Device (LFD) test and the sōna *Aspergillus* GM Lateral Flow Assay (LFA) are promising for the diagnosis of IA in patients with hematologic malignancy, although further evaluation in the non-hematology setting is needed. In addition, a true point-of-care test, particularly for easily obtained specimens like serum or urine that can be done at the bedside or in the Clinic in a matter of minutes is needed, such as the lateral flow dipstick test, which is under current evaluation. Lastly, improved diagnostic algorithms to diagnose IA in non-neutropenic patients is needed.

Key words: Lateral flow device test, Lateral flow assay, lateral flow dipstick, bronchoalveolar lavage, serum, urine, BDG, automated reader.

Article Highlights:

- Conventional Galactomannan ELISA testing is limited by long processing times, and on a global level only a small proportion of mycology laboratories can actually offer GM testing.
- Point-of-care diagnostic test for IPA are now commercially available and two tests are CE-marked, the LFA and LFD tests.
- These tests have been shown to reliably diagnose IPA in patients with hematological malignancies, and performance can be further improved by automatic readout of test results.
- More data for both CE-marked tests is needed outside the hematological malignancy setting, particularly evaluating performance in BALF but also serum, as reliable serum markers for IPA in non-neutropenic patients are lacking.
- Reliable serum markers for IPA in non-neutropenic patients would be particularly valuable for patients with COVID-19, where IPA occurs frequently, but bronchoscopy is rarely performed due to fear of transmission by this droplet-creating procedure.
- Both the LFA and LFD have potential to diagnose IPA on their own and can also complement GM and culture as well as other novel biomarkers for optimizing diagnostic performance for IPA.
- Only the LFD offers true point-of-care diagnosis at the bedside without sample treatment and only for non-hemorrhagic and non-viscous BALF samples. True bedside diagnostic tests that can be performed on a variety of clinical samples are needed.
- Reliable consensus definitions are needed for classifying IA in patients outside the hematology setting.

1. Introduction:

Aspergillus is a genus of ubiquitous, environmental fungi found in soil, water, food, and air that cause a wide spectrum of infections in humans [1]. Of over 250 species of *Aspergillus*, fewer than 40 are thought to cause infections in humans [2], with the spectrum of disease ranging from allergic bronchopulmonary aspergillosis (ABPA) to its most severe manifestation, invasive aspergillosis (IA) [3]. The 2017 global estimates (https://www.gaffi.org) indicate that over 300,000 cases of IA occur annually, with a mortality rate ranging from 30-80% [4,5]. Studies from settings which still perform routine autopsies in hospital deaths indicate that about 50% of IA cases are detected only postmortem [6], although overall autopsy rates in hospital deaths is below 5% in the United States [7] and the true mortality rate from IA is probably trending towards the upper range of the estimate.

The two most important predictors of survival from IA are early diagnosis and prompt initiation of appropriate antifungal therapy [8,9]. Despite significant advances over recent years in the field of non-culture based IA diagnostics, early diagnosis still remains difficult to establish [10]. Currently, the gold standard for the diagnosis of IA is the detection of the fungal cell wall component galactomannan (GM) [11-14], a polysaccharide that primarily exists in the cell wall of *Aspergillus* species [15]. This test (Platelia™, Bio-Rad, Marnes-la-Coquette, France) is approved by the U.S. Food and Drug Administration (FDA) for testing of GM from serum and bronchoalveolar fluid (BALF). More sensitive than culture, the current sensitivity and specificity of GM from blood in neutropenic patients with underlying hematologic malignancy are 82% and 81%, respectively [14]. The optimal optical density index (ODI) threshold is debatable, although the FDA considers an ODI of $\geq$0.5 to be positive for GM in both serum and BALF. GM from BALF has shown better diagnostic performance for invasive aspergillosis than GM from blood, particularly in patients on mold-active antifungal prophylaxis [16], with a sensitivity and specificity of 0.88 and 0.81, respectively, from BALF at an ODI of 0.5 [17].

In non-hematologic patients, IA is emerging in various patient groups including those in the intensive care unit (ICU) with severe influenza [18] or coronavirus disease 2019 (COVID-19) [19-23], but reliable definitions of IA are missing [24-26]. In these settings with non-neutropenic patients the utility of GM is less clear, with a lower sensitivity of GM from serum in non-hematologic patients [27] due to airway invasive growth [24]. While GM testing from BALF is generally preferable [28], there are higher false positive rates of GM from BALF in some studies, possibly due to *Aspergillus* colonization and less invasive *Aspergillus* infection [29,30]. However, GM testing remains limited by the distance/duration of transport between

the clinical setting and the laboratory where the test is performed, as well as by varying turnaround times dependent on the number of specimens to be tested [13,32-35].

Other molecular tests such as polymerase chain reaction (PCR) have emerged as alternative options to diagnose IA and are widely used [32,36,37], although there is a lack of standardization of these assays [38] and a large variation in diagnostic performance across studies and settings [34,39], with particularly poor performance in blood for the diagnosis of breakthrough infections [40] in settings that use mold-active prophylaxis [41,42]. Other fungal diagnostics such as Triacetyfusarinine-C [43] and immune parameters [44] may hold promise, but need further investigation before they can be implemented into clinical routine.

Better and more rapid and easily performed diagnostics are therefore needed to enable early diagnosis, including single sample testing, to enable earlier, targeted treatment of IA. For the following review authors searched Pub Med for literature on "point of care" OR "rapid" tests for aspergillosis/*Aspergillus* published since 2007 and used reference sections of applicable papers to identify more references. The review will give an update on the available point-of-care (POC) tests for the diagnosis of IA, focusing on the IMMY sōna *Aspergillus* Galactomannan Lateral Flow Assay (LFA) (IMMY, Norman, Oklahoma, USA) and the *Aspergillus*-Specific Lateral-Flow Device (LFD) test (OLM Diagnostics, Newcastle Upon Tyne, UK), as well as discuss promising new diagnostic tests that are currently under development.

2. Which point of care diagnostics for invasive aspergillosis are available?

2.1. Aspergillus Galactomannan LFA

The IMMY sōna *Aspergillus* Galactomannan LFA (IMMY, Norman, Oklahoma, USA) is an immunochromatographic test system for the qualitative or quantitative (i.e., when utilizing an automated reader) detection of *Aspergillus* Galactomannan in serum and BAL samples. The LFA is constructed by having Galactomannan-specific antibodies conjugated to colloidal gold that binds to Galactomannan that may be present in the specimen sample as it flows up the test strip. If binding occurs, the antibody-antigen complex will migrate up the strip by capillary flow until it is captured by the Galactomannan specific antibodies in the test line, resulting in the formation of a visible test line. Additionally, control antibodies conjugated to gold are present that wick along with the specimen and will be captured by the control antibodies present on the control line, regardless of positive or negative test results. The test requires a total of 300 µL of BALF or serum sample which are pretreated, heated, and centrifuged, before an aliquot is being transferred to a second tube and mixed with a running buffer. Test strips are then inserted into the sample running buffer aliquot and results read

after 30 minutes [24,45-47]. Positive test results create two lines (test and control lines) and negative results form only one line (control line). If a control line fails to develop, the test is not valid. Depending on the intensity of the test line, scores are given ranging from 0 (i.e. negative), to 4 (highly positive) by manual read (i.e., qualitative detection of GM), or test lines read by an automated cube reader that is now included with the test kits (i.e, quantitative detection of GM) [45]. The test is CE-marked and currently in the process of getting FDA approval.

2.2. *Aspergillus*-specific LFD

The newly formatted and CE-marked *Aspergillus* specific LFD (OLM Diagnostics, Newcastle upon Tyne, UK) detects extracellular antigenic mannoproteins secreted exclusively during active growth of *Aspergillus* species which binds to the JF5 mouse monoclonal antibody [16,48-53]. The same JF5 monoclonal antibody has also been used successfully in antibody-guided PET/magnetic resonance imaging (immunoPET/MRI) of IA [54]. This assay is described in great detail elsewhere [55]. Importantly, the new CE-marked version of the LFD, which became available in late 2017, should not be confused with previous prototypes of the test of which some batches have shown inconsistent test results [49]. In a direct comparison, the newly formatted test has been shown to be more stable and have higher specificity than the previous prototype [49]. The LFD can be used for testing of serum and BALF samples and shows cross-reaction with *Penicillium* spp. only [56]. Serum samples and hemorrhagic or viscous (i.e., due to large amounts of mucus) BALF samples, per the manufacturers recommendations, require heating after adding an EDTA-containing buffer to 150 µL serum or BALF sample, followed by centrifugation before 70 µL of the supernatant can be applied to the LFD. Other BALF samples that are neither vicious nor hemorrhagic can be applied to the LFD without any pre-treatment [24,57]. The test results are read after 15-minutes. The appearance of the control line in the result window shows that the test had run correctly. The appearance of the *Aspergillus*-specific test line determined after exactly 15 min incubation time. Result 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 [58]. In addition, some studies have reported success in setting up an objective readout method and quantification of results using a digital LFD reader, that has to be purchased from a separate company [57,59]. Peak positions were determined using negative and positive controls included in the LFD kit.

Recent studies have noted a delayed appearance of a test line after more than 15 min in some samples that were negative at the 15-min mark. Later read out after 20 or even 30 minutes increased sensitivity, but came at the cost of slightly lower specificity [16,24,46,48].

2.3. Lateral Flow dipstick from Urine

A third point-of-care (POC) assay, the lateral flow dipstick assay, uses the galactofuranose-specific monoclonal antibody (mAb476), which has been shown to recognize urine antigens after *A. fumigatus* pulmonary infection in animal models [60]. Briefly, in a study of this assay in humans, pooled urine samples were obtained from healthy individuals and banked urine samples in patients diagnosed with IA, based on a positive serum GM, were used. Unconjugated mAb476 and a goat anti-mouse IgM were immobilized at the test and control spots, respectively, on HF180 Nitrocellulose membrane strips. Following incubation with the blocking buffer, colloidal Gold-conjugated mAb476 was applied to blocked conjugate pads. The components were enclosed in a plastic cassette with a sample and reaction window to fabricate the lateral flow device. Between 120 – 150 µL of sample was applied dropwise to the sample window and results (spots) were read visually. This assay can be run and visually interpreted within 30 minutes, with little technical laboratory skill required [61].

2.4. Fungitell STAT™ from Serum

Fungitell STAT™ (Associates of Cape Cod, Inc., Falmouth, MA, USA) is a rapid test that can be run on one or more patient specimens that tests for (1→3)-β-D-glucan (BDG) from serum, with results available in approximately one hour. Results are given as “positive”, “negative”, or “indeterminate” based on the serum BDG titer. Results from the Fungitell STAT™ assay showed good reproducibility when serum samples spiked with *Saccharomyces cerevisiae*-derived for (1→3)-β-D-glucan were analyzed at three independent laboratories [62]. The ability of this assay to diagnose IA requires further evaluation as BDG is not specific for *Aspergillus* species and can be positive in patients with a variety of invasive fungal infections and also patients with increased fungal translocation form the gut [63-65].

3. Performance of POC tests by clinical specimen type

3.1. Bronchoalveolar Lavage

Performance of the LFD and the LFA in BALF has been evaluated by a number of studies including multicenter studies and in different patient populations.

The CE-marked IMMY sōna *Aspergillus* Galactomannan LFA (IMMY, Norman, Oklahoma, USA) is a commercially assay, available since 2018, and its performance in BALF has so far been evaluated in 5 studies [24,46-48, 66]. In the first study, performance of the LFA was compared in a variety of respiratory specimens to culture and microscopy, while clinical classification of IPA was not available in that study [47]. A total of 398 respiratory samples from 390 patients were evaluated, of which 52 samples were positive for culture and

microscopy, 254 were positive for either microscopy or culture, and 92 negatives by both culture and microscopy. The LFA showed a diagnostic accuracy of 92% for differentiating samples that were positive by culture and microscopy from those that were negative by both. For differentiating samples that were either positive by culture or microscopy versus negative samples, sensitivity was 90% and specificity 84% [47]. Cross-reactivity was shown in culture-positive respiratory samples with *Scedosporium*, *Fusarium* and other fungi [47]. Since then two single center studies have evaluated performance of the LFA in BALF of patients with and without hematological malignancies, and found high sensitivity and specificity in those with hematological malignancies, while both were lower in those without hematological malignancies where reliable criteria for classification of IPA are lacking [24,46]. Of interest, both studies noted a slightly higher sensitivity of the LFA compared to the newly formatted LFD, while specificity was slightly lower. To date, performance of the LFA in BALF has been evaluated in one multicenter study which was conducted among patients with underlying hematological malignancies in the Netherlands and Belgium [48]. In that study, the LFA showed a sensitivity of 83% and specificity of 87% for differentiating 75 patients with probable/proven IA from 117 patients without IPA. Sensitivity in 11 patients with proven IPA was 91 using the visual readout. Performance increased significantly when a digital reader was used for the readout, with an area under curve (AUC) of 0.92 for differentiating probable/proven IPA versus no IPA and a sensitivity of 87% and specificity of 92% [48]. Interestingly, the LFA optical density index (ODI) showed a better correlation with the LFD ODI than with GM ODI [48].

Overall, sensitivity of the LFA across studies was 77% (85/110) and specificity 81% (147/181) for differentiating probable/proven IPA versus no IPA, with about 70% of all samples that have been evaluated to date stemming from patients with hematological malignancies, where sensitivity was 83% and specificity 87% (**Table 1**). Multicenter studies that evaluated the LFA in other settings, such as ICU patients or solid-organ transplant (SOT) recipients, are lacking.

Since 2012 studies have evaluated the prototype of the *Aspergillus*-specific LFD. These included part retrospective, part prospective multicenter studies which evaluated the LFD prototype in patients with underlying hematological malignancies [52], in SOT recipients [53,67], ICU patients [51,68], and patients with underlying respiratory diseases [28], as well as a number of single center studies [69-72]. Results of the first 4 studies were summarized in a meta-analysis reporting a pooled sensitivity of 86% and specificity of 93% for probable/proven IPA versus no IPA when using BALF samples [73], and results were later updated as part of a review by inclusion of later studies [50,74]. Here in this review we

updated once more the performance of the *Aspergillus*-specific LFD prototype test for probable/proven versus no IA and calculated a 71% overall sensitivity (86/121 samples) and 92% overall specificity (585/634 samples) across studies, with slightly lower specificity in patients with underlying hematological malignancies, and slightly lower specificity in ICU patients (**Table 1**).

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.

The new CE-marked *Aspergillus* specific LFD (OLM Diagnostics, Newcastle upon Tyne, UK) was launched by the end of 2017, and showed in an initial study comparing both versions in a set of BALF samples similar sensitivity as the old prototype but better specificity [49]. Since then the CE-marked *Aspergillus* specific LFD has been evaluated in several single center studies [16,24,46,75], and most recently in a multicenter study including 4 centers in Belgium and the Netherlands. That study included 11 patients with proven IPA, 68 patients with probable IPA, and 124 patients with no signs of IPA and reported 82% sensitivity and 86% specificity for proven IPA versus no IPA, and 71% sensitivity and 86% specificity for proven/probable IPA versus no IPA [57]. This study also utilized a digital reader (aLF reader; Qiagen Lake Constance, Stockach, Germany) to read test line intensities, and found that digital readout further improved the performance (82% sensitivity and 96% specificity for proven versus no IPA) and found an area under curve (AUC) of 0.92 for differentiating proven IPA versus no IPA and 0.825 for differentiating proven/probable IPA versus no IPA [57]. Remaining samples from a subset of that multicenter study were later again thawed and retested with the LFD to compare results with those of the LFA; the study found lower sensitivity for the LFD (69% versus 83% for probable/proven IPA versus no IPA) and exactly the same specificity [48], while two other studies comparing both tests in the same samples also found lower sensitivity for the LFD but higher specificity [24,46]. Overall sensitivity of the newly-formatted CE-marked version of the LFD was 64% (98/152 samples) with an 87% specificity (254/293 samples), and performance was slightly better in those with hematological malignancies (70% sensitivity and 88% specificity (**Table 1**). Outside the hematological malignancy testing the newly formatted test has only been evaluated in single center studies [24,75] and a relative limited number of BALF samples. Larger, multicenter trials are needed that also find a way to work around the problems of IPA classification outside the hematological malignancy setting [24-26]. A study that has evaluated the CE-marked LFD with other biomarkers for IA has confirmed the independent diagnostic potential of the LFD, and found that the LFD was a very valuable addition to diagnostic bundles, and showed close to 100% sensitivity and specificity when combined with serum IL-8 >300 pg/mL

[16], confirming previous studies that found that the LFD prototype showed particularly great performance when combined with other IPA markers such as BALF TAFC [76] or *Aspergillus* PCR from BALF [68].

One study to date has evaluated the CE-marked LFD for diagnosis of chronic pulmonary aspergillosis (CPA) [77], and found a low sensitivity of 11% (3/27) [78].

3.2. Blood

Both the *Aspergillus* Galactomannan LFA and *Aspergillus*-specific LFD tests have shown promise in diagnosing IA from serum samples. This may be particularly relevant now given that bronchoscopy can potentially aerosolize virus [79] in patients with COVID-19 infection, thus posing a risk to patients and personnel from SARS-CoV-2 virus. In many centers the role of bronchoscopy is limited and testing from blood samples may be safer and more optimal.

Earlier studies evaluated the performance of the *Aspergillus*-specific LFD prototype test (**Table 2**). Two studies evaluated the diagnostic performance of the prototype LFD in serum samples from adult patients with hematological malignancies [59,80], reporting sensitivities of 40% and 82% and specificities 87% and 80% for probable/proven IA, respectively. A meta-analysis, which included in addition to those two studies also data from the LFD development study [56], 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 [73].

In a recent single-center retrospective case control study of 179 serum samples from 136 patients with invasive fungal disease, the *Aspergillus* LFA with the handheld digital cube reader had a sensitivity of 96.9% (31/32) (95% CI 94.3 – 99.5) and a specificity of 98% (98/100) (95% CI 93.0 – 99.5) at a positive GM threshold of $\geq$ 0.5, with an AUC of 0.99. Furthermore, the agreement between the LFA and conventional GM test was 89%, with the most common discordance due to false negative conventional GM values that were positive with the LFA [81]. Of note, in this study patients were determined to have invasive fungal disease based on the new revised European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) criteria [82]. Another single center study evaluated both the *Aspergillus* Galactomannan LFA and new CE-marked *Aspergillus*-specific LFD tests from serum samples of 239 hematology patients (41 cases proven/probable IA; 188 controls) using automated readout for both. The *Aspergillus* Galactomannan LFA test had a sensitivity of 49% (95% CI 33 – 65%) and a specificity of 95% (95% CI 91 – 98%) with a negative predictive value of 90% (95% CI 86 – 92%) for probable/proven IA versus controls. The

Aspergillus-specific LFD test had a sensitivity of 24% (95% CI 12 - 40%) and a specificity of 89% (95% CI 84 – 93%) with a negative predictive value of 84% (95% CI 82 – 87%). The conventional GM test performed similar to the *Aspergillus* Galactomannan LFA, with a sensitivity of 44% (95% CI 28 – 60%) and a specificity of 99% (95% CI 96 – 100%) with a negative predictive value of 89% (95% CI 86 – 91%). The AUCs were 0.82 for the LFA and 0.63 for the LFD. After omitting serum galactomannan from the definitions to control for incorporation bias, the sensitivity of the LFA outperformed galactomannan detection (0.41 versus 0.31, p=0.046). The highest negative predictive value was seen in patients with a negative LFA or conventional GM and a negative BDG test [45]. Overall performance of the LFA, the CE-marked LFD and the prototype LFD are displayed in Table 2. Specifically the LFA seems to hold promise for diagnosing IA in serum with a sensitivity of 70% and a specificity of 96%, which seems to outperform the CE-marked LFD particularly in terms of sensitivity.

Although the performance of the *Aspergillus* Galactomannan LFA in serum was comparable to the conventional GM test in these studies, both were single-center studies and multi-centered studies thus far are lacking. In addition, both studies were performed on serum from patients with hematologic malignancy and thus the potential of either the *Aspergillus* Galactomannan LFA or *Aspergillus*-specific LFD test to diagnose IA from serum in non-hematologic patients (such as those in the ICU) is unknown.

The Fungitell STAT™ test has so far been evaluated in only one study. In an analysis of 488 serum samples from patients suspected of having invasive fungal infection, serum samples were analyzed using both the conventional Fungitell assay (Associates of Cape Cod, Inc., Falmouth, MA, USA) and Fungitell STAT™ assay. Overall, there was good concordance between both assays, with 74% positive percent agreement (including indeterminate results) and 99% positive percent agreement (excluding indeterminate results). Negative percent agreement was 91% and 98% with and without indeterminate results, respectively. In total, this test takes about 70 minutes to produce a result. While performance of the test seems to match what has been observed for the conventional BDG testing, the value of BDG testing for diagnosis of IA overall remains unknown, particularly if performed in addition to more specific tests like GM, the GM LFA or the *Aspergillus*-specific LFD [16].

3.3. Urine and other specimens

Aspergillus GM is released throughout the body during IA, including in the urine [83-86]. Given that urine is abundant, readily available, and can be obtained non-invasively, urine is well-suited for a POC test that can diagnose IA, although urine dilution can vary widely and thereby is an important confounder for GM levels [86]. A lateral flow assay detecting mAb476

has been developed and shown to be feasible as a POC test in a proof-of-concept test [60]. This test has shown promising performance in stored human urine samples from 78 patients evaluated for suspected invasive fungal infections [61]. In this study, the sensitivity for the diagnosis of proven or probable IA was 80% (95% CI 61.4 - 92.3%) and specificity 92% (95% CI 74 - 99%). In the subgroup with underlying malignancy, the sensitivity and specificity were similar at 89.5% (95% CI 58.7 – 99.8%) and 90.9% (95% CI 58.7-99.8%), respectively. Semiquantitative urine assay results correlated well with serum GM level. Further validation of this test in larger, multicenter studies is needed to determine its role in the diagnosis of IA.

4. Impact of Neutropenia on Performance of POC tests

IA most frequently occurs in the lungs or sinus tract following inhalation of *Aspergillus* conidia, although it can spread hematogenous to other organ systems such as the gastrointestinal tract or cause infection via direct inoculation. Clinical presentation, symptoms, and also mycological findings in neutropenic and non-neutropenic patients differ markedly as well and may be related to the immunopathology of IPA, which shows primarily angioinvasive growth in neutropenic patients versus airway invasive growth in non-neutropenic patients [24,87-89]. Differences in clinical and radiological presentation of IPA dependent on neutrophil status also warrant different classification criteria. While revised EORTC/MSG criteria define IA in neutropenic patients and other hematological malignancy patients with severe immunosuppression [82], they fail to properly classify IPA in non-neutropenic patients, including those in the ICU where “typical” clinical and radiological signs are mostly lacking [87]. Blot and colleagues have started tackling the problem by creating consensus definitions for the ICU classifying cases as putative IPA, and the work on optimizing these definitions in non-neutropenic patients continues, with a current initiative led by ESCMID in cooperation with the ECMM and MSG currently underway [25,26]. The lack of broadly applicable and reliable consensus definitions for IPA in non-neutropenic patients may also explain why LFD and LFA performances differ in non-neutropenic patients.

5. Conclusions

In conclusion, both the IMMY sōna *Aspergillus* Galactomannan LFA, and the *Aspergillus*-Specific LFD have advanced over recent years, undergoing CE-certification and implementing automatic readout, and an automated cube reader is now included in LFA kits. The LFA is currently in the process of getting FDA approval. These tests can be performed in rudimentary facilities, although only the LFD allows for bedside testing of non-hemorrhagic or viscous BALF samples. Other samples, including those run with the LFA assay, require pretreatment, heating and centrifugation before testing. Both tests allow for reliable diagnosis of IPA in BALF specimens, with the LFA trending towards higher sensitivity, while the LFD

shows in some studies higher specificity. Data is more scarce for serum samples where particularly the LFA test seems to have similar performance as serum GM, while the CE-marked LFD has lower sensitivity. Performance can be further improved by combining the POC tests with other diagnostic tests for IPA, such as PCR, GM, or cytokines. While a number of studies have evaluated both CE-marked tests in patients with hematological malignancies, larger multicenter studies are needed to investigate both tests for diagnosis of IPA in other patient groups, such as solid organ transplant recipients or patients in the ICU.

6. Expert Opinion

Given the limitations of currently available diagnostics for IA, with limited availability in low- and middle-income countries and varying turnaround time without the possibility of single-sample testing, new and better tests are needed. Importantly, on a global level only a small proportion of mycology laboratories can offer GM testing, as shown in a recently published survey of 241 laboratories in Asia (including 71 from China), where only 23% indicated that they performed GM testing [31]. Several new rapid and simple diagnostics for IA are now available that may complement conventional GM testing, including the LFA and LFD tests. Performance of these tests has been markedly improved by introducing automatic read-out, which make the tests comparable across study sites and allow for more investigation of quantitative test performance. However, there is still an unmet need, namely a broadly useable true POC test - particularly for easily obtained specimens such serum or BALF/tracheal aspirate, that can be done at the bedside or in the Clinic in a matter of minutes. POC tests for urine specimens would be a particularly attractive testing for home testing.The LFD for non-hemorrhagic or viscous BALF samples and the lateral flow dipstick test for urine, which is under current evaluation, are both steps in the direction towards a true POC test. Still, the goal would be a true POC test such as the urine pregnancy test, which can be performed at home in a matter of minutes with no technical expertise required. A cheap, easily accessible, simple test that can be done at home between Clinic appointments could be a good option for patients at high risk for IA as a screening modality or to aid in prompt diagnosis of patients with signs or symptoms of IA. Another important factor that will decide on the success of these rapid/POC tests will be pricing, and how prices will compare to GM testing in resource rich settings, and whether these POC tests will be affordable in lower resource settings. An economic analysis would help shedding more light into the questions of cost effectiveness. Also, data is lacking about whether the LFA and LFD can diagnose IA very early in the course of disease, e.g. before GM becomes positive. Studies looking on the temporal relationship between LFA and LFD positivity as well as GM positivity are needed.

Notably, both new CE-marked point-of-care tests for IA have been evaluated, mostly in the hematology setting only. This is particularly true for serum, where the tests have been exclusively evaluated in patients with hematological malignancies. Validation of these tests is therefore needed for the increasing proportion of patients who develop IA outside the hematology setting, including SOT recipients and patients in the ICU. As an important next step, reliable definitions of IA are needed for the non-hematology settings as clinical presentation and radiologic findings differ in these settings. Once consensus definitions are available, future research should focus on diagnostics that allow for early diagnosis of IA in patients outside of the hematology setting.

Five years from now, mycologic diagnosis of IA will be established with true POC tests performed at the bedside, which will be available around the world, in both high and low resource settings. Urine POC tests will allow for self-testing for IA in ambulatory settings or even at home, similar to home urine pregnancy testing. Broadly accepted consensus definitions will be available that reliably classify IPA in non-neutropenic patients, including patients in the ICU with severe influenza or COVID-19. Using these new definitions, a variety of biomarkers will be evaluated in these settings and help enable earlier treatment initiation and improved patient outcomes in those diagnosed with IA. Clinical mycology societies will work closely together, and with the involvement of low and middle-income countries worldwide, applicable guidelines for the diagnosis of fungal infection as part of the "One world – one guideline" initiative [90,91] will be established. This strategy will provide diagnostic guidance for all countries, including those with limited resources.

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References

*Papers of special note have been highlighted as: * of interest ** of considerable interest*

1. Salzer HJF, Prattes J, Hoenigl M. Editorial: Diagnostic Approaches for Aspergillus Infections. Frontiers in microbiology. 2019;10:446.
2. Geiser DM. Sexual structures in Aspergillus: morphology, importance and genomics. Medical mycology. 2009;47 Suppl 1:S21-6.
3. *Latgé JP. Aspergillus fumigatus and aspergillosis. Clin Microbiol Rev. 1999 Apr;12(2):310-50.

*** Important paper reviewing *Aspergillus* disease.**

4. Bongomin F, Gago S, Oladele RO, et al. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. J Fungi (Basel). 2017 Oct 18;3(4).
5. Barton RC. Laboratory diagnosis of invasive aspergillosis: from diagnosis to prediction of outcome. Scientifica (Cairo). 2013;2013:459405.
6. Lewis RE, Cahyame-Zuniga L, Leventakos K, et al. Epidemiology and sites of involvement of invasive fungal infections in patients with haematological malignancies: a 20-year autopsy study. Mycoses. 2013 Nov;56(6):638-45.
7. Shojania KG, Burton EC. The vanishing nonforensic autopsy. N Engl J Med. 2008 Feb 28;358(9):873-5.
8. Patterson TF. Risk stratification for invasive aspergillosis: early assessment of host susceptibility. Med Mycol. 2009;47 Suppl 1:S255-60.
9. Cornely OA, Lass-Florl C, Lagrou K, et al. Improving outcome of fungal diseases - Guiding experts and patients towards excellence. Mycoses. 2017 Jul;60(7):420-425.
10. Jenks JD, Salzer HJF, Hoenigl M. Improving the rates of Aspergillus detection: an update on current diagnostic strategies. Expert review of anti-infective therapy. 2019 Jan;17(1):39-50.
11. Hoenigl M, Salzer HJ, Raggam RB, et al. Impact of galactomannan testing on the prevalence of invasive aspergillosis in patients with hematological malignancies. Med Mycol. 2012 Apr;50(3):266-9.
12. Hoenigl M, Seeber K, Koidl C, et al. Sensitivity of galactomannan enzyme immunoassay for diagnosing breakthrough invasive aspergillosis under antifungal prophylaxis and empirical therapy. Mycoses. 2013 Jul;56(4):471-6.
13. Eigl S, Hoenigl M, Spiess B, et al. Galactomannan testing and Aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. Med Mycol. 2017 Jul 1;55(5):528-534.
14. Rawlings SA, Heldt S, Prattes J, et al. Using Interleukin 6 and 8 in Blood and Bronchoalveolar Lavage Fluid to Predict Survival in Hematological Malignancy Patients With Suspected Pulmonary Mold Infection. Frontiers in immunology. 2019;10:1798.
15. Zhou W, Li H, Zhang Y, et al. Diagnostic Value of Galactomannan Antigen Test in Serum and Bronchoalveolar Lavage Fluid Samples from Patients with Nonneutropenic Invasive Pulmonary Aspergillosis. J Clin Microbiol. 2017 Jul;55(7):2153-2161.
16. *Heldt S, Prattes J, Eigl S, et al. Diagnosis of invasive aspergillosis in hematological malignancy patients: Performance of cytokines, Asp LFD, and Aspergillus PCR in same day blood and bronchoalveolar lavage samples. The Journal of infection. 2018;77(3):235-241.

*** Paper outlining the role of the LFD in combination with other tests for diagnosing breakthrough *Aspergillus* disease.**

17. de Heer K, Gerritsen MG, Visser CE, et al. Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients. Cochrane Database Syst Rev. 2019 May 20;5:Cd012399.
18. Schauwvlieghe AFAD, de Jonge N, van Dijk K, et al. The diagnosis and treatment of invasive aspergillosis in Dutch haematology units facing a rapidly increasing

prevalence of azole-resistance. A nationwide survey and rationale for the DB-MSG 002 study protocol. Mycoses. 2018.
19. Prattes J, Valentin T, Hoenigl M, et al. Invasive pulmonary aspergillosis complicating COVID-19 in the ICU - A case report. Med Mycol Case Rep. 2020 May 11.
20. Verweij PE, Gangneux J-P, Bassetti M, et al. Diagnosing COVID-19-associated pulmonary aspergillosis. The Lancet Microbe.
21. Koehler P, Cornely OA, Böttiger BW, et al. COVID-19 associated pulmonary aspergillosis. Mycoses. 2020 Jun;63(6):528-534.
22. Thompson GR, III, Cornely OA, Pappas PG, et al. Invasive Aspergillosis as an Underrecognized Superinfection in COVID-19. Open Forum Infectious Diseases. 2020.
23. **Arastehfar A, Carvalho A, van de Veerdonk FL, et al. COVID-19 Associated Pulmonary Aspergillosis (CAPA)-From Immunology to Treatment. J Fungi (Basel). 2020 Jun 24;6(2).

**** All you ever wanted to know about CVID-19 associated aspergillosis, from immunology over diagnosis and treatment, and review of the first 35 published cases.**

24. **Jenks JD, Mehta SR, Taplitz R, et al. Point-of-care diagnosis of invasive aspergillosis in non-neutropenic patients: Aspergillus Galactomannan Lateral Flow Assay versus Aspergillus-specific Lateral Flow Device test in bronchoalveolar lavage. Mycoses. 2019 Mar;62(3):230-236.

**** Paper outlining the difficulties in diagnosis of IA in non-neutropenic patients, discussing the inadequacy of current classification systems, and evaluating performances of both the LFA and LFD.**

25. Bassetti M, Giacobbe DR, Grecchi C, et al. Performance of existing definitions and tests for the diagnosis of invasive aspergillosis in critically ill, adult patients: A systematic review with qualitative evidence synthesis. The Journal of infection. 2020 Apr 21.
26. Bassetti M, Scudeller L, Giacobbe DR, et al. Developing definitions for invasive fungal diseases in critically ill adult patients in intensive care units. Protocol of the FUNgal infections Definitions in ICU patients (FUNDICU) project. Mycoses. 2019 Apr;62(4):310-319.
27. Fortun J, Martin-Davila P, Gomez Garcia de la Pedrosa E, et al. Galactomannan in bronchoalveolar lavage fluid for diagnosis of invasive aspergillosis in non-hematological patients. J Infect. 2016 Jun;72(6):738-744.
28. Prattes J, Flick H, Pruller F, et al. Novel tests for diagnosis of invasive aspergillosis in patients with underlying respiratory diseases. American journal of respiratory and critical care medicine. 2014;190(8):922-929.
29. Martinelli AW, Patil P, Wong VK, et al. A positive BAL galactomannan in non-haemato-oncology patients risks harmful overtreatment. J Med Microbiol. 2019 Dec;68(12):1766-1770.
30. Unterman A, Izhakian S, Geffen Y, et al. Routine comprehensive Aspergillus screening of bronchoalveolar lavage samples in lung transplant recipients. Clin Transplant. 2020 Mar;34(3):e13811.
31. Chindamporn A, Chakrabarti A, Li R, et al. Survey of laboratory practices for diagnosis of fungal infection in seven Asian countries: An Asia Fungal Working Group (AFWG) initiative. Medical mycology. 2018;56(4):416-425.
32. Buchheidt D, Reinwald M, Hoenigl M, et al. The evolving landscape of new diagnostic tests for invasive aspergillosis in hematology patients: strengths and weaknesses. Curr Opin Infect Dis. 2017 Dec;30(6):539-544.
33. Hoenigl M, Prattes J, Neumeister P, et al. Real-world challenges and unmet needs in the diagnosis and treatment of suspected invasive pulmonary aspergillosis in patients with haematological diseases: An illustrative case study. Mycoses. 2018 Mar;61(3):201-205.

34. Springer J, Lackner M, Nachbaur D, et al. Prospective multicentre PCR-based Aspergillus DNA screening in high-risk patients with and without primary antifungal mould prophylaxis. Clin Microbiol Infect. 2016 Jan;22(1):80-86.
35. Reinwald M, Hummel M, Kovalevskaya E, et al. Therapy with antifungals decreases the diagnostic performance of PCR for diagnosing invasive aspergillosis in bronchoalveolar lavage samples of patients with haematological malignancies. J Antimicrob Chemother. 2012 Sep;67(9):2260-7.
36. Guenter S, Gorkiewicz G, Halwachs B, et al. Impact of ITS-Based Sequencing on Antifungal Treatment of Patients with Suspected Invasive Fungal Infections. J Fungi (Basel). 2020 Mar 27;6(2).
37. Jenks JD, Spiess B, Buchheidt D, et al. (New) Methods for Detection of Aspergillus fumigatus Resistance in Clinical Samples. Curr Fungal Infect Rep. 2019 Sep;13(3):129-136.
38. White PL, Bretagne S, Klingspor L, et al. Aspergillus PCR: one step closer to standardization. J Clin Microbiol. 2010 Apr;48(4):1231-40.
39. White PL, Wingard JR, Bretagne S, et al. Aspergillus Polymerase Chain Reaction: Systematic Review of Evidence for Clinical Use in Comparison With Antigen Testing. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015 Oct 15;61(8):1293-303.
40. Cornely OA, Hoenigl M, Lass-Florl C, et al. Defining breakthrough invasive fungal infection-Position paper of the mycoses study group education and research consortium and the European Confederation of Medical Mycology. Mycoses. 2019 Sep;62(9):716-729.
41. Egger M, Jenks JD, Hoenigl M, et al. Blood Aspergillus PCR: The Good, the Bad, and the Ugly. Journal of fungi (Basel, Switzerland). 2020;6(1):E18.
42. Arvanitis M, Ziakas PD, Zacharioudakis IM, et al. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. J Clin Microbiol. 2014 Oct;52(10):3731-42.
43. Hoenigl M, Orasch T, Faserl K, et al. Triacetylfusarinine C: A urine biomarker for diagnosis of invasive aspergillosis. The Journal of infection. 2019 Feb;78(2):150-157.
44. Jenks JD, Rawlings SA, Garcia-Vidal C, et al. Immune Parameters for Diagnosis and Treatment Monitoring in Invasive Mold Infection. Journal of fungi (Basel, Switzerland). 2019;5(4):E116.
45. Mercier T, Guldentops E, Lagrou K, et al. Prospective evaluation of the turbidimetric β-D-glucan assay and two lateral flow assays on serum in invasive aspergillosis. Clin Infect Dis. 2020 Mar 19.
46. Jenks JD, Mehta SR, Taplitz R, et al. Bronchoalveolar lavage Aspergillus Galactomannan lateral flow assay versus Aspergillus-specific lateral flow device test for diagnosis of invasive pulmonary Aspergillosis in patients with hematological malignancies. The Journal of infection. 2019 Mar;78(3):249-259.
47. Lass-Flörl C, Lo Cascio G, Nucci M, et al. Respiratory specimens and the diagnostic accuracy of Aspergillus lateral flow assays (LFA-IMMY™): real-life data from a multicentre study. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2019;25(12):1563.e1-1563.e3.
48. **Mercier T, Dunbar A, de Kort E, et al. Lateral flow assays for diagnosing invasive pulmonary aspergillosis in adult hematology patients: A comparative multicenter study. Medical mycology. 2019:myz079.

**** Important paper investigating the performances of both the LFA and LFD in BALF from patients with hematological malignancies and comparing visual with automated read out.**

49. Hoenigl M, Eigl S, Heldt S, et al. Clinical evaluation of the newly formatted lateral-flow device for invasive pulmonary aspergillosis. Mycoses. 2018;61(1):40-43.
50. Heldt S, Hoenigl M. Lateral Flow Assays for the Diagnosis of Invasive Aspergillosis: Current Status. Current fungal infection reports. 2017;11(2):45-51.

51. Eigl S, Prattes J, Lackner M, et al. Multicenter evaluation of a lateral-flow device test for diagnosing invasive pulmonary aspergillosis in ICU patients. Critical Care (London, England). 2015;19:178-015-0905-x.
52. Prattes J, Lackner M, Eigl S, et al. Diagnostic accuracy of the Aspergillus-specific bronchoalveolar lavage lateral-flow assay in haematological malignancy patients. Mycoses. 2015;58(8):461-469.
53. Willinger B, Lackner M, Lass-Florl C, et al. Bronchoalveolar lavage lateral-flow device test for invasive pulmonary aspergillosis in solid organ transplant patients: a semiprospective multicenter study. Transplantation. 2014 Oct 27;98(8):898-902.
54. Rolle AM, Hasenberg M, Thornton CR, et al. ImmunoPET/MR imaging allows specific detection of Aspergillus fumigatus lung infection in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2016;113(8):E1026-33.
55. Thornton CR. Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis. Clin Vaccine Immunol. 2008 Jul;15(7):1095-105.
56. Thornton CR. Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis. Clinical and vaccine immunology : CVI. 2008;15(7):1095-1105.
57. Mercier T, Schauwvlieghe A, de Kort E, et al. Diagnosing Invasive Pulmonary Aspergillosis in Hematology Patients: a Retrospective Multicenter Evaluation of a Novel Lateral Flow Device. J Clin Microbiol. 2019 Apr;57(4).
58. Wiederhold NP, Thornton CR, Najvar LK, et al. Comparison of lateral flow technology and galactomannan and (1->3)-beta-D-glucan assays for detection of invasive pulmonary aspergillosis. Clinical and vaccine immunology : CVI. 2009;16(12):1844-1846.
59. 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. Journal of clinical microbiology. 2013;51(5):1510-1516.
60. Dufresne SF, Datta K, Li X, et al. Detection of urinary excreted fungal galactomannan-like antigens for diagnosis of invasive aspergillosis. PloS one. 2012;7(8):e42736.
61. Marr KA, Datta K, Mehta S, et al. Urine Antigen Detection as an Aid to Diagnose Invasive Aspergillosis. Clin Infect Dis. 2018 Nov 13;67(11):1705-1711.
62. D'Ordine RL, Garcia KA, Roy J, et al. Performance characteristics of Fungitell STAT™, a rapid (1→3)-β-D-glucan single patient sample in vitro diagnostic assay. Med Mycol. 2020 May 13.
63. Hoenigl M. Fungal Translocation: A driving force behind the Occurrence of non-AIDS Events? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2019 Mar 12.
64. Hoenigl M, Perez-Santiago J, Nakazawa M, et al. (1-->3)-beta-d-Glucan: A Biomarker for Microbial Translocation in Individuals with Acute or Early HIV Infection? Frontiers in immunology. 2016;7:404.
65. Hoenigl M, Moser CB, Funderburg N, et al. Soluble Urokinase Plasminogen Activator Receptor Is Predictive of Non-AIDS Events During Antiretroviral Therapy-mediated Viral Suppression. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2019;69(4):676-686.
66. Hoenigl MJ, J; Spiess, B; Buchheidt, D; Prattes, J. Performance of the Bronchoalveolar Lavage Fluid Aspergillus Galactomannan Lateral Flow Assay with Cube Reader for Diagnosis of Invasive Pulmonary Aspergillosis: a Multicenter Mixed Cohort Study. 2020.
67. Eigl S, Prattes J, Reinwald M, et al. Influence of mould-active antifungal treatment on the performance of the Aspergillus-specific bronchoalveolar lavage fluid lateral-flow device test. International journal of antimicrobial agents. 2015;46(4):401-405.
68. Hoenigl M, Prattes J, Spiess B, 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. Journal of clinical microbiology. 2014;52(6):2039-2045.
69. Hoenigl M, Koidl C, Duettmann W, et al. Bronchoalveolar lavage lateral-flow device test for invasive pulmonary aspergillosis diagnosis in haematological malignancy and solid organ transplant patients. The Journal of infection. 2012;65(6):588-591.
70. 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. Journal of clinical microbiology. 2015;53(7):2103-2108.
71. Castillo CG, Kauffman CA, Zhai J, et al. Testing the performance of a prototype lateral flow device using bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in high-risk patients. Mycoses. 2018;61(1):4-10.
72. Miceli MH, Goggins MI, Chander P, 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):368-374.
73. Pan Z, Fu M, Zhang J, et al. Diagnostic accuracy of a novel lateral-flow device in invasive aspergillosis: a meta-analysis. Journal of medical microbiology. 2015;64(7):702-707.
74. Prattes J, Heldt S, Eigl S, et al. Point of Care Testing for the Diagnosis of Fungal Infections: Are We There Yet? Current fungal infection reports. 2016;10:43-50.
75. Linder KA, Kauffman CA, Zhou S, et al. Clinical application of Aspergillus lateral flow device in bronchoalveolar lavage fluid of patients with classic risk factors for invasive pulmonary aspergillosis. Mycoses. 2019 Dec;62(12):1189-1193.
76. Orasch T, Prattes J, Faserl K, et al. Bronchoalveolar lavage triacetylfusarinine C (TAFC) determination for diagnosis of invasive pulmonary aspergillosis in patients with hematological malignancies. The Journal of infection. 2017.
77. Denning DW, Page ID, Chakaya J, et al. Case Definition of Chronic Pulmonary Aspergillosis in Resource-Constrained Settings. Emerging infectious diseases. 2018;24(8):10.3201/eid2408.171312.
78. Salzer HJF, Prattes J, Flick H, et al. Evaluation of Galactomannan Testing, the Aspergillus-Specific Lateral-Flow Device Test and Levels of Cytokines in Bronchoalveolar Lavage Fluid for Diagnosis of Chronic Pulmonary Aspergillosis. Frontiers in microbiology. 2018;9:2223.
79. Wahidi MM, Lamb C, Murgu S, et al. American Association for Bronchology and Interventional Pulmonology (AABIP) Statement on the Use of Bronchoscopy and Respiratory Specimen Collection in Patients with Suspected or Confirmed COVID-19 Infection. J Bronchology Interv Pulmonol. 2020 Mar 18.
80. Held J, Schmidt T, Thornton CR, et al. 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):1163-1169.
81. White PL, Price JS, Posso R, et al. An evaluation of the performance of the IMMY sona Aspergillus Galactomannan Lateral flow assay when testing serum to aid in the diagnosis of invasive aspergillosis. J Clin Microbiol. 2020 Mar 18.
82. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2019 Dec 5.
83. Haynes KA, Latge JP, Rogers TR. Detection of Aspergillus antigens associated with invasive infection. J Clin Microbiol. 1990 Sep;28(9):2040-4.
84. Ansorg R, Heintschel von Heinegg E, Rath PM. Aspergillus antigenuria compared to antigenemia in bone marrow transplant recipients. Eur J Clin Microbiol Infect Dis. 1994 Jul;13(7):582-9.

85. Duettmann W, Koidl C, Troppan K, et al. Serum and urine galactomannan testing for screening in patients with hematological malignancies. Medical mycology. 2014;52(6):647-652.
86. Reischies FM, Raggam RB, Prattes J, et al. Urine Galactomannan-to-Creatinine Ratio for Detection of Invasive Aspergillosis in Patients with Hematological Malignancies. Journal of clinical microbiology. 2016;54(3):771-774.
87. Blot SI, Taccone FS, Van den Abeele AM, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. American journal of respiratory and critical care medicine. 2012 Jul 1;186(1):56-64.
88. Cornillet A, Camus C, Nimubona S, et al. Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2006;43(5):577-584.
89. Bergeron A, Porcher R, Sulahian A, et al. The strategy for the diagnosis of invasive pulmonary aspergillosis should depend on both the underlying condition and the leukocyte count of patients with hematologic malignancies. Blood. 2012;119(8):1831-7; quiz 1956.
90. Cornely OA, Alastruey-Izquierdo A, Arenz D, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. The Lancet Infectious diseases. 2019 Nov 4.
91. Hoenigl M, Gangneux JP, Segal E, et al. Global Guidelines and Initiatives from the European Confederation of Medical Mycology to improve Patient Care and Research Worldwide: New Leadership is about Working Together. Mycoses. 2018.
92. Thornton C, Johnson G, Agrawal S. Detection of invasive pulmonary aspergillosis in haematological malignancy patients by using lateral-flow technology. Journal of visualized experiments : JoVE. 2012;(61). pii: 3721. doi(61):10.3791/3721.
93. Prattes J, Koidl C, Eigl S, et al. Bronchoalveolar lavage fluid sample pretreatment with Sputasol((R)) significantly reduces galactomannan levels. The Journal of infection. 2015;70(5):541-543.
94. Heldt S, Eigl S, Prattes J, et al. Levels of interleukin (IL)-6 and IL-8 are elevated in serum and bronchoalveolar lavage fluid of haematological patients with invasive pulmonary aspergillosis. Mycoses. 2017;60(12):818-825.

Table 1 Performance in BALF of the Aspergillus Galactomannan LFA, the CE-marked *Aspergillus*-specific LFD, and the earlier *Aspergillus*-specific LFD prototype for proven/probable or putative (ICU only) invasive pulmonary aspergillosis versus no evidence for invasive pulmonary aspergillosis in different patient cohorts (percentage and absolute numbers).

Test	Patient group	Sensitivity	Specificity
Aspergillus Galactomannan LFA [24,46,48]	Overall	77% (85/110)	81% (147/181)
	Hematological malignancies	83% (70/84)	87% (109/125)
	Solid Organ Transplantation	50% (4/8)	48% (10/21)
	Intensive care unit and others	61% (11/18)	80% (28/35)
CE marked *Aspergillus*-specific LFD [16,19,24,46,49,57,75]	Overall	64% (98/152)	87% (254/293)
	Hematological malignancies	70% (78/112)	88% (187/212)
	Solid Organ Transplantation	17% (2/12)	80% (28/35)
	Intensive care unit and others	64% (18/28)	85% (39/46)
Aspergillus-specific LFD prototype [28,50-53,58,67-72,76,92-94]	Overall*	71% (86/121)	92% (585/634)
	Hematological Malignancies	67% (36/54)	92% (142/155)
	Solid Organ Transplantation	81% (17/21)	92% (107/116)
	Intensive Care Unit	75% (27/36)	85% (176/206)
	Respiratory Diseases	78% (25/32)	91% (196/215)

Abbreviations: CE: certification mark; LFA: Lateral flow assay; LFD: Lateral flow device

Table 2 Performance in Blood of the Aspergillus Galactomannan LFA, the CE-marked *Aspergillus*-specific LFD, and the earlier *Aspergillus*-specific LFD prototype for proven/probable invasive pulmonary aspergillosis versus no evidence for invasive pulmonary aspergillosis in patients with hematologic malignancy (percentage and absolute numbers).

Test	**Patient group**	**Sensitivity**	**Specificity**
Aspergillus Galactomannan LFA [45,81]	Overall	70% (51/73)	96% (277/288)
	Hematological malignancies	70% (51/73)	96% (277/288)
CE marked *Aspergillus*-specific LFD [16,45]	Overall	20% (10/51)	91% (222/243)
	Hematological malignancies	20% (10/51)	91% (222/243)
Aspergillus-specific LFD prototype [56,59,80]	Overall	68% (30/44)	87% (116/134)
	Hematological Malignancies	69% (22/32)	86% (112/130)

Abbreviations: CE: certification mark that indicates conformity with health, safety, and environmental protection standards for products sold within the European Economic Area; LFA: Lateral flow assay; LFD: Lateral flow device