Allergo J Int
https://doi.org/10.1007/s40629-024-00287-8

Skin tests, serological IgE detection, basophil test—what is available, useful and helps to clarify a mold allergy?

Monika Raulf · Sabine Kespohl

Accepted: 29 February 2024

Abstract The prevalence of sensitization to molds is low in healthy people, but significant in asthmatics. As it has not yet been possible to establish a cause-and-effect relationship between the presence of mold allergens and the occurrence of allergic symptoms, there is a great deal of uncertainty. The update of the S2k guideline "Medical–clinical diagnostics for indoor mold exposure" should help to objectify the topic. Based on the recommendations listed there for the diagnosis of suspected IgE-mediated mold allergy, this article presents the possibilities of skin tests, IgE determinations, and other in vitro test options, but also their limitations in clarifying the cause. Potential possibilities include component-resolved allergy diagnostics, while the limitations include the difficult standardization of test allergen extracts due to the complex allergen source and the insufficient commercial availability of the test extracts. A diagnostic algorithm is presented as a tool for a systematic approach to patients with suspected mold-associated respiratory allergy.

Keywords Component-resolved diagnostic · In vitro allergy tests · Mold allergens · Mold sensitization · Type I allergy

Abbreviations

ABPA	Allergic bronchopulmonary aspergillosis
BAT	Basophil activation tests
CAST-ELISA	Cellular antigen stimulation test
EAA	Exogenous allergic alveolitis
ECP	Eosinophilic cationic protein
ELISA	Enzyme-linked immunosorbent assay
FEIA	Fluorescence enzyme immunoassay
HP	Hypersensitivity pneumonitis
Ig	Immunoglobulin
IUIS	International Union of Immunological Societies
LTT	Lymphocyte transformation test
MMIS	Mucous membrane irritation syndrome
MVOC	Microbial volatile organic compounds
ODTS	Organic dust toxic syndrome
SBS	Sick building syndrome
WBT	Whole blood test
WHO	World Health Organization

Prof. Dr. M. Raulf (✉) · Dr. S. Kespohl
Department Competence Center Allergology/Immunology, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum, Bürkle-de-la-Camp Place 1, 44789 Bochum, Germany
monika.raulf@dguv.de

Introduction

Molds are ubiquitous and the possibilities of exposure are manifold. To date, more than 100,000 mold species have been described [1, 2]. The most common genera in the air include Cladosporium, Penicillium, Aspergillus, Fusarium and Alternaria. Increased exposure to molds can cause a variety of health effects in humans. The hazard potential of molds for humans is based on the spread of spores, which together with mycelial parts can act as carriers of allergens, and on cell wall components such as β-1,3-glucans, mycotoxins and microbial volatile organic compounds (MVOC) [3]. In addition to infections (e.g. mycoses), irritations (e.g. mucous membrane irritation syndrome [MMIS]), intoxications (e.g. organic dust toxic syndrome [ODTS]) and mood disorders (e.g. sick building syndrome [SBS]), sensitization and allergic diseases can also be triggered [3]. Mold allergies are a global health problem and the incidence of mold sensitization is between 1 and 5% in the general popu-

Published online: 04 April 2024

lation, depending on the study [4, 5] and up to 45% in asthmatics [6, 7]. The figures for sensitization in Germany from a population-based adult survey were 2.3% for *Aspergillus fumigatus* and 1.3% for *Cladosporium herbarum* [4]. Another study by Forkel et al. [8] documented an increase in mold sensitization between 1998 and 2017. This may be due to increased mold growth, which is mainly determined by humidity, nutrient supply and temperature and can pose a health risk. As a rule, higher indoor mold concentrations can also be measured with increased outdoor mold exposure. In asthmatics in particular, indoor mold exposure has a serious impact on respiratory symptoms in the form of an exacerbation or worsening of the disease [9]. However, *Alternaria alternata* (*Alternaria tenuis*), which is classified as an outdoor mold in our latitudes, also appears to be particularly significant for the development and severity of asthma [6]. However, the exact prevalence of sensitization to mold allergens remains unclear due to the high variability between different studies with different study populations and different exposure conditions [10]. In addition, due to the lack of standardization, mold extracts with high variability and varying quality in terms of allergen content were used as test solutions for allergy diagnosis [11–13]. It should also be noted that a comprehensive characterization of the individual allergens is only available for a few mold species to date. Extensive allergen characterization on a molecular basis was carried out in particular for *Alternaria alternata*, the most clinically relevant allergen source among molds in outdoor air, and *Aspergillus fumigatus*, the most clinically relevant allergen source for asthmatics or immunodeficient persons in indoor air. For other mold species, the allergenic potential was rather investigated with regard to homologous structures to already known mold allergens [1] without verifying the possibility of clinical relevance in most cases, so that there is a lack of knowledge regarding the clinical manifestation of mold exposure and sensitization. Therefore, it can be assumed that mold sensitization rates tend to be underestimated [10].

Mold allergens occur ubiquitously throughout the year, but overlap in time and space with mite, pollen and/or animal allergens. In polysensitized individuals, hypersensitivity to molds can be masked by sensitization to other allergen sources, which in turn complicates the diagnosis of a mold allergy [14]. Furthermore, it is difficult to establish cause-and-effect relationships between the presence of mold allergens in the environment and the occurrence of allergic symptoms. In addition to other factors, this continues to contribute to a high level of uncertainty among those affected by indoor mold damage. For this reason, the S2k guideline "Medical clinical diagnostics for indoor mold exposure" update 2023 AWMF register no. 161/001 [15] was updated with the aim of improving the objective handling of the problem and providing physicians with assistance in advising and treating patients who are exposed to increased mold exposure in a typical indoor scenario from a medical perspective. Allergological diagnostics with the steps derived from a medical history, such as skin tests, serology and, if necessary, additional tests, play an important role here. The explanations in the guideline [15] on this topic are presented here and supplemented by information on mold allergens and the diagnostic options for suspected mold sensitization.

Mold allergens

Of the more than 600,000 known species of fungi worldwide [16] around 350 species are listed as potentially sensitizing at www.allergome.org. The WHO/IUIS criteria for classifying an allergen are currently met by 113 fungal allergens from 30 species of fungi (www.allergen.org; 01/2024) [17]. Molds are phylogenetically part of the Ascomycota, but Basidiomycota can also induce IgE-mediated diseases [18]. Of the Ascomycota, 95 individual allergens from eleven fungal genera have currently been characterized according to the WHO/IUIS criteria. These include 38 allergens from the genus Aspergillus, 17 from the genus Penicillium and 13 from the genus Alternaria. Numerous fungal allergens belong to typical protein families, such as the subtilisin-like serine proteases or the 60S acidic ribosomal proteins. These protein families are characteristic of fungi and have not been described as pollen or animal allergens [17].

Test allergen extracts and component-resolved allergy diagnostics

Although molds are of allergological importance, the supply of commercially available mold allergen test extracts required for skin testing or provocation testing is currently very limited. Mold test extracts, like all other test extracts, are medicinal products in accordance with the regulations of EU Directive 2001/83/EC, article 1(4b), and must be authorised. The production of mold extracts is very complex and therefore cost-intensive. This is partly due to the fact that molds are extremely complex allergen sources, as they are whole organisms consisting of mycelium, the interconnected network of fungal hyphae that form the basic structural unit in filamentous fungi and spores [10].

As mycelium and spores can differ considerably in their allergen content, test extracts should consist of both fungal components. Detailed biochemical and immunological analyses showed that the mold allergen extracts that were commercially available and used for skin testing exhibited a very high variability in allergen composition and that preparations of one mold species from different manufacturers were not comparable [11]. As part of a multicenter study, the skin prick test extracts from the various manufacturers were tested on 168 patients with mold expo-

sure and/or suspected mold-induced allergic symptoms under standardized conditions. It was found that the biochemical properties, in particular the antigen content, significantly influenced the test sensitivity. The skin test extracts of the mold Alternaria are an exception, as even the smallest amounts of the main allergen Alt a 1 were sufficient for a positive skin reaction. Skin test extracts with a high antigen content were more sensitive than specific IgE measurements; conversely, skin test extracts with a low antigen content showed lower sensitization rates than specific IgE detection [19]. Skin test extracts are sensitive and essential tools in the diagnostic repertoire for the detection of mold sensitization and should be or remain available and can be supplemented by the detection of specific IgE. A further disappearance of the test extracts from the market would severely limit targeted diagnostics [20].

Component-based diagnostics could be a valuable addition. Although—as described—numerous mold allergens have been identified, only a total of eight mold allergens from the species *Alternaria alternata, Aspergillus fumigatus* and *Cladosporium herbarum* are currently commercially available [17]. With rAlt a 1, which is available as an allergen on various test platforms, up to 98% of IgE-mediated *Alternaria alternata* sensitizations can be detected [1]. The Aspergillus allergens rAsp f 1, 2, 3, 4, 6 are also available, although Aspergillus, like all other mold species, lacks a typical major allergen comparable to Alt a 1. The use of recombinant rAsp f allergens in serological IgE testing can be useful for differentiating between allergic bronchopulmonary aspergillosis (ABPA) and allergic asthma [17, 21]. So far, rCla h 8, a dehydrogenase from *Cladosporium herbarum,* is only available on the ISAC chip. Asp o 21, which is available as fungal α-amylase (k87) from *Aspergillus oryzae* in the Immulite and ImmunoCAP systems, is not a typical mold allergen, but a baking agent enzyme used in bakeries. This enzyme is therefore an occupational allergen and important in the diagnosis of bakers with allergic respiratory symptoms [18]. Improved mold IgE diagnostics using commercially available marker allergens for molds (e.g. subtilisin-like proteases) with strong IgE binding would be desirable [17, 22].

Diagnostic test procedures

In principle, the same recommendations and guidelines apply to the diagnosis of a mold allergy as for other allergen sources that are the causes of an immediate-type allergy [23]. The core elements of type I immediate-type diagnostics also correspond here—taking into account individual factors—to the classic step-by-step scheme: medical history/physical findings/clinical examination—skin test—serum analysis or additional in vitro methods—challenge tests [23, 24]. In the case of ABPA, specific IgG antibodies should also be determined. In the case of exogenous allergic alveolitis (EAA; hypersensitivity pneumonitis [HP]), only specific IgG antibodies should be determined serologically [15].

As with other suspected allergy triggers, it is also important to confirm the allergic reaction and identify the allergy trigger for molds in individual cases. There is a wide variety of in vitro tests that record parameters of the cellular and humoral allergic reaction at different levels. Both a positive skin test result and increased specific IgE concentrations can indicate sensitization to mold allergens, but this is not the same as an allergic disease. Only in connection with typical allergic symptoms that are documented in the medical history and/or a positive organ-specific challenge test does a clinically relevant allergy present itself [23, 24].

In the usual routine, skin tests and specific IgE detection are used most frequently. In skin tests, a distinction is made between epicutaneous (patch test, rub test) and cutaneous tests (scratch, prick, intracutaneous test). If an inhalation type I allergy to mold spores is suspected, a prick test is usually carried out depending on the availability of test extracts. It should be noted that a negative skin test result for molds does not rule out the possibility of sensitization to molds per se. Reasons for this include the different composition and quality of test extracts or the absence of relevant allergens [11, 15, 19].

The serological detection of mold-specific IgE antibodies is not only the most practical in vitro test for determining allergen-specific IgE antibodies (sIgE) but is also becoming increasingly important as the only available diagnostic tool, as hardly any mold skin test solutions are currently available. However, this allergen repertoire is also limited, as relevant indoor molds, such as *Aspergillus versicolor* or *Stachybotrys chartarum,* are no longer available for serological determination. According to the study by Kespohl et al. [16], a combination of prick tests with the still available skin test solutions of *Aspergillus fumigatus, Penicillium chrysogenum* and *Alternaria alternata,* which are often more sensitive—and also more informative—than IgE determinations and the use of the mold mixture mx1 (consisting of *Alternaria alternata, Cladosporium herbarum, Aspergillus fumigatus* and *Penicillium chrysogenum*) for IgE determination has proven to be optimal for mold allergy diagnostics. The use of mx1 as a screening tool to support the detection of mold-associated respiratory symptoms has also been confirmed in another study [22]. However, if mold-specific IgE is detectable, a possible exposure history should be checked (indoor or outdoor) and possible co-sensitizations such as grass pollen or house dust mites, which represent an overlapping allergen exposure, should be investigated ([11, 22]; Fig. 1).

There is a large number of tests from different manufacturers that differ not only in the way they are performed (including the use of different detection meth-

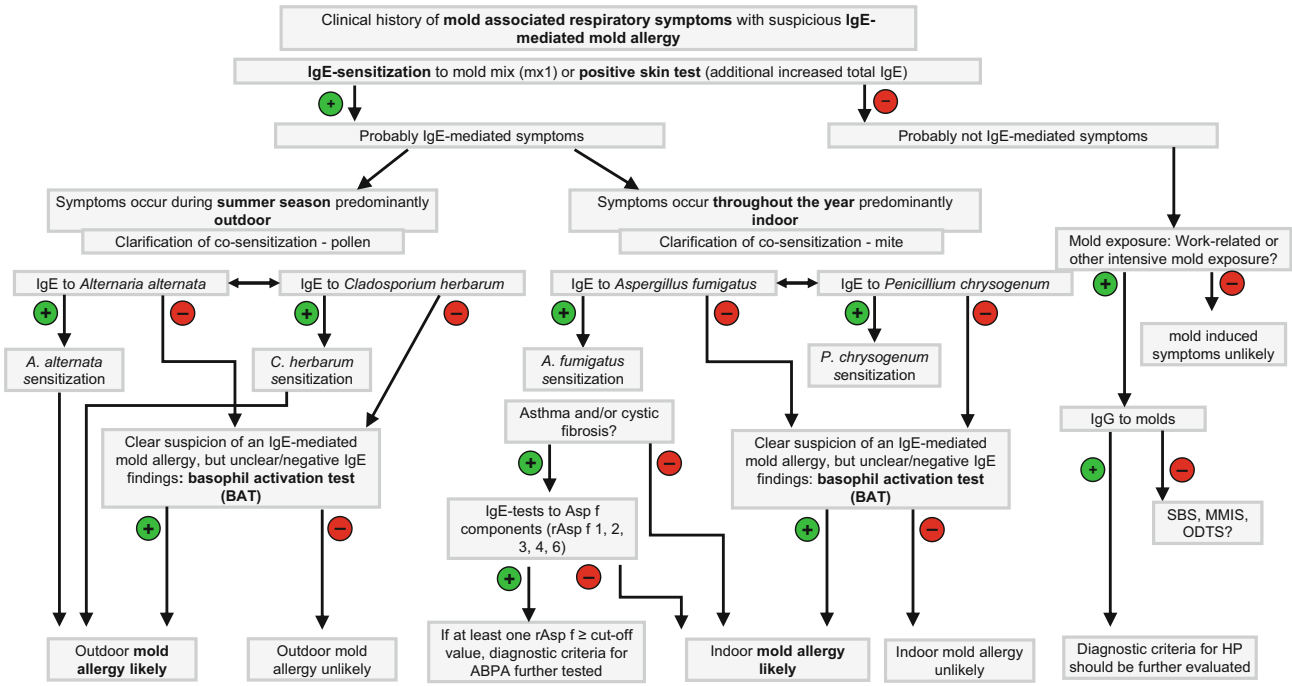

Fig. 1 Diagnostic algorithm for patients with a history of suspected mold-associated respiratory allergy (based on Dramburg et al. [17] from section B07 "Allergy to moulds" by Kespohl & Raulf); mold mixture (mx1): *Aspergillus fumigatus, Penicillium chrysogenum, Cladosporium herbarum, Alternaria alternata*. *SBS* Sick Building Syndrome, *MMIS* Mucos Membrane Irritation Syndrome, *ODTS* Organic Dust Toxic Syndrome (endotoxin, mycotoxins), *HP* Hypersensitivity pneumonitis

ods such as ELISA, FEIA, the use of different allergen carriers such as chemically activated paper disc, microtiter plate, ImmunoCAP, chip technology or the use of liquid allergens), but also due to different allergen raw materials, allergen extract preparations and their standardization [15]. The value of in vitro diagnostics is determined by the diagnostic sensitivity and specificity of the test method, and here too, the validity of allergy diagnostics is heavily dependent on the quality of the allergen extracts used, but also on the method used [19].

As with all other serological allergen specific IgE determinations, the determination of total IgE can be useful as a supplementary parameter for the assessment of sIgE values. However, the determination of total IgE can never rule out or prove specific sensitization and is not useful as the sole determination [25].

The determination of specific IgG antibodies in connection with the diagnosis of an immediate-type mold allergy (type I allergy) has no diagnostic significance. IgG antibodies are a physiological response of the immune system to antigens and are only of pathogenetic significance in special cases, which is why IgG determination is not recommended [25].

Only if allergic bronchopulmonary aspergillosis (type I, type III allergy) or exogenous allergic alveolitis/hypersensitivity pneumonitis (type III, type IV allergy) is suspected does the determination of mold-specific IgG antibodies represent a useful part of the diagnostic procedure and is then also recommended [21, 25].

For the quantitative assessment of specific IgG concentrations (stated in mg_A/l), it must be considered that, in contrast to specific IgE diagnostics, there is no uniform cut-off value, so that a specific reference value or reference range must be determined for each antigen and for each measurement method. Furthermore, there are no fixed cut-off values that clearly indicate pathological changes [26].

Supplementary diagnostic in vitro test procedures

In the case of a clear suspicion of an IgE-mediated mold allergy and unclear previous diagnostic findings, as well as for scientific questions, cellular test systems can be used as a supplement in individual cases. The most important and target-oriented tests are based on basophil granulocytes and are summarized under the term basophil activation tests (BAT). However, it should be noted that the test procedures are methodologically complex, costly and generally unsuitable for sample shipment. Standardized BAT performance is demanding and the evaluation requires appropriate controls. Therefore, this cellular ex vivo diagnostic method with basophil granulocytes is not suitable for routine diagnostics and belongs to specialized allergy diagnostics [15]. Cellular test systems based on basophil granulocytes use various parameters (*read-*

outs) for test evaluation, but are based on allergen-specific in vitro stimulation of the basophil granulocytes. The considerable excess of bound IgE on basophils and its high affinity for the Fc_{ε} RI receptor result in a high analytical sensitivity of these test systems, which can exceed both specific serological IgE methods and skin tests [15]. Compared to other diagnostic in vitro tests, the BAT appears to have better predictive power in terms of clinical relevance. Various basophil markers and parameters have been established that can provide information on the clinical relevance of sensitization, on the development of natural tolerance, on trigger thresholds and on the severity of the allergic reaction, depending on the trigger of the respective allergy [27, 28]. The detection of histamine release (basophil degranulation test and histamine release), sulfidoleukotriene release (cellular antigen stimulation test; CAST ELISA) or the expression of surface molecules (e.g. CD203c, CD63; Flow-CAST or Flow2CAST with CCR3 as an additional selection marker) after in vitro stimulation with different allergen concentrations are possible *readouts* and represent an indirect measure of the cell-bound specific IgE. Compliance with the required pre-analytical conditions is a prerequisite for valid results in all cellular tests [29]. It should be noted that only a few mold test allergens (*Penicillium chrysogenum, Cladosporium herbarum, Aspergillus fumigatus, Alternaria alternata*) are commercially available for these test options and, as with the routine test procedures, the quality of the extracts is a decisive factor for the validity of the method.

Test methods that should not be used with suspected mold allergy

As already mentioned, the determination of specific IgG antibodies and the analysis of immune complexes in connection with the diagnosis of mold allergy of the immediate type (type I allergy) has no diagnostic significance. Another cellular test that is used in particular in immune function diagnostics and for scientific questions is the lymphocyte transformation test or proliferation test (LTT), which is used to detect antigen-specific T lymphocytes, e.g. in type IV reactions. As mold allergens do not lead to type IV sensitization, an LTT for molds is not indicated as a diagnostic procedure [15]. The whole blood test (WBT), which is also used to determine immune reactivity due to mold exposure in research questions [30], is not suitable as an instrument to verify the suspicion of a mold allergy. Furthermore, there is no indication for the serological determination of cytokines or the activation marker for eosinophil granulocytes, the eosinophil cationic protein (ECP), in cases of suspected mold allergy. The detection of galactomannan, a heteropolysaccharide and cell-wall component of the mold genus Aspergillus, in serum also makes no sense for the detection of a mold allergy, but can be used to clarify invasive pulmonary aspergillosis. The same applies to the serological determination of β-1,3-D glucan, a method that is useful in the diagnosis of invasive mycoses, but is not indicated in the diagnosis of an allergy caused by molds.

Conclusion

Compared to other ubiquitous environmental allergens, the sensitizing potential is considered to be lower. If IgE-mediated sensitization due to mold exposure with respiratory symptoms is suspected, a skin prick test or serological IgE determination should be performed first. Studies have shown that a combination of prick tests with the still available skin test solutions of *Aspergillus fumigatus, Penicillium chrysogenum and Alternaria alternata,* which are often more sensitive—and also more informative—than IgE determinations, supplemented by the use of the mold mixture mx1 for IgE determination (consisting of *Alternaria alternata, Cladosporium herbarum, Aspergillus fumigatus* and *Pencillium chrysogenum*) is useful for mold allergy diagnostics. However, negative test results do not rule out IgE-mediated sensitization, as only a very limited number of test allergens are available, and their quality is not optimal in some cases. The determination of specific IgG antibodies in connection with the diagnosis of mold allergy of the immediate type (type I allergy) has no diagnostic significance. Although the basophil activation test can be a useful in special cases as a supplementary test for mold diagnostics due to its high analytical sensitivity, it is not a basic test for allergies and should only be performed in centers with appropriate expertise. The lymphocyte transformation test and the whole blood test for molds are just as unsuitable diagnostic tools for the detection of mold sensitization as the serological determination of cytokines or activation markers for eosinophil granulocytes. The diagnostic algorithm proposed in this article (Fig. 1) provides guidance for further specification in patients with a history of suspected mold-associated respiratory allergy.

Funding Open Access funding enabled and organized by Projekt DEAL.

Conflict of interest M. Raulf received fees for speaking engagements from the following companies and associations between 2020 and 2023 Alk-Abelló Arzneimittel GmbH, Berufsverband Deutscher Baubiologen VDB e.V., Haus der Technik, LetiPharma, ThermoFisher Scientific (Phadia). M. Raulf and S. Kespohl declare that they have no competing interests.

References

1. Simon-Nobbe B, Denk U, Pöll V, Rid R, Breitenbach M. The spectrum of fungal allergy. Int Arch Allergy Immunol. 2008;145:58–86.
2. McLaughlin DJ, Hibbett DS, Lutzoni F, Spatafora JW, Vilgalys R. The search for the fungal tree of life. Trends Microbiol. 2009;17:488–97.
3. Raulf-Heimsoth M, Gabrio T, Lorenz W, Radon K. Vorkommen und gesundheitliche sowie allergologische Relevanz von Schimmelpilzen aus der Sicht der Umwelt- und Arbeitsmedizin, der Innenraumhygiene und der Epidemiologie. Allergo J. 2010; 464–76.
4. Haftenberger M, Laußmann D, Ellert U, Kalcklösch M, Langen U, Schlaud M, et al. Prävalenz von Sensibilisierungen gegen Inhalations- und Nahrungsmittelallergene : Ergebnisse der Studie zur Gesundheit Erwachsener in Deutschland (DEGS1). [Prevalence of sensitisation to aeraoallergens and food allergens: results of the German Health Interview and Examination Survey for Adults (DEGS1). Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2013;56:687–97.
5. Szewzyk R, Becker K, Hünken A, Pick-Fuß H, Kinder-Umwelt-Survey K-GM. 06 Sensibilisierung gegenüber Innenraum Schimmelpilzen. Schriftreihe Umwelt & Gesundheit im Auftrag des. KUS, Vol. 2003. Umweltbundesamt; 2011.
6. O'Driscoll BR, Powell G, Chew F, Niven RM, Miles JF, Vyas A, et al. Comparison of skin prick tests with specific serum immunoglobulin E in the diagnosis of fungal sensitization in patients with severe asthma. Clin Exp Allergy. 2009;39:1677–83.
7. Toppila-Salmi S, Huhtala H, Karjalainen J, Renkonen R, Mäkelä MJ, Wang DY, et al. Sensitization pattern affects the asthma risk in Finnish adult population. Allergy. 2015;70:1112–20.
8. Forkel S, Beutner C, Schröder SS, Bader O, Gupta S, Fuchs T, et al. Sensitization against Fungi in Patients with Airway Allergies over 20 Years in Germany. Int Arch Allergy Immunol. 2021;182:515–23.
9. Valkonen M, Täubel M, Pekkanen J, Tischer C, Rintala H, Zock J-P, et al. Microbial characteristics in homes of asthmatic and non-asthmatic adults in the ECRHS cohort. Indoor Air. 2018;28:16–27.
10. Pfeiffer S, Swoboda I. Problems Encountered Using Fungal Extracts as Test Solutions for Fungal Allergy Diagnosis. JoF. 2023;9:957.
11. Kespohl S, Maryska S, Zahradnik E, Sander I, Bruening T, Raulf-Heimsoth M. Biochemical and immunological analysis of mould skin prick test solution: current status of standardization. Clin Exp Allergy. 2013;43:1286–96.
12. Fukutomi Y, Taniguchi M. Sensitization to fungal allergens: resolved and unresolved issues. Allergol Int. 2015;64:321–31.
13. Crameri R, Garbani M, Rhyner C, Fungi HC. the neglected allergenic sources. Allergy. 2014;69:176–85.
14. Abel-Fernández E, Fernández-Caldas E. Allergy to fungi: Advances in the understanding of fungal allergens. Mol Immunol. 2023;163:216–23.
15. Hurraß J, Heinzow B, Walser-Reichenbach S et al. Guideline "Medical clinical diagnostics of indoor mold exposure"—Update 2023—AWMF Registry No. 161/001. Allergol Select 2024: in press
16. Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B. How many species are there on Earth and in the ocean? PLoS Biol. 2011;9:e1001127.
17. Dramburg S, Hilger C, Santos AF, Las Vecillas L de, Aalberse RC, Acevedo N et al. EAACI Molecular Allergology User's Guide 2.0. Pediatr Allergy Immunol 2023;34 Suppl 28:e13854
18. Kespohl S, Raulf M. Mold allergens: Where do we stand with molecular allergy diagnostics?: Part 13 of the series Molecular Allergologyfor use in clinical routine: Part 18 of the Series Molecular Allergology. Allergo J Int. 2014;23:120–5.
19. Kespohl S, Maryska S, Bünger J, Hagemeyer O, Jakob T, Joest M, et al. How to diagnose mould allergy? Comparison of skin prick tests with specific IgE results. Clin Exp Allergy. 2016;46:981–91.
20. Klimek L, Hoffmann HJ, Kalpaklioglu AF, Demoly P, Agache I, Popov TA, et al. In-vivo diagnostic test allergens in Europe: a call to action and proposal for recovery plan—an EAACI position paper. Allergy. 2020;75:2161–9.
21. Muthu V, Sehgal IS, Dhooria S, Aggarwal AN, Agarwal R. Utility of recombinant Aspergillus fumigatus antigens in the diagnosis of allergic bronchopulmonary aspergillosis: a systematic review and diagnostic test accuracy meta-analysis. Clin Exp Allergy. 2018;48:1107–36.
22. Kespohl S, Liebers V, Maryska S, Meurer U, Litzenberger C, Merget R, et al. What should be tested in patients with suspected mold exposure? Usefulness of serological markers for the diagnosis. Allergol Sel. 2022;6:118–32.
23. Raulf M. Diagnostik der Allergien vom Soforttyp—„State of the Art". Akt Dermatol. 2009;35:385–92.
24. Steiß J-O, Lindemann H, Brosig B, Zimmer KP. Wichtige Aspekte bei der Betreuung chronisch kranker Kinder und Jugendlicher am Beispiel des Asthma bronchiale. [Important aspects in pediatric care of children and adolescents with chronic disease using the example of bronchial asthma. Dtsch Med Wochenschr. 2013;138:2613–8.
25. Renz H, Biedermann T, Bufe A, Eberlein B, Jappe U, Ollert M et al. In-vitro-Allergiediagnostik. Leitlinie der Deutschen Gesellschaft für Allergologie und klinische Immunologie. (DGAKI) unter Beteiligung des Ärzteverbandes Deutscher Allergologen (ÄDA), der Gesellschaft für Pädiatrische Allergologie und Umweltmedizin (GPA) und der Deutschen Dermatologischen Gesellschaft (DDG). Allergo J. 2010;19:110–28.
26. Raulf M, Joest M, Sander I, Hoffmeyer F, Nowak D, Ochmann U, et al. Update of reference values for IgG antibodies against typical antigens of hypersensitivity pneumonitis. Allergo J Int. 2019;28:192–203.
27. Kleine-Tebbe J, Erdmann S, Knol EF, MacGlashan DW, Poulsen LK, Gibbs BF. Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. Int Arch Allergy Immunol. 2006;141:79–90.
28. Eberlein B. Basophil Activation as Marker of Clinically Relevant Allergy and Therapy Outcome. Front Immunol. 2020;11:1815.
29. Pascal M, Edelman SM, Nopp A, Möbs C, Geilenkeuser WJ, Knol EF, et al. EAACI task force report: A consensus protocol for the basophil activation test for collaboration and external quality assurance. Allergy. 2023; https://doi.org/10.1111/all.15907.

30. Liebers V, Kespohl S, Borowitzki G, Stubel H, Raulf M. Is in vitro cytokine release a suitable marker to improve the diagnosis of suspected mold-related respiratory symptoms? A proof-of-concept study. Allergol Sel. 2022;6:133–41.