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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 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 Soňa *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.

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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.

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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

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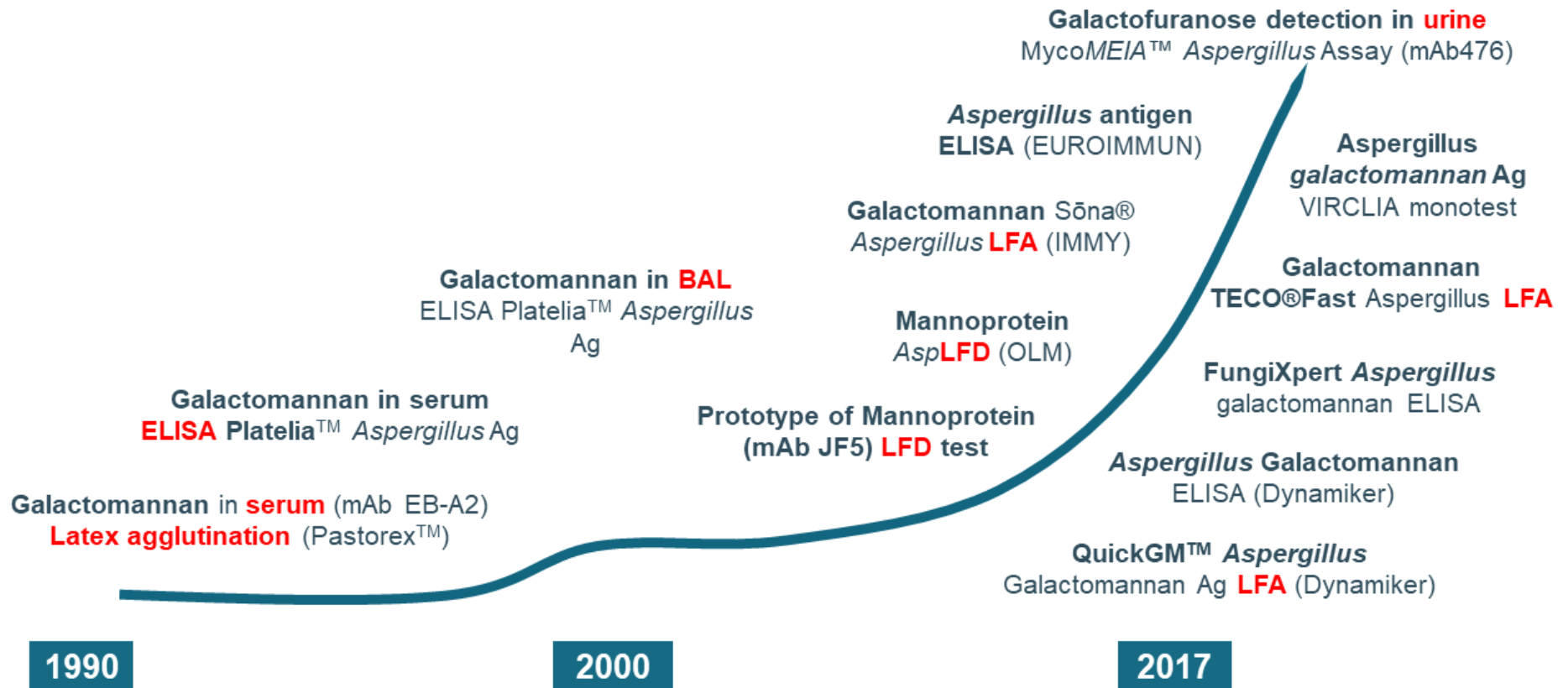
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759 **Figure 1**
760 Antigen detection assays for invasive aspergillosis: a timeline.

Expansion of antigen detection assays for invasive aspergillosis



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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).

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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

Scharmman 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
Scharmman 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 ^u	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
Only 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

Hoeningl 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, 36 -oma	Serum	Mixed (Non Neutro)	74%	84%	NA	NA
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 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.

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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.

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

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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.

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786 X Including possible CAPA

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

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