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Original Article

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Aspergillus antibody detection: diagnostic strategy and technical considerations from the Société Française de Mycologie Médicale (French Society for Medical Mycology) expert committee

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Abstract

Until now, there has been no consensus on the best method for the detection of anti-*Aspergillus* antibodies, a key diagnostic tool for chronic aspergilloses. To better appreciate the usage of and confidence in these techniques, the Société Française de Mycologie Médicale (French Society for Medical Mycology; SFMM) performed a two-step survey of French experts. First, we administered an initial survey to French labs performing *Aspergillus* serology to depict usage of the different techniques available for *Aspergillus* serology. Second, an opinion poll was conducted of 40 experts via an online questionnaire. Each item was rated from 1 to 9 according to the level of agreement. The initial survey revealed that enzyme-linked immunosorbent assay (ELISA) (81%) and immunoelectrophoresis (IEP) (67%) were the most commonly used techniques for screening and confirmation, respectively. The distinction between screening and confirmation techniques was confirmed by the experts (median = 7) with a 44.2% variation coefficient. Only ELISA for screening and IEP and Western blot (WB) for confirmation were clearly considered valuable methods (median $\geq$8 with variation coefficients less than 30%).

The use of a confirmation technique was recommended in the case of a positive result in a compatible clinical context (cystic fibrosis, for example) or during the patient's follow-up. In the case of discordant results between the screening and confirmation techniques, the experts recommended greater confidence in the results obtained with the confirmation technique. All experts emphasized the need to standardize *Aspergillus* serology techniques and to better define the place of each of them in the diagnosis of aspergillosis.

Key words: *Aspergillus*, antibody, serology, techniques, expert opinion.

Introduction

Detection of anti-*Aspergillus* antibodies is essential for the diagnosis of allergic and chronic forms of pulmonary aspergillosis.[1–3] The detection of specific anti-*Aspergillus* IgG and/or precipitins is included in the definition criteria of allergic bronchopulmonary aspergillosis (ABPA)[3–5] and chronic aspergilloses.[3,6–7] Nevertheless, there is no international consensus on the serological test(s) to use in a given clinical context. The French nomenclature of biological tests established in 1995 distinguishes screening tests from confirmatory ones for *Aspergillus* serology (Table 1). This rationale is based on the idea that the screening method should be more sensitive and the confirmation test more specific. However, there is a relative paucity of published reports on the performances of the *Aspergillus* serology tests.[8–12] Moreover, until recently, there was no commercially available or fully standardized confirmation method, leading to the development of a large number of variant techniques to detect anti-*Aspergillus* precipitins.[13–18] Nevertheless, as most French mycologists tend to apply the 2-step strategy, the French experience is quite unique for clarifying the usefulness of the different methods and technical issues in this field. Thus, the "Société Française de Mycologie Médicale" (SFMM, French Society for Medical Mycology) set up a task force of mycologists selected on their experience in *Aspergillus* serology. Data were collected through an initial survey followed by a poll regarding the experts' confidence in the different serological methods used for the detection of anti-*Aspergillus* antibodies.

Table 1. Official French nomenclature of biological analysis: techniques to use for aspergillosis serology.

Phase	Methods (one chosen)
Screening	Electrosyneresis Hemagglutination Immuno enzymatic Double agar gel immunodiffusion test
Confirmatory	Coelectrosyneresis Immunoelectrophoresis Western Blot

Note: "Journal Officiel," 28th April 1995; https://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000352954.

Materials and methods

Initial survey

The first meeting aimed to identify the *Aspergillus* serology practices in 20 tertiary care centres. Each center should report the number of tests they performed per year, the techniques they used, and their exact strategy regarding screening for and confirmation of anti-*Aspergillus* antibodies.

Expert advice

Based on the results of the first survey, an online questionnaire was built and sent to a committee of 40 mycology experts in the field of *Aspergillus* serology. Experts were selected as regional senior advisors for *Aspergillus* serology. As such they are used to routinely validate different techniques, are registered with an external quality assessment program and are involved in the accreditation of those techniques according to the international standard ISO 15189. This online questionnaire contained 37 questions for which an answer was mandatory, and the questionnaire was scored from 1 to 9: from complete disagreement (1) to complete agreement (9) under the link:

https://docs.google.com/forms/d/1-dcrPxXSqf2Bsb7775I4eLPhrrf43r9enmjIASfHlAI/edit.

The results were analyzed blindly using box plots to determine the median value and the dispersion of the values (Prism v. 6d; GraphPad). In addition, the coefficient of variation (CV = standard deviation/mean) was calculated for each item. Strong opinion was considered when a median value of 7 was observed with a %CV inferior to 25%.

Table 2. Serological strategy for anti-*Aspergillus* antibody detection reported by 20 French mycology labs.

Strategy	Technique combination	Number of users, N (%)
Systematic combination of two techniques	ELISA + IEP	5 (25%)
	ELISA + ES	1 (5%)
	IHA + IEP	1 (5%)
One screening technique followed by one confirmation technique (in case of a positive result)	ELISA then IEP	5 (25%)
	ELISA then IEP + DID	2 (10%)
	ELISA then ES	1 (5%)
	ELISA then WB	1 (5%)
Two screening techniques plus one confirmation technique (in case of a positive result)	ELISA + IHA/ES then IEP	2 (10%)
	ES + IHA then CoES	1 (5%)

Abbreviations: CoES, coelectrosyneresis; DID, double agar gel immunodiffusion test; ELISA, enzyme-linked immunosorbent assay; ES, electrosyneresis; IHA, indirect haemagglutination; IEP, immunoelectrophoresis; WB, Western blot.

Results

The first survey conducted among 20 French tertiary care centers revealed that *Aspergillus* serology is quantitatively one of the most important serological tests performed within the mycology labs, with approximately 40,000 tests per year.

Nine center (45%) strictly adopt the 2-step strategy (screening followed by confirmation in the case of positivity) suggested by the nomenclature (Table 2). The other centers directly combined the screening and the confirmations tests in first line (35%) or combined two different screening tests before a confirmation one (15% of the centers). Overall, commercial ELISA kits (81% of the centers) and home-made immunoelectrophoresis (70% of the centers) were the most frequently used techniques for screening and confirmation, respectively (Figure 1). Four commercial ELISA kits were mentioned: Platelia™ *Aspergillus* IgG ELISA (BioRad Laboratories, Marnes La Coquette, France), Serion ELISA IgG classic (Institut Virion/Serion GmbH, Würzburg, Germany), Mastazyme *Aspergillus* IgG ELISA (Mast Diagnostica GmbH, Reinfeld, Germany), and ELISA *Aspergillus fumigatus* IgG Bordier (Bordier Affinity Products SA, Crissier, Switzerland).

Because there was a wide variability of answers, we decided to propose an online survey to a panel of 40 experts. Thirty-six of 40 (90%) questionnaires were completed. Again a large variability in the answers was recorded as 22 (59.5%) of the 37 items of the questionnaire were quoted from a minimum of 1 to a maximum of 9, and a third of these items had a CV greater than 50%.

Nevertheless, the distinction between the screening and confirmation tests suggested by French nomenclature is considered as somewhat relevant by the experts (Table 3), as the median opinion reaches a score of 7 but with a great

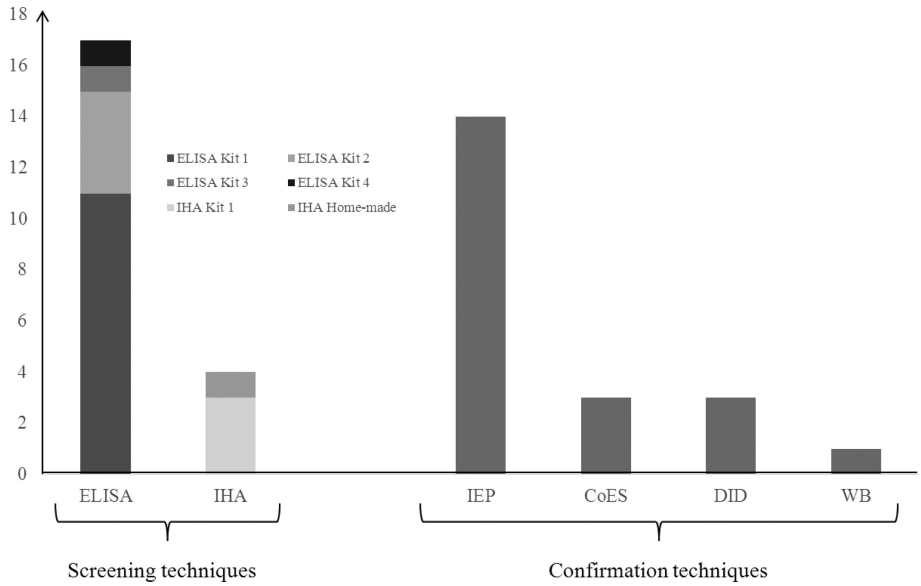

Figure 1. Techniques used for anti-*Aspergillus* antibody detection or confirmation by 20 laboratories. ELISA enzyme-linked immunosorbent assay; IEP immunoelectrophoresis; DID double agar gel immunodiffusion test; ES electrosyneresis; CoES coelectrosyneresis; IHA indirect haemagglutination. ELISA kit: 1 Platelia™ *Aspergillus* IgG ELISA, 2 Serion IgG Serion ELISA classic, 3 Mastazyme *Aspergillus* IgG ELISA, 4 ELISA *Aspergillus fumigatus* IgG Bordier; WB western blot.

Table 3. Expert opinions on anti-*Aspergillus* antibody detection strategies.

Item	Item: Agreement with. . .	Mean	CV* (%)	Median	Number of scores at 1	Number of scores at 9
Use of a screening technique:						
1	Distinguish between screening and confirmation techniques	6.2	44.3	7	3	7
9	Systematic combination of two techniques for screening	6	50.2	7	4	10
Pertinence of manufacturer's inconclusive zone:						
10	For IHA**	4.3	51.1	5	6	1
11	For ELISAs***	5.6	38.7	6	4	2
Strategy regarding inconclusive results of an ELISA or IHA test:						
12	Perform a second screening technique	3. 8	74.9	2.5	10	4
13	Perform a confirmation technique	7.6	31.1	9	2	19
14	Test again using the same technique	4.3	71.7	3.5	10	5
Use of a confirmation technique:						
22	Systematically	3.8	69.6	3	10	3
23	After a positive screening result	8.3	14.3	9	0	22
24	Depending on the clinical context (i.e., cystic fibrosis)	7.3	31.4	8	2	13
25	In cases of the first serum for a new patient (within 6 months)	5.6	51.0	6	4	7
26	During follow up for a previously positive serum sample	6.8	33.1	8	0	9
Strategy when screening and confirmation results are discordant:						
27	Systematically ask for a second serum sample as a control after 15 days	5.4	51.7	5	1	8
28	Directly interpret the results, giving priority to the confirmation results (as a function of the patient's global clinic data)	7.3	28.4	8	1	12

*CV, Coefficient of variation.
**IHA, indirect haemagglutination.
***ELISA, enzyme-linked immunosorbent assay.

variability among the experts (CV = 44.3%). A combination of two tests for screening is considered equally relevant (median = 7, CV = 50.2%). French experts then considered ELISA as the most valuable technique for screening (median = 7; CV = 27.7%) (Figure 2A), IHA being ruled out either for screening (median = 3, CV = 58.5%) or confirmation (median = 1, CV = 78.2%).

According to the experts, a confirmation test should not be systematically performed in parallel with screening, but it is strongly recommended (i) when the screening result is positive (median = 9; CV = 14.3%) (ii) in the case of a negative result that is associated with a compatible clinical presentation or context (median = 8; CV = 31.4%), or (iii) during patient follow-up (median = 8; CV = 33.1%) (Table 3).

For confirmation, IEP and Western blot (WB) are considered the most valuable methods, with a median agreement of 8 for both and CVs at 15 and 28.5%, respectively (Figure 2B). Experts are still relatively confident in the IEP both for diagnosis and patient follow-up (median = 7 for both, with CV at 33.8 and 31.8%, respectively). Historically, a cut-off of three precipitin lines was proposed to consider patients as infected.[18] However, the reliability of this cut-off is now considered weak by French experts (median = 5; CV = 48.7%). This belief may be because the reproducibility of the method is thought overall to be moderate (median = 5.5; CV = 31.6%). In contrast, the detection of enzymatic activity (catalase or chymotrypsin) in precipitin lines, interpreted as a marker of progressive infection and thus important for the final interpretation, is considered valuable (median = 7, CV = 38.4%). Whatever and not surprisingly, we found a high level of agreement in the need to standardize the IEP technique (median = 9, CV = 10.1%). This may be correlated with the emergence of commercial WB kit (LDBIO Diagnostics, Lyon, France) as a potential alternative for the detection of anti-*Aspergillus* antibodies.[12,13]

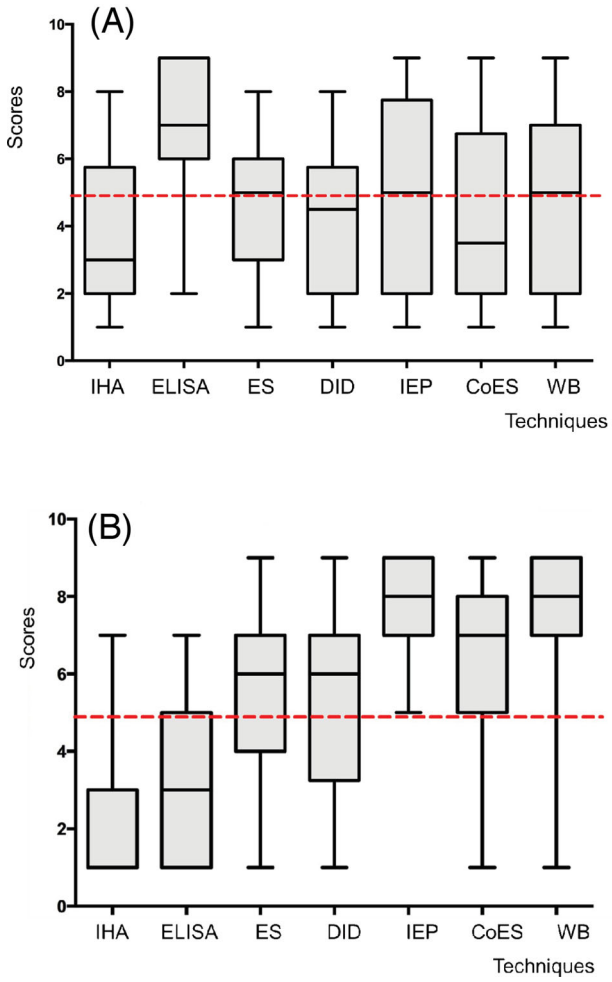

Figure 2. Box plot results on the reliability of expert's opinions (median and dispersion of the answers quoted from 1 to 9) on screening (A) and confirmation (B) tests (see Materials and Methods). ELISA enzyme-linked immunosorbent assay; IEP immunoelectrophoresis; DID double agar gel immunodiffusion test; ES electrosyneresis; CoES coelectrosyneresis; IHA indirect haemagglutination; WB western blot. (The dotted line represents the median value of 5).

This technique was considered reproducible by the experts with a median of 8 and a CV of 26.28% but not yet placed as a reference method (median = 6; CV = 45.5%).

Discussion

Although it is now standard that any patient suspected of having a chronic or a subacute form of pulmonary aspergillosis should be tested for *A. fumigatus* IgG antibody or precipitins, there is no recommendation regarding the technique(s) to use for this purpose even in the most recent reviews.[2,3]

To address this issue, the SFMM organized in 2015 a survey to collect the opinions of a large panel of French experts regarding *Aspergillus* serologic tests and the strategy to use these tests.

The results of our initial survey underline the diversity of strategies and opinions on most of the technical aspects of anti-*Aspergillus* serology. This diversity likely reflects a lack of standardization of in-house techniques, particularly for precipitin detection, and the relative paucity of robust investigations aiming to precisely evaluate the performances of the available commercial techniques.[8–13] This absence itself is due in part to the wide range of clinical forms of noninvasive aspergillosis including colonization, sensitization, *Aspergillus* bronchitis, allergic bronchopulmonary aspergillosis, cavitary, or necrotizing chronic forms. Moreover, there is a large overlap in the clinical and radiological presentations of the clinical entities that are able to convert from one to another.[3,7]

To the best of our knowledge, the distinction between screening and confirmation tests is rarely advised elsewhere as it is in the French practice. Looking at British and French studies comparing precipitin detection and ELISA, the concomitant use of two techniques allows better detection of anti-*Aspergillus* antibodies.[8–12]

Not surprisingly, experts of our panel considered ELISA as the most appropriate and reliable technique for screening. ELISA is supposed to be sufficiently sensitive and useful in high-throughput screens, some of which are fully automated.[9,14–16]

Regarding confirmation techniques, immunoprecipitation (mainly IEP) has been considered as the standard in France for approximately 50 years. Immunoprecipitation techniques were developed in the 1950–1960 s, a time when ELISA tests did not exist,[17–19] and the number of precipitin lines with or without a potential enzymatic activity was globally considered an important criterion for patient follow-up.[19] Since then, antibody detection has been included in different criteria for the diagnosis of chronic forms of aspergillosis.[20–25] Because of this past experience, French experts are still very confident in this method even if they agree for the need of an in-depth standardization. Indeed, these techniques are far more laborious and time-consuming than ELISA tests, and many important variables, including the nature of antigens, the antigen/antibody ratio, and the duration of migration or staining must be taken into account in the analysis of the results, hampering any comparison between centers.

In the United Kingdom, precipitins are mainly detected using coelectrosyneresis with agarose gels or double agar gel immunodiffusion test and are expressed as a titer corresponding to the highest serum dilution for which a precipitation line is still detected.[9] These are also quite laborious techniques and no comparison of performances between all these precipitin-detection methods has been performed until now.

Finally, the favorable opinion of the experts regarding the WB technique is likely related to the recent availability of a marketed kit, already used by some of the questioned laboratories. The main advantages of this test are the ease of performance and the rapid obtaining of results.[12,13] However, its experience of use is still limited, and there is a need for further evaluation.

In conclusion, although some survey items received a clear response enabling clear recommendations, this survey suggests the urgent need for robust studies to standardize a reference method and to harmonize the testing strategies. Such standardization and harmonization are a prerequisite for further multicenter evaluation of the performance and for the interpretation of serological techniques for diagnosis and follow-up of aspergilloses.[26]

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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