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How to diagnose mould allergy? Comparison of skin prick tests with specific IgE results

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Summary

Background Diagnosis of mould allergy is complicated due to the heterogeneity of the test material and the decrease in the number of commercial mould skin test solutions that are currently available.

Objectives The aim of this study was to compare skin prick tests (SPT) from different manufacturers to one another and concurrently with sIgE tests for *Aspergillus fumigatus* (Asp f), *Cladosporium herbarum* (Cla h), *Penicillium chrysogenum* (Pen ch), *Alternaria alternata* (Alt a) and *Aspergillus versicolor* (Asp v) to ascertain a feasible diagnostic procedure for mould sensitization.

Methods In this multi-centre study, 168 patients with mould exposure and/or mould-induced respiratory symptoms were included. Mould SPT solutions were analysed biochemically and tested in duplicate on patients' arms. Specific IgE (sIgE) concentrations to corresponding mould species and mould mix (mx1) were measured by ImmunoCAP. SPTs in accordance with one another and with sIgE were further considered. The test efficiency was calculated using receiver-operating characteristic (ROC) analysis.

Results Mould sensitization was more frequently detected by the SPT (90 of 168) than by the sIgE tests (56 of 168). Concordances of double SPT positives were only sufficient ($\geq$ 80%) for environmental allergens, two Asp f and three Alt a SPT solutions, whereas all other mould solutions revealed concordances $<$ 80%. The antigen content of SPT solutions was positively associated with concordant SPT double values as well as with sIgE. Taking sIgE as the 'positive standard', all mould SPT solutions revealed test efficiencies $>$ 80%, but varied up to 20% in sensitivity and positive predictive value with the exception of Alt a.

Conclusions SPT solutions are sensitive and essential diagnostic tools for the detection of mould sensitization. Our recommendation for diagnosis would be to test at least Alt a, Asp f and Pen ch using SPT and additional sIgE test to mx1.

Keywords *in vitro* diagnosis, mould allergy, multi-centre study, predictive values, sensitivity, skin prick test, specific IgE, specificity

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Introduction

The diagnosis of mould-induced IgE sensitization is often difficult due to the heterogeneity of the test material and missing standardization. Another reason is the drastic reduction in the number of commercially available skin test solutions, especially for allergens with minor therapeutic applications which have also been recognized for several years. Nevertheless, skin tests remain the most often clinically applied techniques used to assess allergic sensitization. Guidelines from different allergy societies, for example, EAACI [1, 2], IAAAI [3] and DGAKI [4] currently exist to ensure the proper conduction of skin prick tests (SPT). The accuracy of these tests depends on a broad range of factors, including allergen potency, allergen content and stability of the allergen test solution, individual skills of the tester (e.g. depth of puncture), status of skin reactivity, application devices [5, 6], patients' medication and others [7, 8]. In our previous study [9], a biochemical analysis of mould SPT solutions revealed both quantitative and qualitative differences depending not only on the mould species, but more specifically on the manufacturer. The questions arising from that analysis were the following: do these differences in extract composition also reveal differences in SPT results, and how good are the correlations/concordances between SPT from different manufacturers and the sIgE test? In 2009, O'Driscoll et al. [7] compared SPT results for different mould species from one manufacturer with the sIgE test (ImmunoCAP). As concordance between the SPT and sIgE for all tested mould species was below 60%, the authors concluded that the diagnosis of mould sensitization should include both SPT and sIgE tests. A similar recommendation was already made 6 years earlier by Smits et al. [8] using the same SPT solutions and sIgE test system. In this study, SPTs with mould allergen solutions from four manufacturers were applied in a multi-centre study that included 168 patients. The obtained results were then compared among the different allergen solutions and with sIgE tests. Each test solution was pricked twice to evaluate the necessity of performing the SPT in duplicate for the diagnosis of mould sensitization.

In addition to commercially available SPT diagnostics for the mould species *Aspergillus fumigatus* (Asp f), *Penicillium chrysogenum* (Pen ch), *Cladosporium herbarum* (Cla h) and *Alternaria alternata* (Alt a), a solution of *Aspergillus versicolor* (Asp v) was also added to the test panel used in our study. *Aspergillus versicolor* is not available in commercial mould allergen panels, probably resulting in a diagnostic gap concerning indoor dampness associated with mould sensitization [10–12]. As it is known that humidity supports the growth of both mould and mites, we also included a SPT to house dust mite (HDM) in our panel. Furthermore, the frequencies of mono- and poly-sensitizations to mould species were investigated, in addition to whether one specific mould species could be used as a marker for mould sensitization. Finally, all SPT results were evaluated for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with sIgE as the 'positive standard' for evaluation.

Material and methods

Study design and subjects

The study was designed as a multi-centre study that included 13 allergy practices and clinics (12 German and 1 Polish) recruiting patients with suspected mould allergy and/or mould exposure. The inclusion criteria were anamnestic or self-reported suspicion and/or diagnosis of mould allergy or mould exposure and/or mould-induced allergic symptoms. Mould-induced symptoms could have occurred occupationally, privately or both. The study consisted of a questionnaire, together with the SPT and sIgE measurement. Mould exposure was documented by questionnaire asking for visual mould formation (bigger/smaller DIN A4) in living areas, at workplaces or during recreation. Altogether, 168 participants were recruited, all of whom signed a consent form before examination. The study was approved by the ethics committee of the Ruhr University Bochum (register no. 4104-11) and was conducted in accordance with the Helsinki Declaration.

Skin prick test (SPT)

Mould SPT solutions of *Aspergillus fumigatus* (Asp f), *Penicillium chrysogenum* (Pen ch), *Alternaria alternata* (Alt a), and *Cladosporium herbarum* (Cla h) were purchased from four different manufacturers: Allergopharma (Reinbek, Germany), ALK-Abelló (Wedel, Germany), HAL (Düsseldorf, Germany) and Lofarma (Willich, Germany). Environmental allergens (grass pollen mix, tree pollen mix II and house dust mite (HDM, *Dermatophagoides pteronyssinus*)) as well as control solutions were used only from one supplier. In Table 1, an overview of analysed SPT solutions is given, displaying a 'key code' for all tables and figures. Extracts for *A. versicolor* (Asp v) were prepared as in-house solutions using allergen material from two manufacturers (Allergon, Ängelholm, Sweden, and Greer Laboratories, Lenoir, NC, USA) as described earlier [9]. Mould SPT solutions were analysed for protein and antigen amount as described previously [9]. The major allergen of *Alternaria alternata* was quantified with an ELISA kit based on mAb to Alt a 1 (Indoor Biotechnologies, Charlottesville, VA, USA). In all other mould SPT

solutions, not allergen but antigen amounts were measured with noncommercial sandwich ELISA based on pAbs (rabbit), recognizing proteins from mould spores/mycel crude extracts [13]. Most likely human IgE-binding proteins are part of these antigens, but also non-allergenic components might be detected. However, quantification of mould antigen is closer to the allergen amount than protein estimation because stabilizing proteins like human serum albumin are not detected.

At each medical centre, all SPT solutions were pricked in duplicate, one on the right and one on the left volar forearm in opposite directions (wrist/elbow) as shown in Fig. 1 according to the current European position paper [1]. Briefly, SPT was performed on untreated skin with a new steel lancet (ALK-Abelló, Madrid, Spain) for each test solution. Test results were taken after 15 min, after which the skin was wiped off with ethanol and wheal sizes were exactly retraced using a ballpoint pen. Subsequently, sticky tape was laid over the forearm to transfer the marks. All measurements of wheal sizes were taken at IPA (Bochum, Germany), by one person (S.K.) as described by van Kampen et al. [14] and calculated as the largest longitudinal diameter plus maximal transverse diameter divided by two. For the evaluation of SPT solutions, the mean of both SPT determinations was used. In case of a reaction to the negative control (NaCl), the mean NaCl-induced wheal size was subtracted from all allergen-induced wheal reactions. SPT testing was performed between January 2012 and February 2014.

Specific IgE (sIgE)

Measurements of total IgE and sIgE to the following single mould species: Pen ch (m1), Cla h (m2), Asp f (m3), Alt a (m6), Asp v (Gm25), to mould mixture mx1 (including m1, m2, m3 and m6) as well as to the HDM allergen d1 (*Dermatophagoides pteronyssinus*), were taken by ImmunoCAP 250 (Thermo Fisher Scientific, Uppsala, Sweden). Additionally, atopy status was determined with the inhalation allergy screening tool, sx1

Table 1. Manufacturer and abbreviations of SPT solutions

	Manufacturer					
Allergen	Allergopharma	ALK-Abelló	HAL	Lofarma	IPA-Allergon	IPA-Greer
Negative control (NaCl)		SPT_1				
Positive control (histamine)		SPT_2				
Aspergillus fumigatus	SPT_3	SPT_4	SPT_5	SPT_6	–	–
Penicillium chrysogenum	SPT_7	SPT_8	SPT_9	SPT_10	–	–
Cladosporium herbarum	SPT_11	SPT_12	–	SPT_13	–	–
Alternaria alternata	SPT_14	SPT_15	SPT_16	SPT_17	–	–
Aspergillus versicolor	–	–	–	–	SPT_18	SPT_19
Gras pollen mix	SPT_20	–	–	–	–	–
Tree pollen mix II	SPT_21	–	–	–	–	–
Dermatophogoides pteronyssinus	SPT_22	–	–	–	–	–

Elbow right arm		Elbow left arm	
Thumb side			Thumb side
SPT_1	SPT_12	SPT_11	SPT_22
SPT_2	SPT_13	SPT_10	SPT_21
SPT_3	SPT_14	SPT_9	SPT_20
SPT_4	SPT_15	SPT_8	SPT_19
SPT_5	SPT_16	SPT_7	SPT_18
SPT_6	SPT_17	SPT_6	SPT_17
SPT_7	SPT_18	SPT_5	SPT_16
SPT_8	SPT_19	SPT_4	SPT_15
SPT_9	SPT_20	SPT_3	SPT_14
SPT_10	SPT_21	SPT_2	SPT_13
SPT_11	SPT_22	SPT_1	SPT_12
Wrist right arm		Wrist left arm	

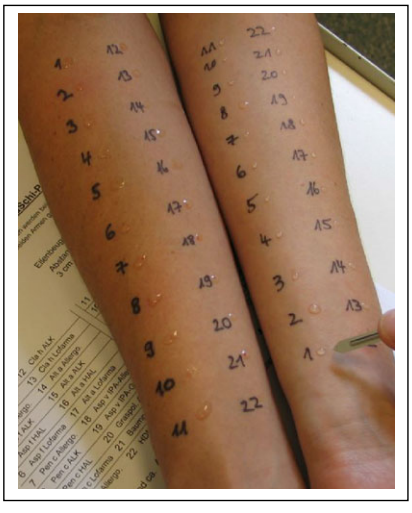

Fig. 1. SPT schema for clinical practice. SPT solutions were numbered according to Table 1.

(including *Dermatophagoides pteronyssinus*, cat and dog dander, timothy grass pollen, rye grass pollen, *Cladosporium herbarum*, birch pollen, mugwort pollen). Specific IgE values ≥ 0.35 kU/L and total IgE values ≥ 150 kU/L were considered positive.

Statistical analysis

To assess the diagnostic agreement of the SPT double test on the right and left arms, the differences of right and left wheel sizes were calculated by Bland–Altman; *P*-values < 0.05 were considered significant.

For the evaluation of the SPT solutions, the results from the sIgE determinations were taken as the 'positive standard' because in most cases specific challenge tests could not be performed due to missing test solutions for some mould species. The Youden index (sensitivity + specificity-1) is generally used to assess the quality of diagnostic tests. For each SPT, the Youden index was calculated for different cut-points (mean value of left and right arm > 0 mm, ≥ 1.5 mm, ≥ 3 mm). True positives (tp) were the subjects with sIgE ≥ 0.35 kU/L to mould and corresponding positive SPT values, whereas true negatives (tn) were subjects with sIgE < 0.35 kU/L to mould and corresponding negative SPT values. False positives (fp) were subjects with sIgE to mould < 0.35 kU/L and positive SPT values, and false-negative (fn) subjects had sIgE ≥ 0.35 kU/L to mould and corresponding negative SPT. Sensitivities [tp/(tp + fn)], specificities [tn/(tn + fp)], positive predictive values (PPV) [tp/(tp + fp)] and negative predictive values (NPV) [tn/(tn + fn)] as well as test efficiencies [(tp + tn)/(tp + fp + tn + fn)] were calculated.

To compare the SPT solutions from different manufacturers, receiver-operating characteristic (ROC) plots were generated by plotting sensitivity vs. 1-specificity over all measured wheal sizes with sIgE values ≥ 0.35 kU/L as the 'positive standard'. The area under curve (AUC) was calculated using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA) and was used as the dimension for test quality. Calculated p values of ROC were tested against the null hypothesis that the area under the curve really equalled 0.50.

Results

Study group

Of 168 participants, 166 reported respiratory symptoms probably by mould. However, the cause for allergic symptoms could not be explicitly due to a single mould species or exclusively to moulds. The median age was 44 years (range: 10–78 years), and about half of the participants (51%) were male. Serological data are summarized in Table 2, showing that most of the subjects were atopic (47% by mean of total IgE ≥ 150 kU/L, 62% by mean of sx1 ≥ 0.35 kU/L). The prevalence of sIgE to any mould (single mould species and/or mould mixture) was 33% (56 of 168). The mould mixture (mx1) covered 55 of 56 serological sensitizations against the included single mould species with only one exception: a monosensitized patient with CAP class 1 to Pen ch was not positive to mx1. About 85% of the patients with a mx1 IgE test ≥ 0.35 kU/L were positive to sx1 as well and 67% of these had a total IgE concentration ≥ 150 kU/L. The highest sensitization prevalence to a single mould species was detected for Alt a with 27%.

SPT- single- vs. double-positive results

Skin prick test quality was analysed with regard to antigen amount and skin reactivity by means of investigating the concordance between single- and double-positive SPTs as shown in Table 3. The measured antigen amount was heterogeneous in SPT solutions depending on mould species as well as the manufacturer and ranged from below 1 μg/mL up to 1287 μg/ml. SPT solutions with the highest antigen amount within one mould species showed usually the highest degree of concordance. Skin prick test reactions were evaluated using histamine as the positive control, which exhibited 100% concordance, but only 166 of 168 patients had a reaction to histamine on both arms with wheals ≥ 3 mm. Comparable results of double-positive SPTs with ≥ 90% concordances were calculated in the current study for grass pollen, tree pollen and HDM, which are all known to be well-standardized allergen solutions. In contrast, double testing with 12 of the 17 (71%) mould SPT solutions resulted in a concordance

Table 2. Serological data of study participants (n = 168)

Allergen/Total IgE (ImmunoCAP)	Median [kU/L]	Range [kU/L]	sIgE sensitization prevalence* n (%)
Total IgE	135	5.06 to > 5000	79 (47)
Environmental aeroallergens (sx1)	1.49	0.05 to > 100	104 (62)
HDM (d1)	0.12	0.01 to > 100	66 (39)
Mould mix (mx1)	0.09	0.03 to 43.6	55 (33)
Asp f (m3)	0.06	0.01 to 67.7	22 (13)
Pen ch (m1)	0.05	0.00 to 18.6	23 (14)
Cla h (m2)	0.03	0.00 to 8.5	14 (8)
Alt a (m6)	0.04	0.00 to 39.3	46 (27)
Asp v (Gm25)	0.00	0.00 to 1.5	4 (2)
Any mould (mx1, m3, m1, m2, m6, Gm25)	0.05	0.00 to 67.7	56 (33)

*Total IgE conc. ≥ 150 kU/L and sIgE conc. ≥ 0.35 kU/L were considered as positive; mould mix (mx1) contains allergens from Asp f, Pen ch, Cla h and Alt a, HDM (house dust mite, *Dermatophagoides pteronyssinus*).

Table 3. Summary of SPT values from 168 Patients

		Antigen amount [μg/ml]	Single right (≥ 3 mm); left = 0 mm (*n* =)	Single left (≥ 3 mm); right = 0 mm (*n* =)	Double (right and left ≥ 3 mm) (*n* =)	Concordance double vs. double- plus single-positive SPTs (%)
Histamine	SPT_2	–	0	0	166	99
Aspergillus fumigatus	SPT_3	250	7	9	18	53
	SPT_4	**345**	2	2	23	85
	SPT_5	< 1	1	4	10	67
	SPT_6	82	1	1	15	**88**
Penicillium chrysogenum	SPT_7	**1287**	4	5	26	**74**
	SPT_8	217	2	9	12	52
	SPT_9	51	7	4	8	42
	SPT_10	190	4	8	15	56
Cladosporium herbarum	SPT_11	8	4	4	12	60
	SPT_12	**20**	3	3	12	**66**
	SPT_13	13	5	2	8	53
Alternaria alternata	SPT_14	**124**	6	1	40	**85**
	SPT_15	79	7	3	39	80
	SPT_16	8	5	2	38	84
	SPT_17	2	7	2	35	76
Aspergillus versicolor	SPT_18	**167**	5	2	10	59
	SPT_19	24	4	4	14	**64**
Grass	SPT_20	–	2	2	70	95
Tree	SPT_21	–	0	3	53	95
HDM	SPT_22	–	1	6	63	90

Bold: Highest antigen amount within one mould species and highest rate of double concordant SPTs; numbers of SPT solutions were according to Table 1.

below 80%. With only five SPT solutions, double-armed-positive results were consistent compared to single-armed-positive results (concordance ≥ 80%).

Dependence of SPT results on arm position (elbow vs. wrist)

To investigate whether the SPT results were different depending on the left/right arm or prick position closer to the elbow/wrist, Bland–Altmann analysis was performed (data not shown). From 22 SPT solutions (Fig. 1) used for double tests in the opposite direction on both arms, only four solutions elicited significant differences ($P < 0.05$). These were histamine (SPT_2) and Alt a (SPT_14) producing bigger wheals on the right arm, in contrast to Pen ch (SPT_8) and HDM (SPT_22) that induced bigger wheals on the left arm, indicating no left/right arm preference per se. Assigning values to prick position (Fig. 1) indicated that wheal sizes located closer to the elbow were slightly bigger (between 0.3 and 0.5 mm) compared with those at the wrist in all four cases.

SPT cut-point – Youden index

To evaluate the optimal cut-point for the SPT results, sIgE measurements were taken as the 'positive standard' and the Youden index was calculated for mean wheal sizes from the right and left arms > 0 mm, ≥ 1.5 mm and ≥ 3 mm as shown in Table 4.

The Youden index was the highest at cut-point > 0 mm for eight mould SPT solutions; at cut-point ≥ 1.5 mm for five mould solutions and at cut-point ≥ 3 mm for five solutions. The differences in the Youden index taking cut-point > 0 mm or ≥ 1.5 mm were below 10%, whereas the difference between cut-point ≥ 1.5 mm and ≥ 3 mm was up to 26%. We decided to use the cut-point ≥ 1.5 mm as the optimal cut-point for all mould SPTs. An advantage of a mean value ≥ 1.5 mm as the cut-point was that the single-armed SPT wheals of ≥ 3 mm were calculated as positive; for example, a wheal size of 0 mm on the left arm and a wheal size of 3 mm on the right arm resulted in a mean wheal size of 1.5 mm.

Sensitization prevalence calculated by SPT and/or sIgE

From 168 tested patients, 90 (54%) exhibited sensitization against at least one mould allergen, either by sIgE or with the SPT. Serological sensitization against any of the five moulds and/or mould mix (mx1) was observed in 56 patients. On the other side, 90 patients had skin reactions to at least one of the tested moulds with MW ≥ 1.5 mm (right/left arm double testing). The SPT was about 30% more sensitive compared with the sIgE test regarding any mould sensitization.

Table 4. Rate of sensitization and Youden index for mould and HDM SPT results at different cut-points

		Rate of sensitization at cut-point among n = 168 patients				Youden Index at cut-point		
	SPT	Mean wheal size > 0 mm n (%)	Mean wheal size ≥ 1.5 mm n = (%)	Mean wheal size ≥ 3 mm n (%)	Maximum wheal size [mm]	> 0 mm	≥ 1.5 mm	≥ 3 mm
Aspergillus fumigatus	SPT_3	42 (25.0)	37 (22.0)	24 (14.3)	11.3	0.601	**0.636**	0.620
	SPT_4	32 (19.0)	30 (17.9)	27 (16.1)	12.0	0.722	0.736	**0.757**
	SPT_5	22 (13.1)	21 (12.5)	13 (7.7)	10.5	0.529	**0.536**	0.434
	SPT_6	22 (13.1)	20 (11.9)	17 (10.1)	13.3	0.582	0.595	**0.616**
Penicillium chrysogenum	SPT_7	40 (23.8)	36 (21.4)	28 (16.7)	8.8	**0.530**	0.507	0.411
	SPT_8	32 (19.0)	26 (15.5)	17 (10.1)	8.3	**0.636**	0.576	0.487
	SPT_9	25 (14.9)	22 (13.1)	13 (7.7)	7.5	0.382	**0.402**	0.313
	SPT_10	30 (17.9)	28 (16.7)	19 (11.3)	12.3	**0.498**	0.462	0.373
Cladosporium herbarum	SPT_11	27 (16.1)	24 (14.3)	18 (10.7)	11.0	0.526	**0.545**	0.429
	SPT_12	22 (13.1)	20 (11.9)	15 (8.9)	8.8	**0.714**	0.649	0.604
	SPT_13	21 (12.5)	16 (9.5)	10 (6.0)	8.8	**0.487**	0.442	0.325
Alternaria alternata	SPT_14	53 (31.6)	49 (29.2)	46 (27.4)	16.5	**0.763**	0.736	**0.761**
	SPT_15	53 (31.6)	52 (31.0)	46 (27.4)	13.8	0.733	0.711	0.761
	SPT_16	50 (29.8)	47 (28.0)	42 (25.0)	13.8	**0.758**	0.722	0.733
	SPT_17	51 (30.4)	46 (27.4)	40 (23.8)	8.5	**0.749**	0.731	0.720
Aspergillus versicolor	SPT_18	20 (11.9)	18 (10.7)	12 (7.1)	11.3	0.646	0.659	**0.695**
	SPT_19	25 (14.9)	22 (13.1)	17 (10.1)	13.5	0.616	0.634	**0.665**
HDM	SPT_22	73 (43.5)	71 (42.3)	65 (38.7)	14.8	0.707	**0.726**	0.710

Bold: Highest Youden index at three different cut-points; numbers of SPT solutions were according to Table 1.

Sensitizations against solutions from *Alternaria alternata* were most frequent (Table 5) when it came to sensitization to a single mould species. In up to 60 subjects, either sIgE or SPT was positive against Alt a. The next frequent was sensitization against *Penicillium chrysogenum* (up to 44 subjects) and *Aspergillus fumigatus* (up to 42 subjects), followed by sensitization against *Cladosporium herbarum* (up to 29 subjects). *Aspergillus versicolor* resulted in a maximum sensitization prevalence of 23 subjects. Manufacturer-dependent differences in rates of sensitization were clearly observed for every mould species. SPT solutions from Allergopharma (SPT_3, SPT_7, SPT_11) resulted in the highest rates of sensitization, whereas the SPT solution from ALK (SPT_15) was most sensitive for Alt a. The best concordance between positive SPT and sIgE results was for all mould species calculated for solutions from ALK (SPT_4, SPT_8, SPT_12), except for *Altanaria alternata*, where SPT solutions from Allergopharma (SPT_14) and Lofarma (SPT_17) showed the best concordance with the sIgE test.

Regarding mono- and poly-sensitizations, it was seen that from 90 patients with mould sensitization in the SPT, only 24 (27%) were sensitized exclusively to one mould species (mono-sensitized), whereas 66 (73%) were sensitized to several mould species (≥ two mould species). Among the mono-sensitized subjects, the most frequently recognized mould species was Alt a (n = 11), followed by Pen ch (n = 7), Asp f (n = 5) and Cla h (n = 1). There was no mono-sensitization to Asp v. From these 24 mould mono-sensitized subjects, seven reacted exclusively to one mould species (three to Alt a and four to Asp f a) without additional sIgE to sx1. In other words, only 8% of all mould sensitizations were exclusively mono-sensitized to one mould species without sensitization to environmental allergens. In poly-sensitized subjects, most often two mould species (n = 27) were recognized, followed by sensitizations against five (n = 14), three (n = 13) or four (n = 12) different mould species. Among these 66 mould poly-sensitized patients 52 subjects were additionally sensitized to other allergens (included in sx1) and 14 subjects to only other mould allergens. Therefore, to obtain a mould panel that would adequately detect all mould-sensitized subjects using SPT, the results indicate that testing for Alt a, Asp f and Pen ch is sufficient to identify all subjects with mould sensitization, with the exception of subjects with a mono-sensitization to Cla h.

The detection rates of mould sensitization were calculated for three SPTs with and without additional sIgE to mx1 (Table 6). Sixty-three to 75% of all mould sensitizations could be detected using a SPT (tested in duplicate) to the three mould species Alt a, Asp f and Pen ch, depending on the manufacturer. Data in Table 2 show that mould mix (mx1) was sufficient to identify almost all (55 of 56) subjects with serological mould sensitizations. Therefore, sIgE measurement of mx1 in

Table 5. Positive SPT (≥ 1.5 mm) and/or sIgE (≥ 0.35 kU/L) results for different moulds in 168 subjects

Mould (CAP)	sIgE (+) (*n* =)	SPT (+) (*n* =)	SPT	SPT (+) and/or sIgE (+) (*n* =)	SPT (+) and sIgE (+) (*n* =)	SPT (+) and sIgE (−) (*n* =)	SPT (−) and sIgE (+) (*n* =)	Concordance SPT and sIgE (%)
Asp f (m3)	22	37	SPT_3	**42**	17	20	5	40
		30	SPT_4	34	18	12	4	**53**
		21	SPT_5	30	13	8	9	43
		20	SPT_6	28	14	6	8	50
Pen ch (m1)	23	36	SPT_7	**44**	15	21	8	34
		26	SPT_8	34	15	11	8	**44**
		22	SPT_9	34	11	11	12	32
		28	SPT_10	38	13	15	10	34
Cla h (m2)	14	24	SPT_11	**29**	9	15	5	31
		20	SPT_12	24	10	10	4	**42**
		16	SPT_13	23	7	9	7	30
Alt a (m6)	46	49	SPT_14	57	38	11	8	**67**
		52	SPT_15	**60**	38	14	8	63
		47	SPT_16	56	37	10	9	66
		46	SPT_17	55	37	9	9	**67**
Asp v (Gm25)	4	18	SPT_18	19	3	15	1	**16**
		22	SPT_19	**23**	3	19	1	13
Der p (d1)	66	71	SPT_22	80	57	14	9	71

Bold: SPT with the highest sensitization rate or SPT with the highest concordance to sIgE within one mould species; numbers of SPT solutions were according to Table 1.

Table 6. Detection rates (%) of mould sensitization combining manufacturer-dependent SPT results and sIgE to mould mix in 90 patients with at least one positive skin or sIgE reaction to moulds

Detection rate (%) of mould sensitizations (total: *n* = 90)	sIgE to mx1	SPT Allergo	SPT ALK	SPT HAL	SPT Lofarma
Only sIgE to mx1	62	–	–	–	–
Only SPT to Pen ch, Asp f, Alt a	–	75	69	63	67
Only SPT to Pen ch, Asp f, Alt a, Cla h	–	78	73	63*	70
sIgE to mx1 + SPT to Pen ch, Asp f, Alt a	+	78	72	70	71
sIgE to mx1 + SPT to Pen ch, Asp f, Alt a, Cla h	+	80	74	70*	73

*Cla h SPT not available by HAL.

addition to SPTs to Alt a, Asp f and Pen ch increased the sensitization rates to 70–78% depending on the SPT manufacturer. Additional SPT with Cla h did not increase detection rates substantially. Consequently, the SPT with Alt a, Asp f and Pen ch plus the sIgE (mx1) would be recommended to diagnose the majority of mould sensitizations.

Comparison of SPT solutions from different manufacturers

The degree of concordance among the SPT solutions from different manufacturers for one mould species was analysed by Venn diagrams (Fig. 2). Sensitization to any *Aspergillus fumigatus* SPT solution was observed in 43 subjects: 14 reacted exclusively with Asp f SPT solution of one manufacturer, eight showed skin reactions with two SPT solutions, six subjects with three different Asp f SPT solutions and 15 subjects with all four SPT solutions for Asp f. The concordance between subjects who reacted positively to all four Asp f SPT solutions (*n* = 15) to those with at least one positive SPT (*n* = 43) was 35%. SPT-based sensitization to *Penicillium chrysogenum* was observed in 54 subjects: 24 were exclusively positive with the Pen ch SPT solution from one specific manufacturer and 11 with all four SPT solutions. The rate of concordance between positive SPT with all Pen ch solutions vs. any SPT solution was 20%. A similar distribution was seen for *Cladosporium herbarum* SPT solutions with a concordance of 20% (*n* = 7 positive with all vs. *n* = 35 with any). Testing with SPT solutions prepared for *Aspergillus versicolor* resulted in 12 of 28 consistent tests (43%). The SPT results for *Alternaria alternata* obtained with solutions from different manufacturers showed the highest concordance. Forty of 58 subjects (69%) with at least one positive SPT to *Alternaria alternata* were positive with all applied Alt a SPT solutions.

Evaluation of SPT wheals by sIgE results

Receiver-operating characteristics (ROC) were calculated for all mould SPT solutions and additionally for HDM (Table 7). The areas under curve (AUCs) were between 0.70 and 0.92 for tested mould SPT solutions, comparable to SPT solution against HDM with AUC of 0.89. With the exception of the two Asp v SPT solutions, one Pen ch and two Cla h SPT solutions, AUC values for all other SPT results correlated highly significant ($P < 0.0001$) with sIgE results. The highest AUC values were in most cases obtained with solutions from ALK. Calculation of sensitivity, specificity, PPV and NPV with a cut-point of 1.5 mm (Table 7) yielded only minimal differences within the SPT solutions against Alt a. Conversely, the differences depending on the manufacturer were high for other mould SPT solutions, varying especially in sensitivity (about 20%) and PPV values (up to 25%) within one mould species. Nevertheless, test efficiency was > 80% for all applied SPT solutions independent of the manufacturer.

Discussion

Previous studies have shown that double testing in SPT improved test reproducibility and reduced the risk of false-negative results [1, 15] at least for occupational allergens. Nevertheless, it is often the case that only single-armed SPTs are performed in both clinical practice and epidemiological studies. Our present results indicate that only well-standardized allergen sources or SPT solutions with an adequate amount of allergen, such as HDM, tree or grass pollen and histamine, had high rates (≥ 90%) of double-positive skin reactions (Table 3). In these cases, single skin prick testing can be sufficient. However, double skin prick testing with mould allergens – with the exception of five SPT solutions (two Asp f and three Alt a) – resulted in a concordance below 80%; and, with six mould SPT solutions, the concordance fell below 60%. Similar results have been described for SPT with occupational allergens [15]. Taking the antigen amount of SPT solutions into account (Table 3), those SPT solutions with the highest

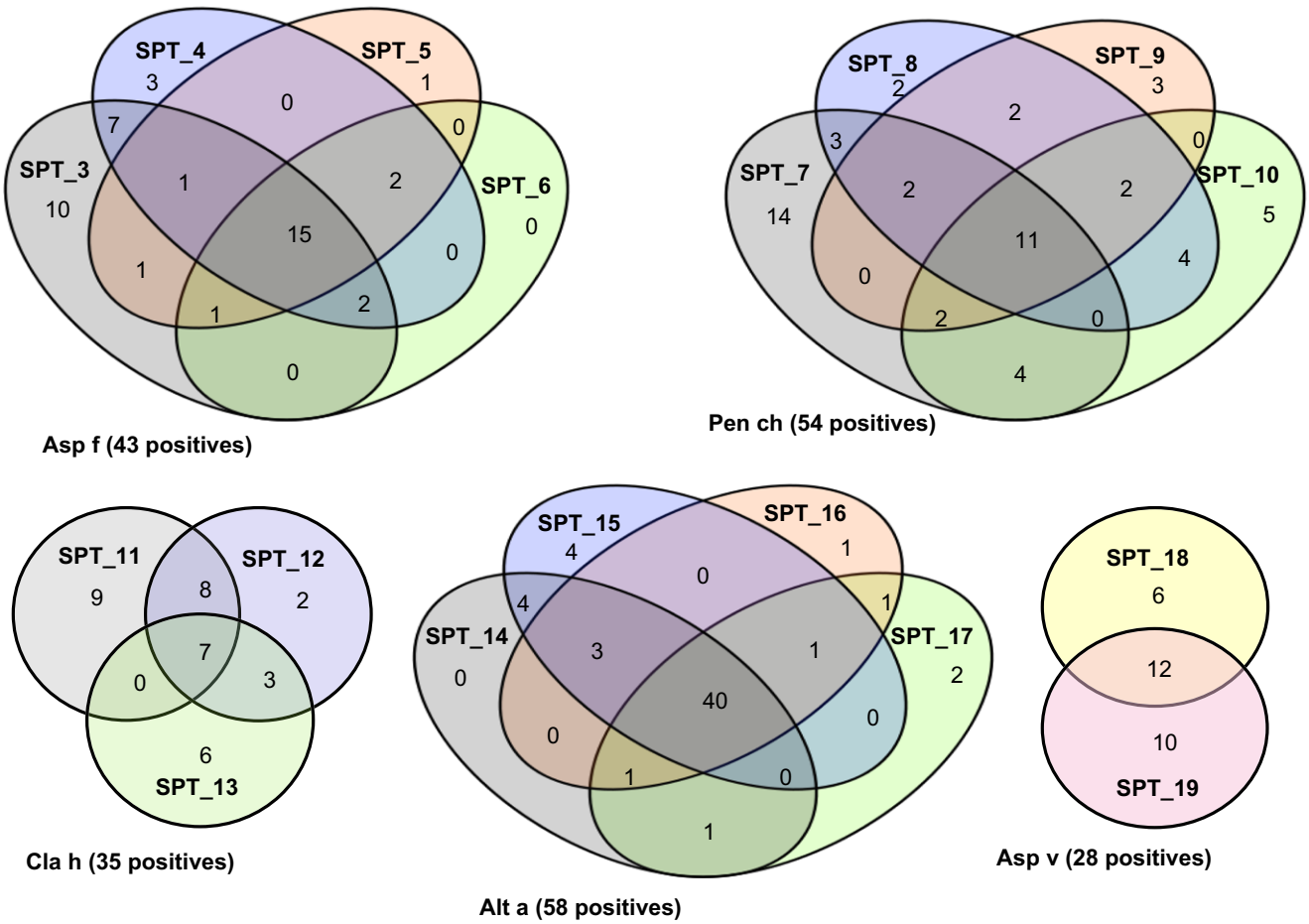

Fig. 2. Venn diagrams of positive mould SPT results n = (mean wheal size ≥ 1.5 mm), obtained with test solutions from different manufacturers (test solutions from each manufacturer in one colour: Allergopharma, ALK, HAL, Lofarma, Allergon-IPA, Greer-IPA). SPT solutions were numbered according to Table 1.

Table 7. Evaluation of SPT wheals with sIgE ≥ 0.35 kU/L as 'positive standard', ROC analysis (independent of wheal size) and sensitivity, specificity, PPV, NPV, test efficiency (SPT wheal cut-point ≥ 1.5 mm)

	SPT	ROC area under curve (AUC)	tp (n =) [+IgE/+ SPT]	tn (n =) [-IgE/- SPT]	fp (n =) [-IgE/+ SPT]	fn (n =) [+IgE/- SPT]	Sensitivity (%) [tp/(tp + fn)]	Specificity (%) [tn/ (tn + fp)]	PPV (%) [tp/(tp + fp)]	NPV (%) [tn/(tn + fn)]	Efficiency (%) [(tp + tn)/ (tp +fp + tn + fn)]
Aspergillus fumigatus	SPT_3	0.8524**	17	126	20	5	77.3	86.3	45.9	96.2	85.1
	SPT_4	0.8887**	18	134	12	4	81.8	91.8	60.0	97.1	90.5
	SPT_5	0.7782**	13	138	8	9	59.1	94.5	61.9	93.9	89.9
	SPT_6	0.8062**	14	140	6	8	63.6	95.9	70.0	94.6	91.7
Penicillium chrysogenum	SPT_7	0.7769**	15	124	21	8	65.2	85.5	41.7	93.9	82.7
	SPT_8	0.8343**	15	134	11	8	65.2	92.4	57.7	94.4	88.7
	SPT_9	0.7046*	11	134	11	12	47.8	92.4	50.0	91.8	86.3
	SPT_10	0.7585**	13	130	15	10	56.5	89.7	46.4	92.9	85.1
Cladosporium herbarum	SPT_11	0.7713*	9	139	15	5	64.3	90.3	37.5	96.5	88.1
	SPT_12	0.8636**	10	144	10	4	71.4	93.5	50.0	97.3	91.7
	SPT_13	0.7512*	7	145	9	7	50.0	94.2	43.8	95.4	90.5
Alternaria alternata	SPT_14	0.9163**	38	111	11	8	82.6	91.0	77.6	93.3	88.7
	SPT_15	0.9022**	38	108	14	8	82.6	88.5	73.1	93.1	86.9
	SPT_16	0.9017**	37	112	10	9	80.4	91.8	78.7	92.6	88.7
	SPT_17	0.9007**	37	113	9	9	80.4	92.6	80.4	92.6	89.3
Aspergillus versicolor	SPT_18	0.8537*	3	149	15	1	75.0	90.9	16.7	99.3	90.5
	SPT_19	0.8476*	3	145	19	1	75.0	88.4	13.6	99.3	88.1
HDM	SPT_22	0.8882**	57	88	14	9	86.4	86.3	80.3	90.7	86.3

Numbers of SPT solutions were according to Table 1.

*$P < 0.05$.

**$P < 0.0001$.

antigen amount also showed the highest concordance between double-positive SPTs on both arms. The ratio between antigen amount and single-/double-positive SPT results seemed not to be linear, suggesting that a minimum antigen amount was necessary to elucidate a skin reaction, and any additional antigen amount only minimally increased the skin reaction. Also, individual factors of the patient (e.g. atopic or nonatopic) could play a role.

Comparing the results obtained using mould SPT solutions from different manufacturers indicated that solutions obtained from Allergopharma produced the most cases with the highest number of positives (cut-off ≥ 1.5 mm). The most overlap was obtained with SPT solutions from Allergopharma and ALK (Fig. 2). As a rule, the SPT solutions from these manufacturers also revealed the highest antigen amounts as well. Further analysis showed that comparing the number of concordant positive test results with all solutions for one mould species to the detection number with any solution (Fig. 2) resulted in a concordance ratio below 50% for Asp f (35%), Pen ch (20%), Cla h (20%) and Asp v (43%), thus emphasizing the large diversity in test solution quality depending on the manufacturer. In contrast, SPTs to Alt a were in 69% positive with all four Alt a solutions, indicating that manufacturer-dependent differences were less distinct for this allergen. It was also observed that sIgE values were positive in almost 90% of the cases where subjects reacted with all SPT solutions for one allergen. However, this was not applicable for Cla h and Asp v, where 71 and 25% of the positive results to all SPT solutions, respectively, were associated with positive sIgE values. These mould solutions were apparently highly heterogeneous and conspicuously different to sIgE antigen spectrum.

To evaluate the clinical impact of the SPT results, a 'gold standard' for classification is needed. Examples of clinically reliable gold standards include a double-blind placebo control provocation challenge conducted according to the standardized guidelines for food allergy [16], or challenge tests with workplace-related inhalation tests for occupational asthma and rhinitis [17]. However, bronchial or nasal challenge tests with mould allergens were only conducted in a minority of patients in our study. Alternatively, a physician's diagnosis of mould allergy was considered as 'gold standard', but was usually limited as one definitive mould species was often not identified. In addition, the diagnosis was partially based on the SPT results which needed to be evaluated, making this parameter inoperative. Other studies [14, 18–21] indicated that sIgE was a valid predictor for allergen-induced allergic symptoms. A recent study reported an excellent correlation for the SPT and bronchial provocation, especially for Alt a, in asthmatic subjects, and that a high sIgE concentration

against Alt a was an effective predictor for a positive challenge [19]. Therefore, sIgE test results were used to evaluate SPT results by ROC analysis. For Alt a, all SPT solutions produced similar AUCs of 90.1–91.6%, and similar rates of sensitization were diagnosed by different manufactures' Alt a SPTs (27–29%) and the Alt a-specific IgE test (27%). In contrast, for the other mould species, most SPT solutions gave much higher sensitization rates than the corresponding sIgE test. Due to missing challenge tests and/or other clinically relevant parameters for validation, it is not a trivial task to decide whether SPTs are unspecific or sIgE tests are insensitive. However, for Asp f, Pen ch and Cla h the SPT solutions that exhibited the lowest sensitization rates also revealed the lowest AUCs in the ROC analysis. Therefore, SPT solutions that yielded higher sensitization rates due to higher antigen content are considered superior, even based on a probably insensitive sIgE test.

Finally, the question remains which tests should be performed if mould sensitization is suspected without prior knowledge of the responsible mould species. In our group of 168 patients with suspected mould allergy, 90 mould sensitizations were detected using either sIgE or SPT. The detection rate of mould sensitizations by sIgE was only 62% (56 of 90), whereas 100% (90 of 90) detection rate was obtained if all 17 SPT results were combined. Thus, serological testing alone does not seem to be sufficient to record mould sensitization; but, testing patients with 17 different solutions is not feasible for daily practice. Therefore, we analysed the data with the aim to minimize the number of tests while simultaneously gathering (nearly) all mould sensitizations. The most frequent mould allergen sources were Alt a, Asp f and Pen ch, which should be included in the SPT panel. Furthermore, sIgE to mould mix, mx1 was sufficient to identify almost all (55 of 56) subjects with serological mould sensitizations. Therefore, serological testing with mx1 is highly recommended. Although sensitization rates to single mould species in SPT differed strongly between the manufacturers, overall sensitization rates of 70 and 78% (Table 6) could be obtained by testing mx1 serologically and in addition three SPT to Alt a, Asp f and Pen ch (independent of manufacturer). It is important to mention that this mould panel is representative for German and probably European patients. The mould flora in other parts of the world is known to be quite different and must be tested accordingly.

In conclusion, this study showed that biochemical parameters like antigen/allergen content of mould SPT solutions influence the quality and the reproducibility of SPT results. When using the most potent SPT solutions, mould sensitizations were more frequently (up to twofold more) detected by SPT than by sIgE. Nevertheless, mould SPTs should be conducted as double tests to improve reproducibility and reliability of results. Skin areas closer to the elbow were slightly more sensitive than the skin areas closer to the wrist. The additionally introduced dampness-related mould, Asp v, did not seem to play an important role in the patient study group. Sensitization rates were low and consistently positive together with Asp f. Therefore, the recommendation for testing mould sensitization would be to perform at least three different SPT solutions, which include Asp f, Alt a and Pen ch in double values (independent from manufacturer), plus an additional serological test (mx1). With these diagnostic tools, up to 80% of all detected mould sensitizations could be screened, but clinical relevance must be verified either by further anamnesis or provocation tests.

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Conflict of interest

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