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Point-of-care testing for viral-associated pulmonary aspergillosis

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Abstract:

Introduction: Over the last years, severe respiratory viral infections, particularly those caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the influenza virus, have emerged as risk factor for viral-associated pulmonary aspergillosis (VAPA) among critically ill patients. Delays in diagnosis of VAPA are associated with increased mortality. Point-of-care-tests may play an important role in earlier diagnosis of VAPA and thus improve patient outcomes

Areas covered: The following review will give an update on point-of-care tests for VAPA, analyzing performances in respiratory and blood specimens.

Expert opinion: Point-of-care tests have emerged, and particularly the IMMY *Aspergillus* galactomannan lateral flow assay (LFA) shows performances comparable to the galactomannan ELISA for diagnosis of VAPA. Notably, nearly all evaluations of POC tests for VAPA have been performed in COVID-19 patients, with very limited data in Influenza patients. For early diagnosis of COVID associated pulmonary aspergillosis (CAPA) the LFA has shown promising performances in respiratory samples, particularly in bronchoalveolar lavage fluid, and may thereby help in improving patient outcomes. In contrast, serum LFA testing may not be useful for early diagnosis of disease, except in cases with invasive tracheobronchial aspergillosis.

Keywords

Lateral flow device test, Lateral flow assay, non-neutropenic, ICU, COVID, Influenza, pulmonary aspergillosis, Aspergillus.

1. Introduction

Over the last decades prevalence of infections caused by *Aspergillus* species continues to increase, triggered by advances in medicine and other factors such as the COVID-19 pandemic [1,2]. As a result *Aspergillus fumigatus*, the most relevant and prevalent species causing infections in humans, was included into the World Health Organization (WHO) priority pathogens list and classified in the highest category of critical importance [3]. *Aspergillus* is found ubiquitous in nature, commonly colonizes the respiratory tract via conidial inspiration and can cause various forms of disease, depending on the hosts immune competence, ranging from allergic disease to invasive pulmonary aspergillosis (IPA) [4].

Over the last years, severe respiratory viral infections have emerged as risk factor for invasive fungal disease among critically ill patients [5-8]. A large and growing body of literature has investigated that although other viral infections may lead to secondary opportunistic infections as well, severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2) and the influenza virus in particular excel in their ability to damage epithelial lung tissue and impair immune response [2,9-14]. Furthermore, corticosteroid and tocilizumab treatment of critically ill COVID-19 patients with respiratory failure may contribute to predisposing immune dysfunction [15-17].

Since clinical and radiological presentation of viral-associated pulmonary aspergillosis (VAPA) are not specific and pulmonary fungal infection may mimic the underlying disease (i.e., pulmonary infiltrates in a patient with respiratory failure due to COVID-19), timely diagnosis is difficult and remains challenging [18-20].

Mycological testing of respiratory specimens, optimally bronchoalveolar lavage fluid (BALF), has become essential for early diagnosis of VAPA, with fungal culture and testing for galactomannan (GM) considered the gold standard tests [21-24]. However, both culture and GM testing are often limited by long turnaround times, with GM sometimes being a send out test, especially in smaller centers [25-27]. Molecular pathogen detection methods like polymerase chain reaction (PCR) assays and next generation sequencing (NGS) have been utilized as well [28-30], While bronchoscopy cannot always be performed, especially in low and middle income countries, it is essential for early diagnosis of VAPA. Unlike fungal infections in neutropenic patients [31], invasive growth in VAPA is primarily limited to lung tissue, with angio-invasion occurring only in later stages of the disease [32]. Therefore, blood-surrogate markers for the diagnosis of VAPA display lower sensitivity than in neutropenic patients [32-36].

All these problems combined result in delayed diagnosis and thereby have a significant impact on morbidity and mortality. These limitations underline the need for more simple diagnostic tests that can be performed at bedside with rapid turnaround time. These point-of-care-tests (POCT) may play an important role in earlier diagnosis of VAPA and thus improve patients outcomes [37,38].

2. Point of care tests for invasive aspergillosis

2.1. Rapid testing

The rat anti-GM monoclonal antibody (mAb) EB-A2, which recognizes the 1→5-β-d-galactofuranoside side chains of the galactomannan (GM) molecule, was first introduced in the early 90's with the Pastorex *Aspergillus* antigen latex agglutination test and later in 1995 with the Platelia GM enzyme immunoassay (EIA) (Bio-Rad, Marnes-la-Coquette, France) [39]. The EIA was first validated in serum and some years later in BALF [40]. The Platelia EIA remained for a long time the technique of choice for the detection of the *Aspergillus* antigen, and due to the fact that histopathology often cannot be obtained in clinic also the primary microbiological criterion for diagnosing invasive aspergillosis.

After introduction of the GM EIA, it took another 13 years until the first rapid POCT for IA was developed (see Fig 1). In 2008, the prototype of a JF5 mouse IgG3 mAb based

immunochromatographic lateral flow device (LFD), detecting an *Aspergillus* mannoprotein, was described [41,42]. JF5 binds to a protein epitope present on an extracellular glycoprotein of *Aspergillus* secreted constitutively during active growth. Introduction of this kind of POCT for IA improved time from sampling to result from multiple hours to less than one hour. After successful clinical performance trials with the prototype LFD in BALF [31,43-53] and serum [54], the test was formatted to its current CE-marked LFD (OLM Diagnostics, Newcastle Upon Tyne, UK) in 2017 [55]. In 2019, a new lateral flow test was introduced, the Soña *Aspergillus* galactomannan lateral flow assay (LFA) (IMMY, Norman, Oklahoma, USA) which detects GM [56]. Studies comparing the performance of the OLM LFD and the IMMY LFA show equal performance of both test or better results for the IMMY LFA [32,57-60]. The IMMY test appears to be an excellent substitute for the GM EIA and in some studies sensitivity was even better than the GM EIA [61]. Cut-off for positivity for both IMMY and OLM tests is an optimal density index (ODI) of 0.5 , which is in agreement with the GM EIA but lower than the BALF cut-offs often used clinically to diagnose invasive aspergillosis with more certainty [19,62,63]. The cut-offs used, and therefore the associated sensitivity and specificity, vary in different performance studies [34]. Performances of the tests are outlined in Table 1, characteristics including cross-reactivity as well as advantages and disadvantages in Table 2.

More recently, other new lateral flow tests were introduced and as a result, the terms LFA and LFD can no longer be used to distinguish specifically the IMMY and OLM test. Currently the QuicGM *Aspergillus* galactomannan Ag LFA (Dynamiker, Tianjin, China), the FungiXpert *Aspergillus* Galactomannan Detection K-set LFA (Genobio [Era Biology Technology], Tianjin, China) and the TECO®*Fast Aspergillus* galactomannan Ag LFA (TECOmedical Group, Sissach, Switzerland; Dynamiker, Tianjin, China) are CE-marked. These new lateral flow tests (LFT) detect GM (Table 2). From these new lateral flow tests (LFTs) only internal validation data are available but no clinical performance studies are published in literature.

LFTs as POCT can be performed on different types of clinical specimens, such as blood, BALF, urine and cerebrospinal fluid (CSF), and can detect the presence of different *Aspergillus* antigens. The level of validation and the clinical performance status of these POCT vary depending on the test, the type of specimen and the patient population. All of the above mentioned lateral flow tests are only CE-marked for testing in serum and BALF. The performance of these tests can also be affected by factors such as the timing of the sample collection and the presence of antifungal prophylaxis or treatment [46,64].

LFTs are most useful when a rapid result is important and the number of samples tested is low. To really speak of point of care testing there must be a very short turnaround time without time lost for transport of the sample or too much pretreatment steps and the possibility to immediately interpret

the result without an additional chain of reporting. Implementation of POCTs bedside is limited because the need of trained personnel, quality control and the need for correct biosafety conditions. Therefore, it is better to speak of LFTs as rapid tests instead of POCT as at the moment they are mostly implemented in microbiology labs [25].

As the IMMY LFA and the new LFTs detect GM or GM-like antigens, in general most of the new POCT detect epitopes very similar to the EB-A2 mAb. There is a void in new diagnostics that employ mAbs directed at epitopes other than those present on GM. In 2012, a new IgM monoclonal antibody (mAb476) that detects small molecular weight galactofuranose-containing antigens in urine was developed [65]. This was further optimized to develop the MycoMEIA® test (Pearl Diagnostics, Baltimore, USA) detecting free glycoproteins and extracellular vesicles in urine [66]. Before, GM testing was assumed to be less sensitive in urine causing LFTs until now not being fully analyzed in urine as a matrix [67-71][1–4]. With the MycoMEIA® test currently being in development as an LFT as well (MycoFLOW®) the possibility of an *Aspergillus* POCT in urine is promising.

Also, other more rapid and single sample non-LFT alternatives, for the Platelia GM EIA are now available. For example the *Aspergillus* Galactomannan VirClia® Monotest (Vircell SL, Granada, Spain) is an automated chemiluminescence immunoassay (CLIA) having a similar performance than the Platelia GM EIA but with a much faster turnaround time because batching is not required [72].

Other more rapid tests are being developed. To detect 1,3-β-D-glucan (BDG) for example, the Wako β-glucan assay (Wako-BDG, Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) provides the possibility for single-sample testing and a faster turnaround time in comparison to the Fungitell assay (Associates of Cape Cod, Massachusetts, USA) that was dominating the market historically [73,74].

2.2. Point of care testing for VAPA

In general, the sensitivity and specificity of these LFTs and other more rapid assays for the diagnosis of VAPA, and particularly influenza associated pulmonary aspergillosis (IAPA) are less well established than in typical immunocompromised populations meeting the host factors of the 2020 European Organisation for Research and Treatment of Cancer (EORTC)/Mycoses Study Group Education and Research Consortium (MSGERC) consensus definitions [62]. While most studies have been performed in the hematology population or in solid organ transplant (SOT) recipients, an increasing number of analyses in patients with viral-associated aspergillosis are becoming available as a consequence of the COVID-19 pandemic. VAPA is currently also more often diagnosed in patients with EORTC/MSGERC host factors.

First studies with the LFTs including patients on ICU only date back from 2014 [45]. Performance studies of LFTs in patients with VAPA are only available on the IMMY LFA and OLM LFD. Of the others

LFTs, including the MycoFLOW test and the VirClia Monotest, a review of literature did not provide any relevant results (Table 2).

In general, for VAPA LFTs were investigated mostly in BALF samples, as serum biomarkers tend to give a false-negative result in critically ill non-neutropenic patients and diagnostic BALF should be performed in suspected cases of IPA [32,45,75,76]. For VAPA, depending on where bronchoscopy is performed, analysis of BALF – in case of a low number of samples – could be performed on-site, at the endoscopy ward or at the intensive care unit (ICU). However, in reality, the personal in charge for endoscopy often does not have the time resources nor possibility to perform the analysis themselves and the sample is in fact sent to the lab, making the testing flow not very different from other microbiological and immunological analyses. This could limit its use as a POCT.

3. Performance in respiratory specimens from patients with severe COVID-19 or Influenza infection

3.1. Bronchoalveolar Lavage

While broadly accepted consensus criteria for classification of IA in non-neutropenic patients are still in the works [77,78], Blot at al., and Schauwvlieghe et al. proposed two versions of a specific algorithm for ICU patients [8,79]. In some other publications, diagnosis of IA in ICU patients is also made based on a proposal from the EORTC/MSG, which classify “proven” and “probable” cases of IA [80]. For CAPA specifically, Koehler et al. have proposed specific criteria in 2021 for classification [63], which are now widely used whereas for IAPA expert case definitions are often used [19]. In both of them, detection of GM antigen in BALF or serum samples, as a “mycological evidence” of *Aspergillus* infection, allows classification as “probable” cases of IA. Both IMMY LFA and OLM LFD were evaluated for detection of GM in BALF in mixed cohorts, which included variable proportions of ICU patients (7-52%) [32,43,46,49,55,81,82]. In all of them except two, it was not specified whether VAPA cases were included [46,81]. Sensitivity in those cohorts was slightly higher for the IMMY LFA (62-98%) [32,56,81-83] compared to the OLM LFD (69-80%) (Table 1) [32,55,84,85]. In two studies sensitivity was very low, one OLM LFD evaluation (38% sensitivity in a small cohort of 8 IA [43]) and one IMMY LFA evaluation (40% sensitivity in a cohort presenting unusual discrepancies with other mycological assays [86]). Specificity was highly variable (44-100%), depending on the used definition criteria and the cohort composition. Among these studies, only one compared LFA and LFD performance in ICU patients, reporting sensitivities of 69% and 65%, and specificities of 71% and 68%, for IMMY LFA and OLM LFD respectively [32].

To date, only two studies focusing on the performance of OLM LFD in BALF were restricted to ICU patients [45,60]. In these studies, sensitivity and specificity were 83-89% and 55-79%, respectively, using the Blot et al. definition of IA. The few other studies that focused on ICU patients evaluated the

IMMY LFA, either in a mixed VAPA/non-VAPA cohort [81,87] or specifically in COVID-19 patients [34,88]. All showed good sensitivity, ranging from 58% to 82%, and excellent specificity, ranging from 75% to 98% (Table 1). No study was restricted to IAPA patients, nor detailed the results for them. Only one study from Mercier et al. (2020), included a significant proportion of IAPA patients (36% of all patients; n=64 with n=17 IAPA cases and n=47 influenza cases without IAPA). This study showed both high sensitivity (87-94% depending on the classification criteria) and high specificity (81%) of the IMMY LFA; specifically for patients with IAPA sensitivity was 94% [87]. The digital readout using the Cube Reader proposed by the manufacturer was associated to a higher sensitivity when compared to a visual readout [87]. Diagnostic value of IMMY LFA was highly comparable to GM EIA Platelia™ assay [81,87], with the obvious benefit of a short handling time. Receiver Operating Characteristics (ROC) curve analysis showed AUCs between 0.754 and 0.920, which corresponds to a good discrimination between IA and non-IA patients. In COVID-19 patients, this was probably due to the impossibility to reach >90% sensitivity without dramatically lowered specificity [34,88]. All together, these data suggest that IMMY LFA detection in BALF trigger other diagnostics for invasive aspergillosis (culture and PCR in BALF and GM in serum) and also lead to initiationof antifungal treatment for CAPA in COVID-19 patients with acute respiratory failure, as it is highly specific of IA at the 1.0 ODI cutoff.

3.2. Other respiratory samples

Several other respiratory samples can be used for diagnosis of invasive pulmonary aspergillosis, such as sputum, tracheal aspiration (TA) or the non-directed bronchoalveolar lavage (NBL). As some of those are sampled in the upper respiratory tract, these are considered to be more at risk for false positivity due to colonization with *Aspergillus*, thus associated with lowered specificity. However, they are still of interest because of their less invasive sampling procedure, allowing repeated samples on contrary to BALF. Few studies evaluated the performance of GM detection in these samples for diagnosis of IA and even more so VAPA patients and the studies evaluating a POCT in these samples are extremely limited. IMMY LFA detection (>1 ODI cutoff) in TA seems to have a good sensitivity (64-81%) for CAPA diagnosis, with an expected low specificity (67-68%), which makes it a good screening test [34,89]. Only one study reported performance of OLM LFD in TA, with 100% (35/35) sensitivity among a cohort of CAPA patients, however the used cutoff was not specified [90]. Considering the severity of VAPA, positivity of a GM rapid test in TA should motivate BALF sampling and further initiation of antifungal therapy if confirmed.

Although positivity of NBL allows classification of “possible CAPA”, according to Koehler et al., only one study evaluated the diagnostic value of GM detection, using LFA in NBL [34]. Sensitivity was comparable to TA (80%), but with far higher specificity (88%).

To date, the only study on GM detection performance in sputum for diagnosis of IA focused on hematology patients and used the GM EIA assay [91]. It showed interesting results as sputum GM had 100% sensitivity and 62% specificity, but this was the study was limited by a small cohort of IA patients (n=6) and these findings have to be validated also in ICU patients. To summarize, it seems clear that there is insufficient data to recommend POCT from non-BALF respiratory samples although such testing could allow for more rapid management adaptations.

4. Performance in Blood

Data on the performance of LFTs in blood from patients with VAPA is scarce, exclusively focusing on CAPA, with not much more than a handful of studies evaluating the test in serum samples from mixed cohorts that included VAPA patients and only 4 studies which focused exclusively on patients with COVID-19 (Table 1). In contrast to CAPA, to the best of our knowledge, no data on performance of serum LFT testing in patients with IAPA, including influenza associated tracheobronchial aspergillosis [92], has been reported to date.

In a large European multicenter study in four countries (148 serum samples; including samples from 48 cases with CAPA) performance of serum IMMY LFA was evaluated specifically for CAPA patients in the ICU. Sensitivity in serum was low with 20% at the 0.5 ODI cutoff, with a specificity of 93% and a non-discriminatory AUC of 0.512 [34], with significantly better performances observed when testing respiratory specimens. While several mixed cohort studies evaluating the serum IMMY LFA or OLM LFD exist in cohorts of ICU or non-neutropenic patients that also include patients with CAPA (sensitivities between 69% and 79%, specificities 79% to 84% in the overall cohorts, Table 1) [37,93,94], only one of these studies reported performance for CAPA specifically (0% sensitivity, 96% specificity), but was limited by including only 2 patients with CAPA [95].

The low sensitivities of serum IMMY LFA for early diagnosis of CAPA can primarily explained by the distinct pathophysiological features of aspergillosis in patients with viral induced acute respiratory failure, characterized by several days of tissue invasion in the lungs, before angioinvasion occurs at a later stage of disease [2,29,96]. In contrast, testing of blood samples for fungal antigens is a common and well-established approach in the hematological malignancy setting, particularly neutropenic patients. The hallmark of invasive aspergillosis in the neutropenic host is angio-invasion and consequently the dissemination of *Aspergillus* antigens into the bloodstream [75,97,98]. Thus for the serum IMMY LFA test higher sensitivities of approximately 50 to 97% were reported in hematological patients [37,58,61], outperforming the OLM LFD in a direct comparison (24% sensitivity) [58].

In contrast to neutropenic patients, angio-invasion in patients with viral induced acute respiratory failure is an indicator for late-stage disease associated with devastating mortality rates in this setting [32,99-101]. This may also explain the higher sensitivity of serum IMMY LFA testing for CAPA

diagnosis in studies from settings with more limited resources for early diagnosis, including more limited access to bronchoscopies (Table 1). In an Iranian study, where most CAPA patients had positive serum GM results indicating late stages of disease, a sensitivity of 56% was observed for the IMMY LFA in serum [88]. Even higher sensitivities were observed in a study from Turkey (80% sensitivity for 74 CAPA cases; 94% specificity) [102] and Argentina (92% sensitivity in 12 CAPA cases; positive serum IMMY LFA results also in 7 patients without any other mycological evidence for CAPA) [103], again likely driven by late stage disease and positivity of serum GM at the time of sampling.Thus, the reported sensitivity may, at least partly, based on advanced stages of CAPA. However, generally speaking, the limited sensitivity of Aspergillus LFTs limit their use in serum as a screening or diagnostic tool for VAPA in the ICU. This is especially true when we target to diagnose VAPA early in the course of the disease.

Even though, there is not a whole lot of data on the diagnostic performance of all different LFTs in blood samples for diagnosis of VAPA out there, the available data indicate, that the performance of the newly introduced LFTs serum samples seems to be similar to that of the established biomarkers like GM detection by ELISA [104-106]. The most important drawback of application of the new LFTs in serum samples in the non-neutropenic cohort is the limited sensitivity. Thus, physicians should keep in mind that a negative test result from a blood sample is not sufficient to rule out VAPA and further diagnostic interventions may be indicated.

5. Conclusions

We have reviewed POCT for VAPA, analyzing performances in respiratory and blood specimens, and have found that most data is available for the IMMY LFA with performances similar to the GM ELISA test. Clinical performance was particularly promising in BALF and to a lesser extend other respiratory specimens, while performance in blood was indiscriminatory for early diagnosis of VAPA. When compared to the OLM LFD the IMMY LFA provided better sensitivity while maintaining good specificity when testing BAL fluid samples. Based on the merged data of these studies, we calculated overall sensitivity and specificity for OLM LFD in BALF and IMMY LFA in BALF, serum, and TA, respectively, for diagnosis of IPA in ICU patients (Table 3). This allowed to estimate Positive and Negative Predictive Values (PPV and NPV, respectively) depending on the IPA incidence. The median prevalence of VAPA is estimated to be between 10% and 20% [5,6]. In this range, while the overall test performance was best in BALF, the best PPV and NPV were obtained in serum (53%-72% PPV) and TA (96%-98% NPV), respectively, with the IMMY LFA device. This suggests that, in ICU patients suffering from VAPA, a positive serum is strongly evocative of an IPA, whereas a negative result in TA almost excludes IPA. However, the value of TA has to be relativized, as the few studies performed only focused on CAPA, and did not include IAPA.

6. Expert opinion

Sensitive and rapid diagnosis of fungal infections improves mortality but remains challenging. Particularly in non-neutropenic patients in the ICU, clinical and radiographic signs are often non-specific, and therefore mycological testing must be performed with a low threshold in case of suspicion of aspergillosis. Conventional classification criteria for IA in neutropenic patients have limited applicability in the heterogenous ICU population where evolving risk factors including new treatments have often replaced classical host factors [107]. Clinical signs of VAPA are often indiscriminatory from viral associated acute respiratory failure, and there are logistical difficulties in performing a chest CT in intubated patients on machines. When chest CT is performed, it often produces non-specific findings in patients with viral associated acute respiratory failure [20].

Therefore new algorithms have been developed to classify VAPA in the ICU [108]. Those algorithms strongly rely on microbiologic findings, which are most reliable when tests are performed in samples from the lower respiratory tract. While bronchoscopy is generally safe for patients on ICU, the diagnostic merits must be evaluated against the status of the patient on a case-by-case basis in those suffering from hypoxemia, organ failure and coagulopathy. Reducing the volume installed during bronchoscopy can lower the risk of complications but may also have an influence on test results, such as antigen levels. Lack of standardization of BALF samples, and the fact that fungal elements are not always homogeneously distributed across the lungs, further complicate the issue. Also timing of the bronchoscopy will have an impact on the yield of microbiological diagnostic tests, with positive results in general observed earlier in IAPA than CAPA, particularly in those with influenza associated tracheobronchial aspergillosis [109].

Given the paramount importance of microbiologic testing on early detection and successful treatment of VAPA POCT have been introduced in various settings, allowing for faster turnaround times and potentially serving a role in antifungal stewardship in settings where GM ELISA results are not rapidly available [110,111]. LFTs have shown promising performances for invasive aspergillosis in the hematology and ICU populations [36,112]. The LFT technology is particularly attractive because of its potential for single sample testing, ease of use and short turnaround time, which may inform early clinical decision making. For early diagnosis of CAPA particularly the IMMY LFA has shown promise when testing of samples from the lower respiratory tract[34]; in contrast limited (OLM LFD) to no data (all other tests) exists for other LFTs outlining that those tests should be used with care. LFTs will not resolve the issues of GM ELISA testing when testing blood samples from patients with CAPA, due to the pathophysiology of the disease with angioinvasion only occurring at a late stage. In contrast to CAPA, literature on performance of LFTs for diagnosis of IAPA is very limited.

Five years from now, mycologic diagnosis of invasive aspergillosis in the ICU will be established with POCT at the bedside, in combination with other diagnostic tests, with positive results and negative results informing clinical decision making in terms on initiation and discontinuation of antifungal treatment. These POC tests will then be validated not only for serum and BALf, but also for CSF and plasma [113,114].

Currently, data on POCT performance for diagnosing VAPA is still limited, especially for IAPA and for testing other specimens than BALF. Also, performance data for POC diagnosis of CAPA is sometimes difficult to interpret due to potential incorporation bias (presence of the evaluated laboratory test and the closely related GM ELISA in the reference mycological criteria) which may lead to an overestimation of the diagnostic accuracy.

For broad implementation of these tests in diagnosing VAPA more studies are therefore necessary. While many POCT are currently available, many of which have not been properly evaluated in terms of their performance, 5 years from now it will not be possible to use POCT in clinical routine without proper validation.

To further improve diagnostic performance, taking into account not only the fungal pathogen but also the host response, novel biomarkers evaluating the host immune response could be implemented or combined in a POCT format with currently available tests. The more distant future will tell, whether the turnaround time of next generation sequencing will significantly improve, allowing for panfungal / panmicrobial testing in the form of a POCT.

Article highlights

- Early diagnosis from samples of the lower respiratory tract and treatment of invasive aspergillosis is an important predictor of survival in patients with VAPA.
- Conventional culture and - to a lesser extent - galactomannan testing is limited by long processing times in some settings.
- Point-of-care diagnostic tests for invasive aspergillosis (IA) are now commercially available and will complement GM and culture in diagnosis of VAPA.
- Particularly the IMMY LFA has shown promise for diagnosis of CAPA, while data on other POC tests are limited to non-existing.
- Serum testing with POCT has shown limited sensitivity for early diagnosis of VAPA.
- Data on diagnostic performance of POCT for IAPA are very limited.

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* Of interest

** Of considerable interest

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Figure 1

Antigen detection assays for invasive aspergillosis: a timeline.

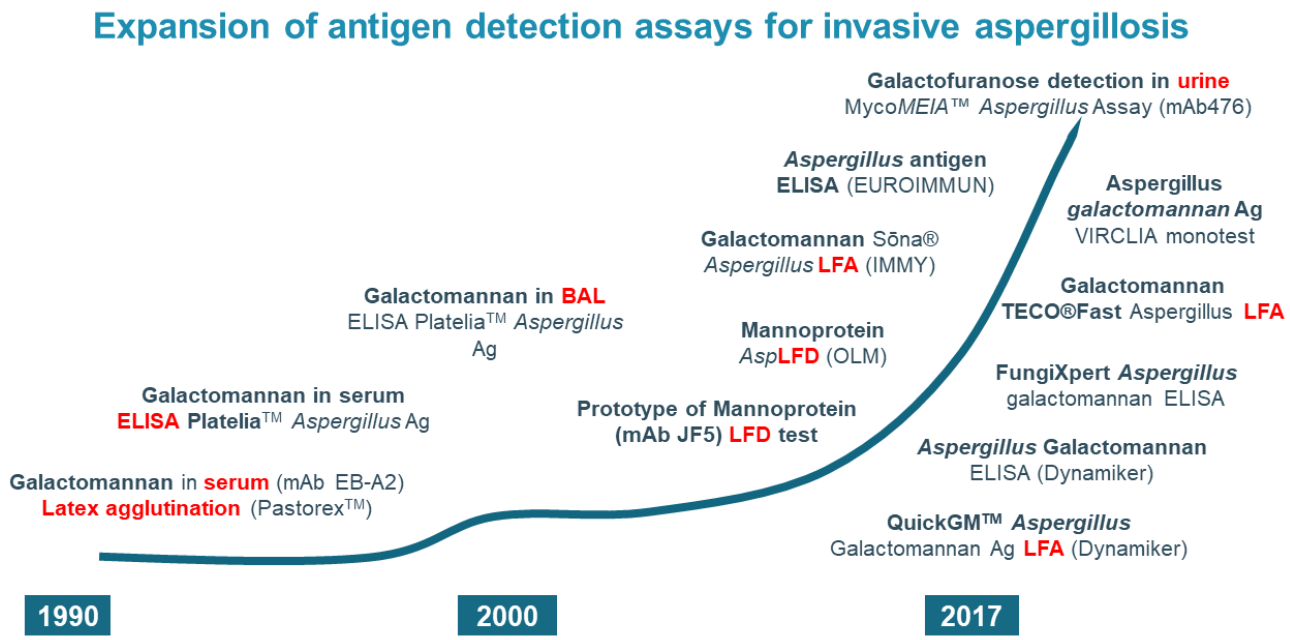

Table 1

Performance of different point-of-care tests (POCTs) for diagnosing VAPA in respiratory and blood specimens. Only studies analyzing performance of lateral flow tests (LFTs) including patients on ICU and/or patients with viral-associated aspergillosis are included.

Studies on LFT in respiratory samples including patients in ICU and/or patients with VAPA:									
Study	**Test**	**Total # of patients n =**	**# Cases Proven and Probable IA***	**Matrix**	**Cohort # on ICU / VAPA**	**Sensitivity entire cohort ******	**Specificity entire cohort ******	**Sensitivity ICU / VAPA**	**Specificity ICU / VAPA**
Hoenigl et al 2014[49]	OLM	78	17	BALF	4 ICU patients	80%	95%	NA	NA
Eigl et al 2015[45]	OLM	133	16	BALF	133 ICU patients, 4 with influenza A	80%	81%	80%	81%
Eigl et al 2015[46]	OLM	60	60	BALF	4 with influenza A	75%	NA	NA	NA
Castillo et al 2018[43]	OLM	106	8	BALF	9 critically ill patients (burn, trauma, ICU)	38%	94%	NA	NA
Hoenigl et al 2018[55]	OLM	28	14	BALF	5 ICU patients	71%	100%	NA	NA
Jenks et al 2019[32]	IMMY	82	13 EORTC / 26 AspICU $	BALF	20 ICU patients	62% EORTC	63% EORTC	69% AspICU+	71% AspICU+

Jenks et al 2019[32]	OLM	82	13 EORTC / 26 AspICU $	BALF	20 ICU patients	69% EORTC	62% EORTC	65% AspICU+	68% AspICU+
Linder et al 2020[86]	IMMY		20	BALF	4 critically ill patients	40%	80%	NA	NA
Mercier et al 2020[87]	IMMY	178	55: 32 EORTC + 23 mAspICU° (17 IAPA)	BALF	178 ICU patients, 64 with influenza	88% EORTC	81% EORTC	94% AspICU 87% mAspICU	81% AspICU 81% mAspICU
Scharmann et al 2020[60]	OLM	200	30 EORTC + mAspICU $$	BALF	200 ICU patients	73.3% EORTC	49.3 EORTC	88.9% AspICU 60.7% AspICU+ 68.5% mAspICU	55.1% AspICU 47.1% AspICU+ 67.2% mAspICU
Scharmann et al 2020[60]	IMMY	200	30 EORTC + mAspICU $$	BALF	200 ICU patients	87.1% EORTC	50.6 EORTC	93.3% AspICU 79.7% AspICU+ 85.3% mAspICU	46.1% AspICU 45.0% AspICU+ 72.9% mAspICU
Jenks et al 2021[81]	IMMY	296	58 EORTC + mAspICU $ (+ 30 putative)	BALF	153 ICU patients (+ other non-Hem and non-SOT), 9 with influenza	89%	44%	86%	48%
Roman-Montes et al 2021[89]	IMMY	144	14 CAPA$^{\mu}$	TA	14 ICU patients	60%	72.6%	60%	72.6%

Autier et al 2022[34]	IMMY	196	48 CAPA^	BALF NBL TA	48 CAPA patients (29 of which BALF tested)	72% [x] 90% [x] 100% [x]	79% [x] 83% [x] 44% [x]	72%	79%
Ghazanfari et al 2022[88]	IMMY	105	33 CAPA^	BALF	105 ICU patients (mechanical ventilation ≥4 days)	60.6%	88.9%	60.6%	88.9%
Marta et al 2022[90]	OLM	300	35 CAPA^^ (8 BALF, 35 TA)	BALF TA	300 ICU patients with COVID-19	87.5% 100%	/	87.5% 100%	/
Egger et al 2022[82]	IMMY	115	43	BALF	52 ICU patients	98%	52%	NA	NA
Studies on LFT in blood including patients in ICU and/or patients with VAPA:									
Study	**Test**	**Total # of patients n =**	**# Cases Proven and Probable IA***	**Matrix**	**Cohort # on ICU / VAPA**	**Sensitivity entire cohort ******	**Specificity entire cohort ********	**Sensitivity ICU / VAPA**	**Specificity ICU / VAPA**
Hoenigl et al 2021[37]	IMMY	122	28 (2 CAPA^)	Serum	59 ICU patients with COVID-19, 28 with no other underlying disease	78.6%	80.5%	0%	96%
Almeida-Paes et al 2022[93]	IMMY	200	2 + 24 CAPA^, 38 CPA,	Serum	Mixed (Non Neutro)	74%	84%	NA	NA

			36 -oma						
Autier et al 2022[34]	IMMY	196	48 CAPA^	Serum	67 CAPA patients (37 proven or probable of which BALF tested)	20% [x]	93% [x]	20%	93%
Ghazanfari et al 2022[88]	IMMY	105	33 CAPA^	Serum	105 ICU patients (mechanical ventilation ≥4 days, 32 CAPA patients of which serum tested)	56.3%	94.2%	56.3%	94.2%
Hsiao et al 2022[94]	OLM	91	29	Serum	35 ICU patients	68.96%	78.67%	NA	NA
Serin et al 2022[102]	IMMY	174	74 CAPA^	Serum	174 ICU-pandemic patients	80% [x]	94% [x]	80% [x]	94% [x]
Giusiano et al 2022[103]	IMMY	185	12 CAPA when LFA was excluded as mycological	Serum	185 ICU patients with COVID-19	92%	/	92%	/

			criterion; 19 probable CAPA when LFA was used as mycological criterion						

OLM = Aspergillus lateral flow device (LFD, OLM Diagnostics, Newcastle Upon Tyne, UK), IMMY = Aspergillus galactomannan lateral flow assay (LFA, Norman, Oklahoma, USA)

* EORTC-MSGERC criteria according to De Pauw et al. Clin Infect Dis. 2008 Jun 15;46(12):1813-21. doi: 10.1086/588660; and Donnelly et al. Clin Infect Dis. 2020 Sep 12;71(6):1367-1376. doi: 10.1093/cid/ciz1008, depending on timing, unless mentioned otherwise.

** Unless mentioned otherwise.

° AspICU criteria according to Blot et al. Am J Respir Crit Care Med. 2012 Jul 1;186(1):56-64. doi: 10.1164/rccm.201111-1978OC; and modified AspICU (mAspICU) criteria according to Schauwvlieghe et al. Lancet Respir Med. 2018 Oct;6(10):782-792. doi: 10.1016/S2213-2600(18)30274-1.

$ AspICU criteria° broadened by adding BALF GM >1.0 ODI as entry criterion (AspICU+).

$$ AspICU criteria° broadened by adding BALF GM >1.0 ODI as entry criterion with a few modifications (AspICU+): 'semi-quantitative Aspergillus-positive culture of BAL fluid (+ or ++), without bacterial growth together with a cytological smear showing branching hyphae' was not included.

µ According to the mAspICU criteria. None of the 14 patients with CAPA met the EORTC/MSG host criteria.

^ According to the ECMM/ISHAM criteria, Koehler et al. Lancet Infect Dis 21: e149–e162. doi.org/10.1016/S1473-3099(20)30847-1, excluding LFA as criterion.

^^ According to the ECMM/ISHAM criteria, Koehler et al. Lancet Infect Dis 21: e149–e162. doi.org/10.1016/S1473-3099(20)30847-1.

X Possible CAPA cases are also included

Abbreviations: ICU = intensive care unit, Hem = hematology, SOT = solid organ transplantation.

Table 2

Composition of the different lateral flow tests for detection of *Aspergillus* antigens mentioned in the text

Lateral flow test	**Composition**	***Aspergillus* antigen detecting**	**Advantages**	**Limitations**	**Test development time**	**Sample volume needed**
Lateral flow device (LFD), OLM Diagnostics, Newcastle Upon Tyne, UK	JF5 mouse IgG3 monoclonal antibody conjugated to nitrocellulose beads (immunochromatography, cassette type)	*Aspergillus* mannoprotein (extracellular glycoprotein)	- Together with IMMY LFA most performance information available - Based on a different antibody than Galactomannan, broadening diagnostic range - Visual readout possible - Pretreatment not required for clean BALF - High specificty when compared to IMMY LFA	- Pretreatment indicated for serum and bloody BALFs - Cross-reactivity with some *Penicillium* species and *Paecilomyces variotii* - Some of the performance data published over the last decade does come from the prototype test which does differ from the current version	30 min. serum 15 min. BALF	150 µL serum 70 µL clean BALF
Soña *Aspergillus* galactomannan lateral flow assay (LFA),	Combination of two monoclonal antibodies conjugated to colloidal gold (immunochromatography,	Galactomannan like antigens	- High sensitivity when compared to OLM LFD, overall best clinical performance - Together with OLM LFD most	- Pretreatment indicated for serum and BALF - Crossreactivity with *Fusarium* spp., *Histoplasma* spp.	30 min.	300 µL

IMMY, Norman, Oklahoma, USA	strip type)		performance information available - Visual readout possible - Cube reader provided by manufacturer for quantitative results	- Longer time to results for BALFs than the OLM LFD - Relatively expensive		
QuicGM *Aspergillus* galactomannan Ag LFA, Dynamiker, Tianjin, China	Monoclonal antibody and Europium nanoparticles (fluorescence immunochromatography, cassette type)	Galactomannan like antigens	- Likely y lower cost per test compared to IMMY LFA - Digital reader available providing quantitative results	- Limited published data, ideal cut-offs to be further investigated, no evaluation of cross-reactivities - Visual readout not possible - Pretreatment indicated for serum and BALF - Different standard curve for serum and BALF with the aim to use 1 threshold (but different thresholds in definitions)	20 min.	300 µL
FungiXpert *Aspergillus* Galactomannan Detection K-set	Antibodies conjugated to colloidal gold (immunochromatography, cassette and strip type)	Galactomannan like antigens	- Likely lower cost per test compared to IMMY LFA - Digital reader available providing quantitative results	- No external/independent (clinical) validation studies published, no evaluation of cross-reactivities	15 min.	300 µL

LFA, Genobio [Era Biology Technology], Tianjin, China			- Visual readout possible - Pretreatment not required for BALF	- Pretreatment indicated for serum		
TECO®*Fast Aspergillus* galactomannan Ag LFA, TECOmedical Group, Sissach, Switzerland; Dynamiker, Tianjin, China	Fluorescence-labeled monoclonal antibodies (fluorescence immunochromatography, cassette type)	Galactomannan like antigens	- Likely lower cost per test compared to IMMY LFA - Digital reader available providing quantitative results	- No external/independent (clinical) validation studies published, no evaluation of cross-reactivities - Visual readout not possible - Pretreatment indicated for serum and BALF	20 min.	300 µL
MycoFLOW® test, Pearl Diagnostics, Baltimore, USA	IgM monoclonal antibody (mAb476)	Small molecular weight galactofuranose-containing antigens (free glycoproteins and extracellular vesicles) in urine	- Based on a different antibody broadening diagnostic range - Only LFT that can be used for urine samples - May allow for home testing in the future	- Still under development, not yet commercially available - Only few performance data available (mostly about the EIA test version) - Cross-reactivity with *Histoplasma* spp. and *Blastomyces* spp.	NA	NA

BALF = bronchoalveolar lavage fluid, LFT = lateral flow test, EIA = enzyme immunoassay; for pretreatment: buffer, centrifuge and heater necessary; caution in case quantitative results are available: it is not yet known how these quantitative results can be compared to the ones from GM EIA; all LFTs are transportable and storable at room temperature

Table 3

Estimation of PPV and NPV of lateral flow tests for aspergillosis diagnosis in VAPA patients

Test	Clinical specimen	Overall sensitivity: % (n/N)	Overall specificity: % (n/N)	PPV/NPV for 1% IPA prevalence	PPV/NPV for 5% IPA prevalence	PPV/NPV for 10% IPA prevalence	PPV/NPV for 20% IPA prevalence	PPV/NPV for 40% IPA prevalence	Merged data from
OLM	BALF	71%	75%	2.8%/99.6%	13.1%/98.0%	24.2%/95.9%	41.8%/91.3%	65.7%/79.7%	[32,45,46,60]

		(152/213)	(178/237)						
IMMY	BALF	76% (206/271)	80% (421/523)	3.8%/99.7%	17.0%/98.5%	30.2%/96.8%	49.4%/93.1%	72.2%/83.4%	[32,34,60,81,87,88]
IMMY	Serum	55% (92/166)	96% (289/300)	9.4%/99.6%	35.2%/97.7%	53.4%/95.3%	72.1%/90.1%	87.3%/77.3%	[34,37,88,102,103]
IMMY	Tracheal aspirate	90% (56/62)	51% (68/133)	1.8%/99.8%	8.9%/99.0%	17.0%/97.9%	31.6%/95.5%	55.2%/88.8%	[34,89,90]

PPV = Positive Predictive Value, NPV = Negative Predictive Value, ICU = Intensive Care Unit, VAPA = Viral-Associated Pulmonary Aspergillosis, OLM = *Aspergillus* lateral flow device (LFD, OLM Diagnostics, Newcastle Upon Tyne, UK), IMMY = *Aspergillus* galactomannan lateral flow assay (LFA, Norman, Oklahoma, USA), BALF = Bronchoalveolar Lavage Fluid