



**HAL**  
open science

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

R Aerts, Brice Autier, Maximilian Gornicec, Juergen Prattes, Katrien Lagrou, Jean-Pierre Gangneux, Martin Hoenigl

► **To cite this version:**

R Aerts, Brice Autier, Maximilian Gornicec, Juergen Prattes, Katrien Lagrou, et al.. Point-of-care testing for viral-associated pulmonary aspergillosis. *Expert Review of Molecular Diagnostics*, 2023, pp.1-13. 10.1080/14737159.2023.2257597 . hal-04228387

**HAL Id: hal-04228387**

**<https://hal.science/hal-04228387v1>**

Submitted on 11 Dec 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

Robina Aerts <sup>1 2</sup>, Brice Autier <sup>3 4</sup>, Maximilian Gornicec <sup>5</sup>, Juergen Prattes <sup>5 6</sup>, Katrien Lagrou <sup>2 7</sup>, Jean-Pierre Gangneux <sup>3 4</sup>, Martin Hoenigl <sup>5 6 8</sup>

- 1 - Department of Internal Medicine, University Hospitals Leuven, Leuven, Belgium.
- 2 - Department of Microbiology, Immunology and Transplantation, Laboratory of Clinical Microbiology, KU Leuven, Leuven, Belgium.
- 3 - Univ Rennes, CHU Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail), UMR\_S 1085, Rennes, France.
- 4 - Centre Hospitalier Universitaire de Rennes, Laboratory of Parasitology and Mycology, European Excellence Center in Medical Mycology (ECMM-EC), National Reference Center on mycology and antifungals (LA-AspC Chronic aspergillosis and *A. fumigatus* resistance), Rennes, France.
- 5 - Division of Infectious Diseases, ECMM Excellence Center for Medical Mycology, Department of Medicine, Medical University of Graz, Graz, Austria.
- 6 - Translational Medical Mycology Research Unit, Medical University of Graz, Graz, Austria.
- 7 - Department of Laboratory Medicine, National Reference Center for Mycosis, University Hospitals Leuven, Leuven, Belgium.
- 8 - BioTechMed, Graz, Austria.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31

**Abstract:**

**Introduction:** Over the last years, severe respiratory viral infections, particularly those caused by SARS-COV-2 and Influenza, 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 patients 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 commentary:** 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 bronchioalveolar 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.

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

32 **1. Introduction:**

33 Over the last decades prevalence of infections caused by *Aspergillus* species continues to  
34 increase, triggered by advances in medicine and other factors such as the COVID-19  
35 pandemic [1,2]. As a result *Aspergillus fumigatus*, the most relevant and prevalent species  
36 causing infections in humans, was included into the World Health Organization (WHO) priority  
37 pathogens list and classified in the highest category of critical importance [3].

38 *Aspergillus* is found ubiquitous in nature, commonly colonizes the respiratory tract via conidial  
39 inspiration and can cause various forms of disease, depending on the hosts immune  
40 competence, ranging from allergic disease to invasive pulmonary aspergillosis (IPA) [4].

41 Over the last years, severe respiratory viral infections have emerged as risk factor for invasive  
42 fungal disease among critically ill patients [5-8]. A large and growing body of literature has  
43 investigated that although other viral infections may lead to secondary opportunistic infections  
44 as well, SARS-COV-2 and Influenza in particular excel in their ability to damage epithelial lung  
45 tissue and impair immune response [2,9-14]. Furthermore, corticosteroid and tocilizumab  
46 treatment of critically ill COVID-19 patients with respiratory failure may contribute to  
47 predisposing immune dysfunction[15-17].

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

52 Mycological testing of respiratory specimens, optimally bronchoalveolar lavage fluid (BALF)  
53 has become essential for early diagnosis of VAPA, with fungal culture and testing for  
54 galactomannan (GM) considered the gold standard tests [21-24]. However, both culture and  
55 GM testing are often limited by long turnaround times, with GM sometimes being a send out  
56 test, especially in smaller centers [25-27]. Molecular pathogen detection methods like  
57 polymerase chain reaction (PCR) assays and next generation sequencing (NGS) have been  
58 utilized as well [28-30], While bronchoscopy cannot always be performed, especially in low  
59 and middle income countries it is essential for early diagnosis of VAPA. Unlike fungal infections  
60 in neutropenic patients[31], invasive growth in VAPA is primarily limited to lung tissue, with  
61 angio-invasion occurring only in later stages of the disease[32]. Therefore, blood -surrogate  
62 markers for the diagnosis of VAPA display lower sensitivity than in neutropenic patients [32-  
63 36].

64 All these problems combined, result in delayed diagnosis and thereby have a significant impact  
65 on morbidity and mortality. These limitations underline the need for more simple diagnostic  
66 tests that can be performed at bedside with rapid turnaround time. These point-of-care-tests  
67 (POCT) may play an important role in earlier diagnosis of VAPA and thus improve patients  
68 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 Soñia *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 0.5 ODI, 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]. More recently, other new lateral flow tests were introduced implementing the terms LFA and LFD can no longer be used to distinguish specifically the IMMY and OLM test in the future. 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. Of these new lateral flow tests (LFTs) only

105 internal validation data are available but no clinical performance studies are published in  
106 literature.

107

108 LFTs as POCT can be performed on different types of clinical specimens, such as blood, BALF,  
109 urine and cerebrospinal fluid (CSV), and can detect the presence of different *Aspergillus*  
110 antigens. The level of validation and the clinical performance status of these POCT vary  
111 depending on the test, the type of specimen and the patient population. The performance of  
112 these tests can also be affected by factors such as the timing of the sample collection and the  
113 presence of antifungal prophylaxis or treatment[46,64].

114

115 LFTs are most useful when a rapid response is important and the number of samples is low.  
116 To really speak of point of care testing there must be no sample transportation, a very short  
117 turnaround time without too much pretreatment steps and the possibility to immediately  
118 interpret the result without an additional chain of reporting. Implementation of POCTs bedside  
119 is limited because the need of trained personnel, quality control and the need for correct  
120 biosafety conditions. Therefore, it is better to speak of LFTs as rapid tests instead of POCT as  
121 at the moment they are mostly implemented in microbiology labs[25].

122

123 As the IMMY LFA and the new LFTs detect GM, in general most of the new POCT detect  
124 epitopes very similar to the EB-A2 mAb. There is a void in new diagnostics that employ mAbs  
125 directed at epitopes other than those present on GM. In 2012, a new IgM monoclonal antibody  
126 (mAb476) that detects small molecular weight galactofuranose-containing antigens in urine  
127 was found[65]. This was further optimized to develop the MycoMEIA® test (Pearl Diagnostics,  
128 Baltimore, USA) detecting free glycoproteins and extracellular vesicles in urine[66]. Before,  
129 GM testing was assumed to be less sensitive in urine causing LFTs until now not being fully  
130 analyzed in urine as a matrix [67-71][32-35]. With the MycoMEIA® test currently being in  
131 development as an LFT as well (MycoFLOW®) the possibility of an *Aspergillus* POCT in urine  
132 is promising.

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

138

139

140 Also, other more rapid tests are being developed. To detect 1,3-β-D-glucan (BDG) for example,  
141 the Wako β-glucan assay (Wako-BDG, Fujifilm Wako Pure Chemical Corporation, Osaka,

142 Japan) provides the possibility for single-sample testing and a faster turnaround time in  
143 comparison to the Fungitell assay (Associates of Cape Cod, Massachusetts, USA) that was  
144 dominating the market historically[73,74].

145

## 146 **2.2. Point of care testing for VAPA**

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

156

157 First studies with the LFTs including patients on ICU only date back from 2014[45].  
158 Performance studies of LFTs in patients with VAPA are only available on the IMMY LFA and  
159 OLM LFD. Of the others LFTs, including the MycoFLOW test and the VirClia Monotest, a  
160 review of literature did not provide any relevant results.

161

162 In general, for VAPA LFTs are investigated especially in BALF as serum biomarkers tend to  
163 give a false-negative result in critically ill non-neutropenic patients and diagnostic BALF should  
164 be performed in suspected cases of IPA [32,45,75,76]. For VAPA, depending on where  
165 bronchoscopy is performed, analysis of BALF – in case of a low number of samples – could  
166 be performed on-site, at the endoscopy ward or at the intensive care unit (ICU). However, in  
167 reality, the personal in charge for endoscopy often does not have the time nor possibility to  
168 add the analysis to its work packet and the sample must be sent to the lab for other  
169 microbiological and immunological analysis anyway. This could limit its use as a POCT.

170

171

172

## 173 **3. Performance in respiratory specimens from patients with severe COVID-19 or** 174 **Influenza infection**

175

### 176 **3.1. Bronchoalveolar Lavage**

177 While broadly accepted consensus criteria for classification of IA in non-neutropenic patients  
178 are still in the works[77,78], Blot at al., and Schauwvlieghe et al. proposed 2 versions of a

179 specific algorithm for ICU patients[8,79]. In some other publications, diagnosis of IA in ICU  
180 patients is also made based on a proposal from the EORTC/MSG, which classify “proven”  
181 and “probable” cases of IA [80]. For CAPA specifically, Koehler et al. have proposed specific  
182 criteria in 2021 for classification[63], which are now widely used whereas for IAPA expert case  
183 definitions are often used[19]. In both of them, detection of GM antigen in BALF or serum  
184 samples, as a “mycological evidence” of *Aspergillus* infection, allows classification as  
185 “probable” cases of IA. Both IMMY LFA and OLM LFD were evaluated for detection of GM in  
186 BALF in mixed cohorts, which included variable proportions of ICU patients (7-52%)  
187 [32,43,46,49,55,81,82]. In all of them except two, it was not specified whether VAPA cases  
188 were included [46,81]. Sensitivity in the whole cohorts was slightly higher for IMMY LFA (62-  
189 98%) [32,56,81-83] compared to OLM LFD (69-80%) (Table 1) [32,55,84,85]. In two studies  
190 sensitivity was unusually low, one OLM LFD evaluation (38% sensitivity in a small cohort of 8  
191 IA[43]) and one IMMY LFA evaluation (40% sensitivity in a cohort presenting unusual  
192 discrepancies with other mycological assays[86]). Specificity was highly variable (44-100%),  
193 depending on the used definition criteria and the cohort composition. Among these studies,  
194 only one detailed LFA and LFD performance in ICU patients, reporting sensitivity of 69% and  
195 65%, and specificity of 71% and 68%, for IMMY LFA and OLM LFD respectively [32].

196  
197 To date, only two studies focusing on the performance of OLM LFD in BALF were restricted to  
198 ICU patients[45,60]. In these studies, sensitivity and specificity were 83-89% and 55-79%,  
199 respectively, using the Blot et al. definition of IA. The few other studies that focused on ICU  
200 patients evaluated the IMMY LFA, either in a mixed VAPA/non-VAPA cohort [81,87] or  
201 specifically in COVID-19 patients [34,88]. All showed good sensitivity, ranging from 58% to  
202 82%, and excellent specificity, ranging from 75% to 98% (Table 1). No study was restricted to  
203 IAPA patients, nor detailed the results for them. Only one study from Mercier et al. (2020),  
204 included a significant proportion of IAPA patients (36% of all patients; n=64 with n=17 IAPA  
205 cases and n=47 influenza cases without IAPA). This study showed both high sensitivity (87-  
206 94% depending on the classification criteria) and high specificity (81%) of the IMMY LFA;  
207 specifically for patients with IAPA sensitivity was 94%[87]. The digital readout using the Cube  
208 Reader proposed by the manufacturer was associated to a higher sensitivity when compared  
209 to a visual readout[87]. Diagnostic value of IMMY LFA was highly comparable to GM EIA  
210 Platelia™ assay [81,87], with the obvious benefit of a short handling time. Receiver Operating  
211 Characteristics (ROC) curve analysis showed AUCs between 0.754 and 0.920, which  
212 correspond to a good discrimination between IA and non-IA patients, although not perfect. In  
213 COVID-19 patients, this was probably due to the impossibility to reach >90% sensitivity without  
214 dramatically lowered specificity[34,88]. All together, these data suggest that IMMY LFA  
215 detection in BALF should motivate for a consolidated diagnosis of invasive aspergillosis



216 (culture and PCR in BALF and GM in serum) and incites to initiate an antifungal treatment in  
217 CAPA patients, as it is highly specific of IA at the 1.0 ODI cutoff.

218

### 219 **3.2. Other respiratory samples**

220 Several other respiratory samples can be used for diagnosis of invasive pulmonary  
221 aspergillosis, in increasing rank of deepness: sputum, tracheal aspiration (TA) or the non-  
222 directed bronchoalveolar lavage (NBL). As they are sampled in the upper respiratory tract,  
223 these are considered to be more at risk for colonization with *Aspergillus*, thus with lowered  
224 specificity. However, they also are of interest because of their less invasive sampling  
225 procedure, allowing repeated samples on contrary to BALF. Few studies evaluated the  
226 performance of GM detection in these samples for diagnosis of IA, moreover in VAPA patients  
227 or using a POCT. IMMY LFA detection (>1 ODI cutoff) in TA seems to have a good sensitivity  
228 (64-81%) for CAPA diagnosis, with an expected low specificity (67-68%), which makes it a  
229 good screening test [34,89]. Only one study reported performance of OLM LFD in TA, with  
230 100% (35/35) sensitivity among a cohort of CAPA patients, however the used cutoff was not  
231 specified[90]. Considering the severity of VAPA, positivity of a GM rapid test in TA should  
232 motivate BALF sampling and further initiation of antifungal therapy if confirmed.

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

236 To date, the only study on GM detection performance in sputum for diagnosis of IA focused on  
237 hematology patients and used GM EIA assay[91]. It showed interesting results as sputum GM  
238 had 100% sensitivity and 62% specificity, but this was determined on a small cohort of IA  
239 patients (n=6) and has to be validated on ICU patients. Finally, it seems clear that the value of  
240 GM detection in non-BALF respiratory samples should not be neglected, although it could be  
241 performed easily and quickly using a POCT to allow rapid management adaptations.

242

243

### 244 **4. Performance in Blood**

245

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

252 In a large European multicenter study in four countries (148 serum samples; including samples  
253 from 48 cases with CAPA) performance of serum IMMY LFA was evaluated specifically for  
254 CAPA patients in the ICU. Sensitivity in serum was low with 20% at the 0.5 ODI cutoff, with a  
255 specificity of 93% and a non-discriminatory AUC of 0.512 [34], with significantly better  
256 performances observed when testing respiratory specimens. While several mixed cohort  
257 studies evaluating the serum IMMY LFA or OLM LFD exist in cohorts of ICU or non-neutropenic  
258 patients that also include patients with CAPA (sensitivities between 69% and 79%, specificities  
259 79% to 84% in the overall cohorts, Table 1)[37,93,94], only one of these studies reported  
260 performance for CAPA specifically (0% sensitivity, 96% specificity), but was limited by  
261 including only 2 patients with CAPA[95].

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

272 In contrast to neutropenic patients, angio-invasion in patients with viral induced acute  
273 respiratory failure is an indicator for late-stage disease associated with devastating mortality  
274 rates in this setting [32,99-101]. This may also explain the higher sensitivity of serum IMMY  
275 LFA testing for CAPA diagnosis in studies from settings with more limited resources for early  
276 diagnosis, including more limited access to bronchoscopies (Table 1). In an Iranian study,  
277 where most CAPA patients had positive serum GM results indicating late stages of disease, a  
278 sensitivity of 56% was observed for the IMMY LFA in serum [88]. Even higher sensitivities  
279 were observed in a study from Turkey (80% sensitivity for 74 CAPA cases; 94% specificity)  
280 [102] and Argentina (92% sensitivity in 12 CAPA cases; positive serum IMMY LFA results also  
281 in 7 patients without any other mycological evidence for CAPA ) [103], again likely driven by  
282 late stage disease and positivity of serum GM at the time of sampling.

283 Thus, the reported sensitivity may, at least partly, based on advanced stages of CAPA.  
284 However, generally speaking, the limited sensitivity of *Aspergillus* LFTs limit their use in serum  
285 as a screening or diagnostic tool for VAPA in the ICU. This is especially true when we target  
286 to diagnose VAPA early in the course of the disease.

287 Even though, there is not a whole lot of data on the diagnostic performance of all different LFTs  
288 in blood samples for diagnosis of VAPA out there, the available data indicate, that the

289 performance of the newly introduced LFTs serum samples seems to be similar to that of the  
290 established biomarkers like GM detection by ELISA [104-106]. The most important drawback  
291 of application of the new LFTs in serum samples in the non-neutropenic cohort is the limited  
292 sensitivity. Thus, physicians should keep in mind that a negative test result from a blood  
293 sample is not sufficient to rule out VAPA and further diagnostic interventions may be indicated.  
294

## 295 **5. Conclusions**

296  
297 We have reviewed POCT for VAPA, analyzing performances in respiratory and blood  
298 specimens, and have found that most data is available for the IMMY LFA with performances  
299 similar to the GM ELISA test. Clinical performance was particularly promising in BALF and to  
300 a lesser extend other respiratory specimens, while performance in blood was indiscriminatory  
301 for early diagnosis of VAPA. When compared to the OLM LFD the IMMY LFA provided better  
302 sensitivity while maintaining good specificity when testing BAL fluid samples.  
303

## 304 **6. Expert Commentary**

305 Sensitive and rapid diagnosis of fungal infections improves mortality but remains challenging.  
306 Particularly in non-neutropenic patients in the ICU, clinical and radiographic signs are often  
307 non-specific, and therefore mycological testing must be performed with a low threshold in case  
308 of suspicion of aspergillosis. Conventional classification criteria for IA in neutropenic patients  
309 have limited applicability in the heterogenous ICU population where evolving risk factors  
310 including new treatments have often replaced classical host factors [107]. Clinical sings of  
311 VAPA are often indiscriminatory from viral associated acute respiratory failure, and there are  
312 logistical difficulties in performing a chest CT in intubated patients on machines. When chest  
313 CT is performed, it often produces non-specific findings in patients with viral associated acute  
314 respiratory failure[20].  
315

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

326 earlier in IAPA than CAPA, particularly in those with influenza associated tracheobronchial  
327 aspergillosis [109].

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

341

342

343

#### 344 **7. 5-year view**

345 Five years from now, mycologic diagnosis of invasive aspergillosis in the ICU will be  
346 established with POCT at the bedside, in combination with other diagnostic tests, with positive  
347 results and negative results informing clinical decision making in terms on initiation and  
348 discontinuation of antifungal treatment. Data on POCT performance for diagnosing VAPA is  
349 still limited, especially for IAPA and for testing other specimens than BALF. Also performance  
350 evaluation for diagnosis of CAPA is sometimes due to incorporation bias (presence of the  
351 evaluated laboratory test and the closely related GM ELISA in the reference mycological  
352 criteria) which may lead to an overestimation of the diagnostic accuracy.

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

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

362

363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383

**8. Key issues:**

- 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 are limited by long processing times in some settings.
- Point-of-care diagnostic test 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.

384 **References**

385 Reference annotations

386 \* Of interest

387 \*\* Of considerable interest

388

- 389 1. Bongomin F, Gago S, Oladele RO, et al. Global and Multi-National Prevalence of  
390 Fungal Diseases-Estimate Precision. *Journal of fungi* (Basel, Switzerland).  
391 2017;3(4):10.3390/jof3040057.
- 392 2. Hoenigl M, Seidel D, Sprute R, et al. COVID-19-associated fungal infections. *Nat*  
393 *Microbiol.* 2022 Aug 2.
- 394 3. Fisher MC, Denning DW. The WHO fungal priority pathogens list as a game-  
395 changer. *Nat Rev Microbiol.* 2023 Apr;21(4):211-212.
- 396 4. Latgé JP, Chamilos G. *Aspergillus fumigatus* and Aspergillosis in 2019. *Clin*  
397 *Microbiol Rev.* 2019 Dec 18;33(1).
- 398 5. Jenks JD, Nam HH, Hoenigl M. Invasive aspergillosis in critically ill patients: Review  
399 of definitions and diagnostic approaches. *Mycoses.* 2021 Mar 24.
- 400 6. Hoenigl M, Seidel D, Carvalho A, et al. The emergence of COVID-19 associated  
401 mucormycosis: a review of cases from 18 countries. *Lancet Microbe.* 2022 Jan 25.
- 402 7. Özbek L, Topçu U, Manay M, et al. COVID-19-associated mucormycosis: a  
403 systematic review and meta-analysis of 958 cases. *Clin Microbiol Infect.* 2023 Mar  
404 13.
- 405 8. Schauwvlieghe AFAD, Rijnders BJA, Philips N, et al. Invasive aspergillosis in  
406 patients admitted to the intensive care unit with severe influenza: a retrospective  
407 cohort study. *The Lancet Respiratory medicine.* 2018.
- 408 9. Salazar F, Bignell E, Brown GD, et al. Pathogenesis of Respiratory Viral and Fungal  
409 Coinfections. *Clin Microbiol Rev.* 2022 Jan 19;35(1):e0009421.
- 410 10. Feys SG, SM; Khan, M; CHoi, S; Boeckx, B; Chatelain, D; Cunha, C; Davaveye, Y;  
411 Hermans, G; Hertoghs, M; Humblet-Baron, S; Jacobs, C; Lagrou, K; Marcelis, L. An  
412 observational study of lung epithelial and myeloid immunity in influenza- and  
413 COVID-19-associated pulmonary aspergillosis. *Lancet Resp Med.* 2022.
- 414 11. Reizine F, Pinceaux K, Lederlin M, et al. Influenza- and COVID-19-Associated  
415 Pulmonary Aspergillosis: Are the Pictures Different? *J Fungi* (Basel). 2021 May  
416 15;7(5).
- 417 12. Gangneux JP, Hoenigl M, Papon N. How to lose resistance to *Aspergillus* infections.  
418 *Trends Microbiol.* 2023 Mar;31(3):222-224.
- 419 13. Arastehfar A, Carvalho A, van de Veerdonk FL, et al. COVID-19 Associated  
420 Pulmonary Aspergillosis (CAPA)-From Immunology to Treatment. *J Fungi* (Basel).  
421 2020 Jun 24;6(2).
- 422 14. Arastehfar A, Carvalho A, Houbraken J, et al. *Aspergillus fumigatus* and  
423 aspergillosis: From basics to clinics. *Stud Mycol.* 2021 Sep;100:100115.
- 424 15. Bartoletti M, Pascale R, Cricca M, et al. Epidemiology of invasive pulmonary  
425 aspergillosis among COVID-19 intubated patients: a prospective study. *Clin Infect*  
426 *Dis.* 2020 Jul 28.
- 427 16. Janssen NAF, Nyga R, Vanderbeke L, et al. Multinational Observational Cohort  
428 Study of COVID-19-Associated Pulmonary Aspergillosis(1). *Emerg Infect Dis.* 2021  
429 Nov;27(11):2892-2898.
- 430 17. Gangneux JD, E; Fekkar, A; Luyt, CE; Botterel, FDe Prost, N. Fungal infections in  
431 mechanically ventilated COVID-19 patients in the ICU during the 1 first wave: The  
432 French multicenter MYCOVID study. *Lancet Resp Med.* 2021.

- 433 \* Large multicenter study investigating the CAPA  
434
- 435 18. Koehler P, Denis B, Denning DW, et al. European confederation of medical  
436 mycology expert consult-An ECMM excellence center initiative. *Mycoses*. 2020 Mar  
437 17.
- 438 19. Verweij PE, Rijnders BJA, Brüggemann RJM, et al. Review of influenza-associated  
439 pulmonary aspergillosis in ICU patients and proposal for a case definition: an  
440 expert opinion. *Intensive care medicine*. 2020;46(8):1524-1535.
- 441 20. Hong W, White PL, Backx M, et al. CT findings of COVID-19-associated pulmonary  
442 aspergillosis: a systematic review and individual patient data analysis. *Clin  
443 Imaging*. 2022 Oct;90:11-18.
- 444 21. Vanderbeke L, Spriet I, Breynaert C, et al. Invasive pulmonary aspergillosis  
445 complicating severe influenza: epidemiology, diagnosis and treatment. *Curr Opin  
446 Infect Dis*. 2018 Dec;31(6):471-480.
- 447 22. Giacobbe DR, Prattes J, Wauters J, et al. Prognostic Impact of Bronchoalveolar  
448 Lavage Fluid Galactomannan and Aspergillus Culture Results on Survival in  
449 COVID-19 Intensive Care Unit Patients: a Post Hoc Analysis from the European  
450 Confederation of Medical Mycology (ECMM) COVID-19-Associated Pulmonary  
451 Aspergillosis Study. *J Clin Microbiol*. 2022 Mar 24:e0229821.
- 452 23. Koehler P, Cornely OA, Böttiger BW, et al. COVID-19 Associated Pulmonary  
453 Aspergillosis. *Mycoses*. 2020 Apr 27.
- 454 24. Prattes J, Wauters J, Giacobbe DR, et al. Diagnosis and treatment of COVID-19  
455 associated pulmonary aspergillosis in critically ill patients: results from a European  
456 confederation of medical mycology registry. *Intensive Care Med*. 2021 Jul 16.
- 457 25. Salmanton-García J, Hoenigl M, Gangneux JP, et al. The current state of laboratory  
458 mycology and access to antifungal treatment in Europe: a European Confederation  
459 of Medical Mycology survey. *Lancet Microbe*. 2023 Jan;4(1):e47-e56.
- 460 \*\* Survey investigating availability of POCT across Europe
- 461 26. Chindamporn A, Chakrabarti A, Li R, et al. Survey of laboratory practices for  
462 diagnosis of fungal infection in seven Asian countries: An Asia Fungal Working  
463 Group (AFWG) initiative. *Medical mycology*. 2018;56(4):416-425.
- 464 \* Study outlining the long turnaround times and delay from GM ELISA testing
- 465 27. Salmanton-García J, Au WY, Hoenigl M, et al. The current state of laboratory  
466 mycology in Asia/Pacific: A survey from the European Confederation of Medical  
467 Mycology (ECMM) and International Society for Human and Animal Mycology  
468 (ISHAM). *Int J Antimicrob Agents*. 2023 Jan 11:106718.
- 469 \*\* Survey investigating availability of POCT across Asia Pacific.
- 470 28. Mikulska M, Furfaro E, Dettori S, et al. Aspergillus-PCR in bronchoalveolar lavage -  
471 diagnostic accuracy for invasive pulmonary aspergillosis in critically ill patients.  
472 *Mycoses*. 2022 Apr;65(4):411-418.
- 473 29. Hoenigl M, Egger M, Price J, et al. Metagenomic Next-Generation Sequencing of  
474 Plasma for Diagnosis of COVID-19-Associated Pulmonary Aspergillosis. *Journal of  
475 Clinical Microbiology*. 2023;0(0):e01859-22.
- 476 30. Gangneux JP, Reizine F, Guegan H, et al. Is the COVID-19 Pandemic a Good Time to  
477 Include Aspergillus Molecular Detection to Categorize Aspergillosis in ICU  
478 Patients? A Monocentric Experience. *J Fungi (Basel)*. 2020 Jul 10;6(3).
- 479 31. Heldt S, Prattes J, Eigl S, et al. Diagnosis of Invasive Aspergillosis in Hematological  
480 Malignancy Patients: Performance of Cytokines, Asp LFD, and Aspergillus PCR in  
481 Same Day Blood and Bronchoalveolar Lavage Samples. *J Infect*. 2018.

- 482 32. Jenks JD, Mehta SR, Taplitz R, et al. Point-of-care diagnosis of invasive aspergillosis  
483 in non-neutropenic patients: Aspergillus Galactomannan Lateral Flow Assay  
484 versus Aspergillus-specific Lateral Flow Device test in bronchoalveolar lavage.  
485 *Mycoses*. 2019 Mar;62(3):230-236.
- 486 \*\* Study investigating the IMMY LFA and OLM LFD in non-neutropenic patients
- 487 33. Lass-Flörl C, Samardzic E, Knoll M. Serology anno 2021—fungal infections: from  
488 invasive to chronic. *Clinical Microbiology and Infection* 2021. p. 1230-1241.
- 489 34. Autier B, Prattes J, White PL, et al. Aspergillus Lateral Flow Assay with Digital  
490 Reader for the Diagnosis of COVID-19-Associated Pulmonary Aspergillosis (CAPA):  
491 a Multicenter Study. *Journal of clinical microbiology*. 2022 Jan 19;60(1):e0168921
- 492 \*\* Multicenter study investigating the IMMY LFA in different patient specimens for  
493 diagnosis of CAPA
- 494 35. Egger M, Prüller F, Krause R, et al. Utility of Serum 1,3-β-d-Glucan Testing for  
495 Diagnosis and Prognostication in COVID-19-Associated Pulmonary Aspergillosis.  
496 *Microbiol Spectr*. 2022 May 31:e0137322.
- 497 36. Jenks JD, Prattes J, Frank J, et al. Performance of the Bronchoalveolar Lavage Fluid  
498 Aspergillus Galactomannan Lateral Flow Assay with Cube Reader for Diagnosis of  
499 Invasive Pulmonary Aspergillosis: a Multicenter Cohort Study. *Clinical Infectious  
500 Diseases*. 2021.
- 501 \*\* Multicenter study investigating the IMMY LFA in BAL specimens for diagnosis of IA
- 502 37. Hoenigl M, Egger M, Boyer J, et al. Serum Lateral Flow Assay with Digital Reader  
503 for the Diagnosis of Invasive Pulmonary Aspergillosis: A Two Center Mixed Cohort  
504 Study. *Mycoses*. 2021 Jul 12.
- 505 38. Mercier T, Dunbar A, de Kort E, et al. Lateral flow assays for diagnosing invasive  
506 pulmonary aspergillosis in adult hematology patients: A comparative multicenter  
507 study. *Medical mycology*. 2019:myz079.
- 508 39. Swanink CM, Meis JF, Rijs AJ, et al. Specificity of a sandwich enzyme-linked  
509 immunosorbent assay for detecting Aspergillus galactomannan. *Journal of clinical  
510 microbiology*. 1997;35(1):257-260.
- 511 40. Verweij PE, Dompeling EC, Donnelly JP, et al. Serial monitoring of Aspergillus  
512 antigen in the early diagnosis of invasive aspergillosis. Preliminary investigations  
513 with two examples. *Infection*. 1997;25(2):86-89.
- 514 41. Thornton CR. Development of an immunochromatographic lateral-flow device for  
515 rapid serodiagnosis of invasive aspergillosis. *Clinical and vaccine immunology :  
516 CVI*. 2008;15(7):1095-1105.
- 517 42. Wiederhold NP, Thornton CR, Najvar LK, et al. Comparison of lateral flow  
518 technology and galactomannan and (1->3)-beta-D-glucan assays for detection of  
519 invasive pulmonary aspergillosis. *Clinical and vaccine immunology : CVI*.  
520 2009;16(12):1844-1846.
- 521 43. Castillo CG, Kauffman CA, Zhai J, et al. Testing the performance of a prototype  
522 lateral flow device using bronchoalveolar lavage fluid for the diagnosis of invasive  
523 pulmonary aspergillosis in high-risk patients. *Mycoses*. 2018;61(1):4-10.
- 524 44. Delama I, Legarraga P, González T, et al. [Evaluation of the lateral flow Aspergillus  
525 assay for the diagnosis of invasive aspergillosis, experience in a university  
526 hospital]. *Rev Chilena Infectol*. 2018 2018;35(5):574-579.
- 527 45. Eigl S, Prattes J, Lackner M, et al. Multicenter evaluation of a lateral-flow device test  
528 for diagnosing invasive pulmonary aspergillosis in ICU patients. *Critical Care  
529 (London, England)*. 2015;19:178-015-0905-x.



- 530 46. Eigl S, Prattes J, Reinwald M, et al. Influence of mould-active antifungal treatment  
531 on the performance of the Aspergillus-specific bronchoalveolar lavage fluid  
532 lateral-flow device test. *International journal of antimicrobial agents*. 2015.
- 533 47. Willinger B, Lackner M, Lass-Flörl C, et al. Bronchoalveolar Lavage Lateral-Flow  
534 Device Test for Invasive Pulmonary Aspergillosis in Solid Organ Transplant  
535 Patients: A Semi-Prospective Multicenter Study. *Transplantation*. 2014;accepted  
536 manuscript.
- 537 48. Prattes J, Lackner M, Eigl S, et al. Diagnostic accuracy of the Aspergillus-specific  
538 bronchoalveolar lavage lateral-flow assay in haematological malignancy patients.  
539 *Mycoses*. 2015;58(8):461-469.
- 540 49. Hoenigl M, Prattes J, Spiess B, et al. Performance of galactomannan, beta-d-glucan,  
541 Aspergillus lateral-flow device, conventional culture, and PCR tests with  
542 bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis.  
543 *Journal of clinical microbiology*. 2014;52(6):2039-2045.
- 544 50. Prattes J, Flick H, Prüller F, et al. Novel tests for diagnosis of invasive aspergillosis  
545 in patients with underlying respiratory diseases. *American Journal of Respiratory  
546 and Critical Care Medicine*. 2014 2014/10/15/;190(8):922-929.
- 547 51. Hoenigl M, Koidl C, Duettmann W, et al. Bronchoalveolar lavage lateral-flow device  
548 test for invasive pulmonary aspergillosis diagnosis in haematological malignancy  
549 and solid organ transplant patients. *J Infect*. 2012 2012/12//;65(6):588-591.
- 550 52. Johnson GL, Sarker SJ, Nannini F, et al. Aspergillus-Specific Lateral-Flow Device and  
551 Real-Time PCR Testing of Bronchoalveolar Lavage Fluid: a Combination Biomarker  
552 Approach for Clinical Diagnosis of Invasive Pulmonary Aspergillosis. *Journal of  
553 clinical microbiology*. 2015;53(7):2103-2108.
- 554 53. Miceli MH, Goggins MI, Chander P, et al. Performance of lateral flow device and  
555 galactomannan for the detection of Aspergillus species in bronchoalveolar fluid of  
556 patients at risk for invasive pulmonary aspergillosis. *Mycoses*. 2015;58(6):368-  
557 374.
- 558 54. White PL, Parr C, Thornton C, et al. Evaluation of real-time PCR, galactomannan  
559 enzyme-linked immunosorbent assay (ELISA), and a novel lateral-flow device for  
560 diagnosis of invasive aspergillosis. *Journal of clinical microbiology*.  
561 2013;51(5):1510-1516.
- 562 55. Hoenigl M, Eigl S, Heldt S, et al. Clinical evaluation of the newly formatted lateral-  
563 flow device for invasive pulmonary aspergillosis. *Mycoses*. 2018;61(1):40-43.
- 564 56. Jenks JD, Mehta SR, Taplitz R, et al. Bronchoalveolar lavage Aspergillus  
565 Galactomannan lateral flow assay versus Aspergillus-specific lateral flow device  
566 test for diagnosis of invasive pulmonary Aspergillosis in patients with  
567 hematological malignancies. *Journal of Infection*. 2019 2019/03/01/;78(3):249-  
568 259.
- 569 57. Jenks JD, Mehta SR, Taplitz R, et al. Bronchoalveolar lavage Aspergillus  
570 Galactomannan lateral flow assay versus Aspergillus-specific lateral flow device  
571 test for diagnosis of invasive pulmonary Aspergillosis in patients with  
572 hematological malignancies. *J Infect*. 2019 Mar;78(3):249-259.
- 573 58. Mercier T, Guldentops E, Lagrou K, et al. Prospective Evaluation of the  
574 Turbidimetric beta-D-Glucan Assay and 2 Lateral Flow Assays on Serum in  
575 Invasive Aspergillosis. *Clin Infect Dis*. 2021 May 4;72(9):1577-1584.
- 576 59. Mercier T, Dunbar A, de Kort E, et al. Lateral flow assays for diagnosing invasive  
577 pulmonary aspergillosis in adult hematology patients: A comparative multicenter  
578 study. *Medical Mycology*. 2020 2020/06/01/;58(4):444-452.

- 579 60. Scharmman U, Verhasselt HL, Kirchhoff L, et al. Evaluation of two lateral flow assays  
580 in BAL fluids for the detection of invasive pulmonary aspergillosis: A retrospective  
581 two-centre study. *Mycoses*. 2020 2020/12//;63(12):1362-1367.
- 582 61. White PL, Price JS, Posso R, et al. Evaluation of the Performance of the IMMY sona  
583 Aspergillus Galactomannan Lateral Flow Assay When Testing Serum To Aid in  
584 Diagnosis of Invasive Aspergillosis. *J Clin Microbiol*. 2020 May 26;58(6).
- 585 62. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and Update of the Consensus  
586 Definitions of Invasive Fungal Disease From the European Organization for  
587 Research and Treatment of Cancer and the Mycoses Study Group Education and  
588 Research Consortium. *Clinical Infectious Diseases*. 2020  
589 2020/09/15//;71(6):1367-1376.
- 590 63. Koehler P, Bassetti M, Chakrabarti A, et al. Defining and managing COVID-19-  
591 associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for  
592 research and clinical guidance. *The Lancet Infectious Diseases*. 2021  
593 2021/06//;21(6):e149-e162.
- 594 \* Consensus definitions for CAPA which include the IMMY LFA as mycological evidence.
- 595 64. Wiederhold NP, Najvar LK, Bocanegra R, et al. Interlaboratory and interstudy  
596 reproducibility of a novel lateral-flow device and influence of antifungal therapy  
597 on detection of invasive pulmonary aspergillosis. *Journal of clinical microbiology*.  
598 2013;51(2):459-465.
- 599 65. Dufresne SF, Datta K, Li X, et al. Detection of urinary excreted fungal  
600 galactomannan-like antigens for diagnosis of invasive aspergillosis. *PloS one*.  
601 2012;7(8):e42736.
- 602 66. Marr KA, Datta K, Mehta S, et al. Urine Antigen Detection as an Aid to Diagnose  
603 Invasive Aspergillosis. *Clinical infectious diseases : an official publication of the  
604 Infectious Diseases Society of America*. 2018.
- 605 67. Kriegl L, Havlicek V, Dichtl K, et al. Siderophores: a potential role as a diagnostic  
606 for invasive fungal disease. *Curr Opin Infect Dis*. 2022 Aug 4.
- 607 68. Hoenigl M, Orasch T, Faserl K, et al. Triacetylfulvarinine C: A urine biomarker for  
608 diagnosis of invasive aspergillosis. *J Infect*. 2019 Feb;78(2):150-157.
- 609 69. Reischies FM, Raggam RB, Prattes J, et al. Urine Galactomannan-to-Creatinine Ratio  
610 for Detection of Invasive Aspergillosis in Patients with Hematological  
611 Malignancies. *Journal of clinical microbiology*. 2016;54(3):771-774.
- 612 70. Salonen J, Lehtonen OP, Terasjarvi MR, et al. Aspergillus antigen in serum, urine  
613 and bronchoalveolar lavage specimens of neutropenic patients in relation to  
614 clinical outcome. *Scandinavian Journal of Infectious Diseases*. 2000;32(5):485-  
615 490.
- 616 71. Duettmann W, Koidl C, Troppan K, et al. Serum and urine galactomannan testing  
617 for screening in patients with hematological malignancies. *Medical mycology*.  
618 2014;52(6):647-652.
- 619 72. Buil JB, Huygens S, Dunbar A, et al. Retrospective Multicenter Evaluation of the  
620 VirClia Galactomannan Antigen Assay for the Diagnosis of Pulmonary Aspergillosis  
621 with Bronchoalveolar Lavage Fluid Samples from Patients with Hematological  
622 Disease. *Journal of Clinical Microbiology*. 2023 2023/04/25//;e0004423.
- 623 73. Singh S, Kanaujia R, Agnihotri S, et al. The Comparative Evaluation of the Fujifilm  
624 Wako  $\beta$ -Glucan Assay and Fungitell Assay for Diagnosing Invasive Fungal Disease.  
625 *Journal of Fungi (Basel, Switzerland)*. 2022 2022/12/20//;9(1):6.
- 626 74. Mercier T, Guldentops E, Patteet S, et al. Beta-d-Glucan for Diagnosing  
627 Pneumocystis Pneumonia: a Direct Comparison between the Wako  $\beta$ -Glucan Assay

- 628 and the Fungitell Assay. *Journal of Clinical Microbiology*. 2019  
629 2019/05/24/;57(6).
- 630 75. Bergeron A, Porcher R, Sulahian A, et al. The strategy for the diagnosis of invasive  
631 pulmonary aspergillosis should depend on both the underlying condition and the  
632 leukocyte count of patients with hematologic malignancies. *Blood*.  
633 2012;119(8):1831-7; quiz 1956.
- 634 76. Mercier T, Castagnola E, Marr KA, et al. Defining Galactomannan Positivity in the  
635 Updated EORTC/MSGERC Consensus Definitions of Invasive Fungal Diseases.  
636 *Clinical Infectious Diseases*. 2021 2021/03/15/;72(Supplement\_2):S89-S94.
- 637 77. Bassetti M, Giacobbe DR, Grecchi C, et al. Performance of existing definitions and  
638 tests for the diagnosis of invasive aspergillosis in critically ill, adult patients: A  
639 systematic review with qualitative evidence synthesis. *J Infect*. 2020 Apr 21.
- 640 78. Bassetti M, Scudeller L, Giacobbe DR, et al. Developing definitions for invasive  
641 fungal diseases in critically ill adult patients in intensive care units. Protocol of the  
642 FUNgal infections Definitions in ICU patients (FUNDICU) project. *Mycoses*. 2019  
643 Apr;62(4):310-319.
- 644 79. Blot SI, Taccone FS, Van den Abeele A-M, et al. A clinical algorithm to diagnose  
645 invasive pulmonary aspergillosis in critically ill patients. *American Journal of  
646 Respiratory and Critical Care Medicine*. 2012 2012/07/01/;186(1):56-64.
- 647 80. Bassetti M, Azoulay E, Kullberg BJ, et al. EORTC/MSGERC Definitions of Invasive  
648 Fungal Diseases: Summary of Activities of the Intensive Care Unit Working Group.  
649 *Clin Infect Dis*. 2021 Mar 12;72(Supplement\_2):S121-s127.
- 650 81. Jenks JD, Prattes J, Frank J, et al. Performance of the Bronchoalveolar Lavage Fluid  
651 Aspergillus Galactomannan Lateral Flow Assay With Cube Reader for Diagnosis of  
652 Invasive Pulmonary Aspergillosis: A Multicenter Cohort Study. *Clinical Infectious  
653 Diseases*. 2021 2021/10/01/;73(7):e1737-e1744.
- 654 82. Egger M, Penziner S, Dichtl K, et al. Performance of the Euroimmun Aspergillus  
655 Antigen ELISA for the Diagnosis of Invasive Pulmonary Aspergillosis in  
656 Bronchoalveolar Lavage Fluid. *J Clin Microbiol*. 2022 Apr 20;60(4):e0021522.
- 657 83. Jenks JD, Hoenigl M. Point-of-care diagnostics for invasive aspergillosis: nearing  
658 the finish line. *Expert Rev Mol Diagn*. 2020 Sep 14:1-9.
- 659 84. Hoenigl M, Prattes J, Spiess B, et al. Performance of galactomannan, beta-d-glucan,  
660 Aspergillus lateral-flow device, conventional culture, and PCR tests with  
661 bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis.  
662 *Journal of Clinical Microbiology*. 2014 2014/06//;52(6):2039-2045.
- 663 85. Eigl S, Prattes J, Reinwald M, et al. Influence of mould-active antifungal treatment  
664 on the performance of the Aspergillus-specific bronchoalveolar lavage fluid  
665 lateral-flow device test. *International Journal of Antimicrobial Agents*. 2015  
666 2015/10//;46(4):401-405.
- 667 86. Linder KA, Kauffman CA, Miceli MH. Performance of Aspergillus Galactomannan  
668 Lateral Flow Assay on Bronchoalveolar Lavage Fluid for the Diagnosis of Invasive  
669 Pulmonary Aspergillosis. *Journal of Fungi (Basel, Switzerland)*. 2020  
670 2020/11/18/;6(4):E297.
- 671 87. Mercier T, Dunbar A, Veldhuizen V, et al. Point of care aspergillus testing in  
672 intensive care patients. *Critical Care (London, England)*. 2020  
673 2020/11/10/;24(1):642.
- 674 \*\* Largest study on POCT testing for IAPA.
- 675 88. Ghazanfari M, Yazdani Charati J, Davoodi L, et al. Comparative analysis of  
676 galactomannan lateral flow assay, galactomannan enzyme immunoassay and BAL

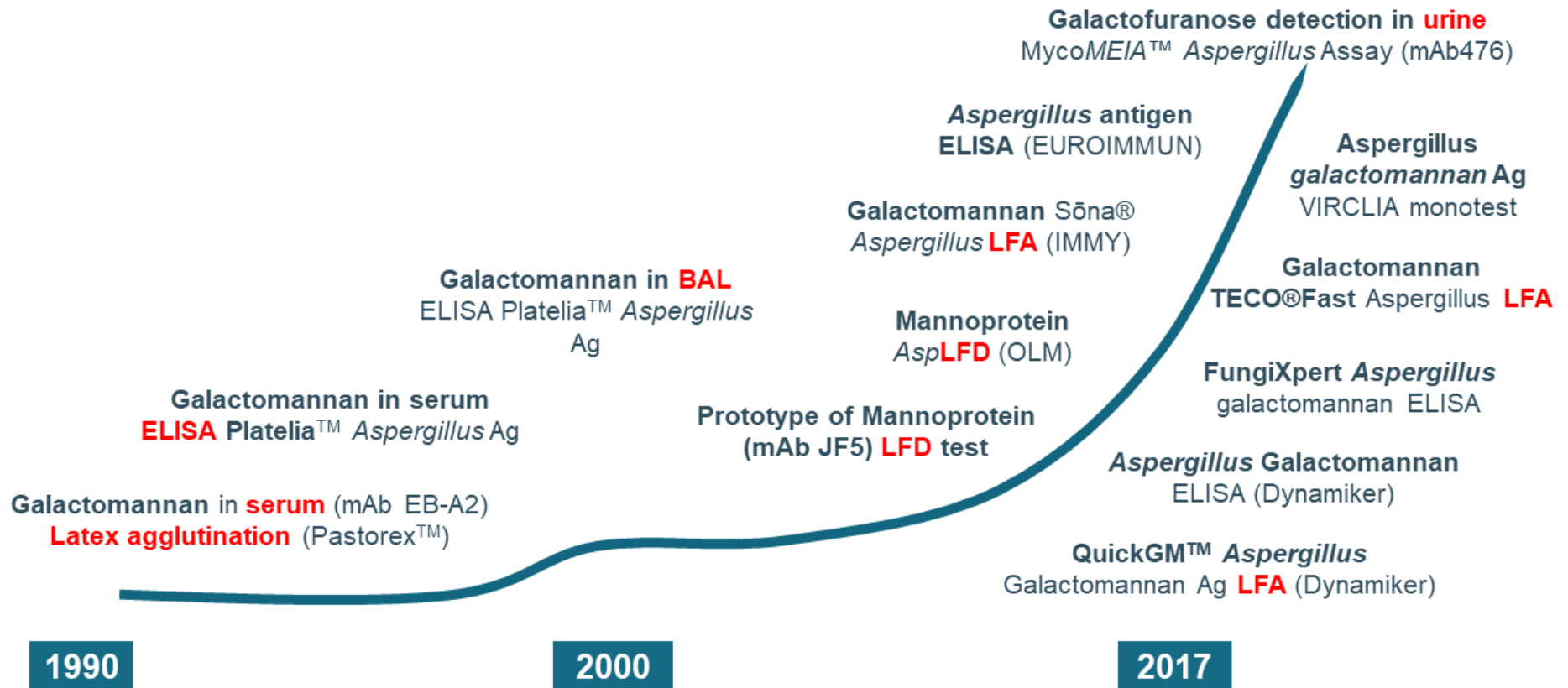
- 677 culture for diagnosis of COVID-19-associated pulmonary aspergillosis. *Mycoses*.  
678 2022 Oct;65(10):960-968.
- 679 89. Roman-Montes CM, Martinez-Gamboa A, Diaz-Lomeli P, et al. Accuracy of  
680 galactomannan testing on tracheal aspirates in COVID-19-associated pulmonary  
681 aspergillosis. *Mycoses*. 2021 2021/04//;64(4):364-371.
- 682 90. Marta G-C, Lorena F-E, Laura M-V, et al. COVID-19-Associated Pulmonary  
683 Aspergillosis in a Tertiary Hospital. *Journal of Fungi*. 2022 2022/02//;8(2):97.
- 684 91. Kimura S-I, Odawara J, Aoki T, et al. Detection of sputum *Aspergillus*  
685 galactomannan for diagnosis of invasive pulmonary aspergillosis in  
686 haematological patients. *International Journal of Hematology*. 2009  
687 2009/11//;90(4):463-470.
- 688 92. Nyga R, Maizel J, Nseir S, et al. Invasive Tracheobronchial Aspergillosis in Critically  
689 Ill Patients with Severe Influenza. A Clinical Trial. *Am J Respir Crit Care Med*. 2020  
690 Sep 1;202(5):708-716.
- 691 93. Almeida-Paes R, Almeida MA, de Macedo PM, et al. Performance of Two  
692 Commercial Assays for the Detection of Serum *Aspergillus Galactomannan* in Non-  
693 Neutropenic Patients. *J Fungi (Basel)*. 2022 Jul 18;8(7).
- 694 94. Hsiao HH, Liu YC, Wang HC, et al. Comparison of a novel lateral-flow device to  
695 galactomannan assay at different time periods for detections of invasive  
696 aspergillosis. *J Formos Med Assoc*. 2022 Oct;121(10):2123-2129.
- 697 95. Hoenigl M, Egger M, Boyer J, et al. Serum Lateral Flow assay with digital reader for  
698 the diagnosis of invasive pulmonary aspergillosis: A two-centre mixed cohort  
699 study. *Mycoses*. 2021 Oct;64(10):1197-1202.
- 700 96. Prattes J, Wauters J, Giacobbe DR, et al. Risk factors and outcome of pulmonary  
701 aspergillosis in critically ill coronavirus disease 2019 patients- a multinational  
702 observational study by the European Confederation of Medical Mycology. *Clin  
703 Microbiol Infect*. 2021 Aug 25.
- 704 97. Cordonnier C, Botterel F, Ben Amor R, et al. Correlation between galactomannan  
705 antigen levels in serum and neutrophil counts in haematological patients with  
706 invasive aspergillosis. *Clinical microbiology and infection : the official publication  
707 of the European Society of Clinical Microbiology and Infectious Diseases*. 2009  
708 Jan;15(1):81-6.
- 709 98. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in Invasive  
710 Aspergillosis. *Clin Microbiol Rev*. 2009 Jul;22(3):447-65.
- 711 99. Ergun M, Brüggemann RJM, Alanio A, et al. *Aspergillus* Test Profiles and Mortality  
712 in Critically Ill COVID-19 Patients. *Journal of clinical microbiology*. 2021 Nov  
713 18;59(12):e0122921.
- 714 100. Ergün M, Brüggemann RJM, Alanio A, et al. *Aspergillus* test profiles and mortality  
715 in critically-ill COVID-19 patients. *J Clin Microbiol*. 2021 Sep 8;Jcm0122921.
- 716 101. Dellièrè S, Dudoignon E, Voicu S, et al. Combination of mycological criteria: a better  
717 surrogate to identify COVID-19 associated pulmonary aspergillosis patients and  
718 evaluate prognosis? *J Clin Microbiol*. 2022 Jan 5;Jcm0216921.
- 719 102. Serin I, Baltali S, Cinli TA, et al. Lateral flow assay (LFA) in the diagnosis of COVID-  
720 19-associated pulmonary aspergillosis (CAPA): a single-center experience. *BMC  
721 Infect Dis*. 2022 Nov 8;22(1):822.
- 722 103. Giusiano G, Fernández NB, Vitale RG, et al. Usefulness of Sōna *Aspergillus*  
723 Galactomannan LFA with digital readout as diagnostic and as screening tool of  
724 COVID-19 associated pulmonary aspergillosis in critically ill patients. Data from a  
725 multicenter prospective study performed in Argentina. *Med Mycol*. 2022 May  
726 18;60(5).

- 727 104. Prattes J, Wauters J, Giacobbe DR, et al. Diagnosis and treatment of COVID-19  
728 associated pulmonary aspergillosis in critically ill patients: results from a European  
729 confederation of medical mycology registry. *Intensive Care Med.* 2021  
730 Oct;47(10):1158-1160.
- 731 105. Bartoletti M, Pascale R, Cricca M, et al. Epidemiology of Invasive Pulmonary  
732 Aspergillosis Among Intubated Patients With COVID-19: A Prospective Study. *Clin  
733 Infect Dis.* 2021 Dec 6;73(11):e3606-e3614.
- 734 106. Prattes J, Wauters J, Giacobbe DR, et al. Risk factors and outcome of pulmonary  
735 aspergillosis in critically ill coronavirus disease 2019 patients-a multinational  
736 observational study by the European Confederation of Medical Mycology. *Clinical  
737 microbiology and infection : the official publication of the European Society of  
738 Clinical Microbiology and Infectious Diseases.* 2021 Aug 26.
- 739 107. Jenks JD, Nam HH, Hoenigl M. Invasive aspergillosis in critically ill patients: Review  
740 of definitions and diagnostic approaches. *Mycoses.* 2021 Sep;64(9):1002-1014.
- 741 108. Koehler P, Bassetti M, Chakrabarti A, et al. Defining and managing COVID-19-  
742 associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for  
743 research and clinical guidance. *The Lancet Infectious Diseases.* 2020  
744 2020/12/14/.
- 745 109. Verweij PE, Brüggemann RJM, Azoulay E, et al. Taskforce report on the diagnosis  
746 and clinical management of COVID-19 associated pulmonary aspergillosis.  
747 *Intensive Care Med.* 2021 Aug;47(8):819-834.
- 748 110. De Pascale G, Martin-Loeches I, Nseir S. Antifungal stewardship in critically ill  
749 patients. *Intensive Care Medicine.* 2023 2023/03/24.
- 750 111. Aerts R, Cuypers L, Mercier T, et al. Implementation of Lateral Flow Assays for the  
751 Diagnosis of Invasive Aspergillosis in European Hospitals: A Survey from Belgium  
752 and a Literature Review of Test Performances in Different Patient Populations.  
753 *Mycopathologia.* 2023 2023/05/20.
- 754 \* Recently published survey on implementation of LFTs in laboratories across Belgium.
- 755 112. Mercier T, Guldentops E, Lagrou K, et al. Prospective evaluation of the  
756 turbidimetric  $\beta$ -D-glucan assay and two lateral flow assays on serum in invasive  
757 aspergillosis. *Clin Infect Dis.* 2020 Mar 19.
- 758

759 **Figure 1**

760 Antigen detection assays for invasive aspergillosis: a timeline.

## Expansion of antigen detection assays for invasive aspergillosis



761

762 **Table 1**

763 Performance of different point-of-care tests (POCTs) for diagnosing VAPA in respiratory and blood specimens. Only studies analyzing performance  
 764 of lateral flow tests (LFTs) including patients on ICU and/or patients with viral-associated aspergillosis are included; OLM = *Aspergillus* lateral flow  
 765 device (LFD, OLM Diagnostics, Newcastle Upon Tyne, UK), IMMY = *Aspergillus* galactomannan lateral flow assay (LFA, Norman, Oklahoma, USA).

766

Only 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
Hoeningl 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
Hoeningl 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 <sup>u</sup>	TA	14 ICU patients	60%	72.6%	60%	72.6%
Autier et al 2022[34]	IMMY	196	48 CAPA <sup>^</sup>	BALf NBL TA	48 CAPA patients (29 of which BALf tested)	72% <sup>x</sup> 90% <sup>x</sup> 100% <sup>x</sup>	79% <sup>x</sup> 83% <sup>x</sup> 44% <sup>x</sup>	72%	79%
Ghazanfari et al 2022[88]	IMMY	105	33 CAPA <sup>^</sup>	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 <sup>^^</sup> (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
<b>Only studies on LFT in blood including patients in ICU and/or patients with VAPA:</b>									
<b>Study</b>	<b>Test</b>	<b>Total # of patients n =</b>	<b># Cases Proven and Probable IA*</b>	<b>Matrix</b>	<b>Cohort # on ICU / VAPA</b>	<b>Sensitivity entire cohort **</b>	<b>Specificity entire cohort **</b>	<b>Sensitivity ICU / VAPA</b>	<b>Specificity ICU / VAPA</b>



Hoeningl et al 2021[37]	IMMY	122	28 (2 CAPA <sup>^</sup> )	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 <sup>^</sup> , 38 CPA, 36 -oma	Serum	Mixed (Non Neutro)	74%	84%	NA	NA
Autier et al 2022[34]	IMMY	196	48 CAPA <sup>^</sup>	Serum	67 CAPA patients (37 proven or probable of which BALf tested)	20% <sup>x</sup>	93% <sup>x</sup>	20%	93%
Ghazanfari et al 2022[88]	IMMY	105	33 CAPA <sup>^</sup>	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 <sup>^</sup>	Serum	174 ICU-pandemic patients	80% <sup>x</sup>	94% <sup>x</sup>	80% <sup>x</sup>	94% <sup>x</sup>
Giusiano et al 2022[103]	IMMY	185	12 CAPA when LFA was excluded as mycological criterion; 19 probable CAPA when LFA was used as mycological criterion	Serum	185 ICU patients with COVID-19	92%	/	92%	/

\* 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.

767  
768  
769  
770  
771  
772

773 ° 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  
774 according to Schauwvlieghe et al. Lancet Respir Med. 2018 Oct;6(10):782-792. doi: 10.1016/S2213-2600(18)30274-1.  
775

776 \$ AspICU criteria° broadened by adding BALF GM >1.0 ODI as entry criterion (AspICU+).  
777

778 \$\$ 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  
779 ++), without bacterial growth together with a cytological smear showing branching hyphae' was not included.  
780

781 μ According to the mAspICU criteria. None of the 14 patients with CAPA met the EORTC/MSG host criteria.  
782

783 ^ 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.  
784 ^^ According to the ECMM/ISHAM criteria, Koehler et al. Lancet Infect Dis 21: e149–e162. doi.org/10.1016/S1473-3099(20)30847-1.  
785

786 X Including possible CAPA  
787

788 Abbreviations: ICU = intensive care unit, Hem = hematology, SOT = solid organ transplantation.  
789

791  
792  
794