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POSITION PAPER

EAACI position paper: skin prick testing in the diagnosis of occupational type I allergies

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Abstract

Skin prick testing (SPT) in combination with the clinical history of the patient is one important step in the diagnosis of IgE-mediated occupational allergies. However, skin test performance is related to the quality of allergen extracts. The present consensus document was prepared by an EAACI Task Force consisting of an expert panel of allergologists and occupational physicians from Germany, Italy, Spain, France, Austria, and Poland. All members of the panel were also involved in the data collection within the European multicentre study STADOCA (Standard diagnosis for occupational allergy). The aim of this Task Force was the assessment of the quality of commercially available SPT solutions for selected occupational allergens under standardized procedure conditions in different European centres and institutes of Occupational Medicine. The data evaluation shows a wide variability among SPT solutions and also indicates that the sensitivity of several SPT solutions is low. Therefore, improvement and standardization of SPT solutions for occupational allergens is highly recommended. Clinical practitioners should also not presume that their SPT solutions are fully reliable. The main objective of the document is to issue consensus suggestions for the use of SPT with occupational allergens based on the European multicentre study STADOCA, on existing scientific evidence and the expertise of a panel of allergologists.

Skin prick testing (SPT) in combination with the clinical history of the patient is one important step in the diagnosis of occupational IgE-mediated allergies. Often, it is a relevant outcome for compensation and thus also has socioeconomic consequences.

Guidelines for allergy diagnosis have been recommended using standardized SPT solutions (1, 2). Unfortunately, these guidelines do not explicitly mention occupational allergens (1), nor do they exclude them concretely (2). In addition, a survey of different allergy centres at the beginning of this study showed that the performance of SPT often differs, and the quality of several commercially available SPT solutions for occupational allergens remains inadequate. In two further studies, the sensitivities of flour SPT solutions that are used in the diagnosis of baker's asthma have been described as variable and partially low (3, 4).

Methods

This consensus document was prepared by an EAACI Task Force consisting of an expert panel of allergologists and occupational physicians from Germany, Italy, Spain, France, Austria, and Poland. All members (co-authors of this manuscript) were also involved in the data collection within the European multicentre study STADOCA (Standard diagnosis for occupational allergy) that was funded by the German Social Accident Insurance. The results of this study have been published in detail (5). A meeting was held to review

the data and to reach consensus. The aim of this Task Force was to assess the quality of commercially available SPT solutions for selected occupational allergens under standardized procedure conditions in different European centres and institutes of Occupational Medicine. It was an original aim to fix recommendations concerning the use of SPT with occupational allergens. These recommendations should lead to an improvement of the diagnosis of occupational allergy, not only in clinical-oriented occupational centres, but also in practice, for example, in cases where compensation is demanded. However, no further evidence-based recommendations could be provided and instead, 'key messages' or 'suggestions' (see Key message box) are given based on the data of the STADOCA study (5) and from the consensus of the expert panel members. The document is not intended to be a formal evidence-based guideline, but is instead written to provide occupational allergologists and physicians involved in the diagnostic work-up of occupational allergies with useful information on the use of SPT. In addition to these recommendations, the general guidelines (1, 2) covering SPT methodology (e.g., SPT should be carried out by trained health professionals) and interpretation as well as indication and contraindication in a variety of settings have to be considered.

The suggestions and key messages were based on the SPT data from 116 bakers, 47 farmers, and 33 persons occupationally exposed to natural rubber latex (NRL) (details in Ref (5)). All patients suffered from work-related symptoms like rhinitis, conjunctivitis, cough, chest tightness, shortness of breath or wheezing and have been examined within the scope of claims for compensation due to occupational asthma. Whereas SPTs and challenge tests (challenge tests in bakers: wheat flour, $n = 70$; rye flour, $n = 54$) were performed in the different centres, specific IgE (sIgE) measurements using ImmunoCAP (Phadia, Uppsala, Sweden; CAP values ≥ 0.35 kU/l: positive) were performed centrally in one facility (IPA).

Biochemical *in vitro* analysis of SPT solutions

Thirty commercially available SPT solutions for wheat and rye flour, soy, cow hair/dander, storage mites as well as NRL from seven manufacturers (ALK-Abelló, Hørsholm, Denmark; Allerbio, Varennes-en-Argonne, France; Allergopharma, Reinbek, Germany; Bencard Munich, Germany; Hal, Duesseldorf, Germany; Lofarma, Milan, Italy; Stallergènes, Antony, France) were analyzed *in vitro* for protein and antigen content. Independent of the allergens, all SPT solutions from different manufacturers showed variability in protein and even greater variability in antigen content (Table 1). For example, differences in antigen content of SPT solutions for rye flour, cow, and *Tyrophagus putrescentiae* are more than 100-fold. It is assumed that solutions with higher protein and antigen content showed a higher potency in SPT. However, nonallergenic proteins like human serum albumin (HSA) sometimes were added to the SPT solutions for stabilization (3) and should be considered when evaluating protein content. Therefore, the determination of protein content alone is not a feasible predictive marker for the quality of a SPT solution.

Table 1 Protein and antigen contents (min. – max.) of commercial SPT solutions for different occupational allergens [Correction made after online publication on 20 March 2013: units of protein content changed to μg/ml.]

Allergen (number of tested SPT solutions)	Protein content* (μg/ml)	Antigen content† (U/ml)
Wheat flour ($n = 4$)	191–538	88–1845
Rye flour ($n = 4$)	183–1037	21–2721
Soy ($n = 3$)	1114–1631	n.d.
Cow ($n = 5$)	43–659	3–1439
T. putrescentiae ($n = 3$)	b.d.-588	37–1597
L. destructor ($n = 3$)	b.d.-205	15–266
A. siro ($n = 3$)	b.d.-192	34–429
		Allergen content‡ (μg/ml)
NRL ($n = 5$)	b.d.-65	0.6–11.7

b.d., below detection limit; n.d., not done; NRL, natural rubber latex.

*Measured by Bradford assay (6)

†Measured by rabbit IgG sandwich enzyme-linked immunosorbent assays (ELISAs); wheat flour (7), rye flour (3), cow hair (8), *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, and *Acarus siro* (9).

‡Measured by CAP inhibition (10).

Performance of SPT

In each allergy centre, identical solutions were used for SPT, as outlined in the European position papers (11, 12), according to a standardized procedure - twice in a predetermined order on the untreated skin on both volar forearms in opposite direction. Skin prick testing panel for bakers included cereal flours and soy. The SPT panel for farmers included SPT solutions for cow hair/dander and three different storage mites, whereas NRL-exposed subjects were pricked with five NRL SPT solutions from different manufactures. Histamine (10 mg/ml) and saline were used as positive and negative controls, respectively, in each patient.

In a recently published study, it was demonstrated that metal lancets (or needles) are the tool of choice for SPT (13). Thus, in all centres, a new commercial steel lancet (ALK-Abelló) was used for each allergen. After 15 min, test solutions were wiped off with alcohol; contours of wheals were drawn with a ballpoint pen and transferred to a blank sheet of paper using transparent tape. Assessment of SPT results was carried out centrally by a single person. Wheal sizes (mean value of the largest diameter and the diameter at the midpoint, at a right angle) were recorded in mm.

For all tested allergens, the number of patients showing at least one wheal reaction in SPT depended on the manufacturer of the SPT solution (Table 2). For example, in the case of rye flour, the number of positive reactions was highly variable, whereas results with the different NRL SPT solutions were similar.

Table 2 Concordance (min. – max.) of SPT double estimations using different commercial SPT solutions

Allergen (number of tested SPT solutions)	At least one wheal >0 mm n (%)	Both wheals >0 mm n (%)	Concordance
Histamine	196 (100%)	195 (99%)	99.5%
Wheat flour (n = 4)	33 (29)–50 (43)	25 (22)–38 (33)	74–82
Rye flour (n = 4)	22 (19)–67 (58)	15 (13)–64 (55)	68–96
Soy (n = 3)	27 (23)–31 (27)	21 (18)–26 (22)	78–86
Cow (n = 5)	5 (14)–15 (41)	5 (14)–13 (35)	60–100
Tp (n = 3)	6 (21)–11 (38)	3 (10)–9 (31)	50–82
Ld (n = 3)	5 (17)–12 (41)	5 (17)–12 (41)	73–100
As (n = 3)	3 (10)–11 (38)	1 (3)–8 (28)	33–88
NRL (n = 5)	11 (33)–12 (36)	9 (27)–12 (36)	82–100

Tp, *Tyrophagus putrescentiae;* Ld, *Lepidoglyphus destructor;* As, *Acarus siro*; NRL, natural rubber latex.

Using histamine resulted in one positive test result in 99.5% of cases that could be confirmed by the second test. This high degree of concordance in the SPT reactions with histamine was previously shown by other authors (14). Using the 30 SPT solutions for occupational allergens, a median degree of concordance of 82% was achieved. However, in some cases, concordance of SPT double estimation was much lower (Table 2). As mentioned in the European position paper, it is possible that single negative tests are obtained in sensitive patients, even by skilled technicians (11). There is also the risk of false-negative tests due to technical problems in patients with low skin sensitivity. The degree of reproducibility with allergen SPT solutions seemed to be dependent on the potency of SPT solution. Therefore, it would be desirable to perform SPTs with occupational allergens at least in duplicate. However, from the practical point of view in cases testing a huge panel of different allergens, duplicate testing seems not realistic.

Interpretation of SPT

For evaluation of SPT solutions, the results of sIgE determinations were taken as the gold standard, because challenge tests were not performed in all cases. Evaluation of SPT results in a subgroup of 70 (wheat flour) and 54 (rye flour) bakers, in relation to the two gold standards – sIgE and challenge test – resulted in the same ranking of flour SPT solutions (data not shown). Of 116 bakers, 71 (61.2%) showed sIgE to wheat and 75 (64.7%) to rye flour. Twenty-seven (13.3%) were also positive to soy. Of the 43 farmers tested to cow-sIgE, twelve (27.9%) were positive; whereas out of those tested to storage mite-sIgE (n = 35), nine (25.7%) were positive to *Tyrophagus putrescentiae* and *Lepidoglyphus destructor* and eight (22.9%) to *Acarus siro*. Nine (27.3%) of the 33 HCWs showed sIgE to NRL.

True positives (tp) were subjects with positive sIgE and positive SPT; true negatives (tn) were subjects with negative sIgE and negative SPT; false positives (fp) were subjects with negative sIgE and positive SPT; false negatives (fn) were subjects with positive sIgE and negative SPT. Two-by-two tables were used to calculate sensitivities [tp/(tp + fn)], specificities [tn/(tn + fp)], positive (PPV) [tp/(tp + fp)] and negative [tn/(tn + fn)] predictive values (NPV), as well as test efficiencies [(tp+tn)/(tp + fp + tn + fn)]. To identify the optimal cut-point for the different SPT solutions, Youden Index (sensitivity + specificity-1) was calculated for each SPT solution at four different wheal sizes. Whereas PPV and NPV depend largely on the prevalence rates, sensitivity and specificity are presumably inherent properties of the test. Thus, Youden Index, which equally considers sensitivity and specificity, was used to define the optimal cut-point, because this index should be independent of selection bias.

Table 3 Optimal cut-points of SPT solutions for different occupational allergens

Allergen (number of tested SPT solutions)	Optimal cut-point at wheal size			
	≥1.5 mm	≥2.0 mm	≥2.5 mm	≥3.0 mm
Wheat flour (n = 4)	4/4*	–	–	–
Rye flour (n = 4)	4/4	–	–	–
Soy (n = 3)	1/3	1/3	1/3	–
Cow (n = 5)	4/5	1/5	–	–
Tp (n = 3)	2/3	1/3	–	–
Ld (n = 3)	–	1/3	1/3	1/3
As (n = 3)	–	1/3	–	2/3
NRL (n = 5)	1/5	1/5	1/5	2/5

Tp, *Tyrophagus putrescentiae*; Ld, *Lepidoglyphus destructor*; As, *Acarus siro*; NRL, natural rubber latex.
*For all four tested wheat flour SPT solutions, the optimal cut-point was ≥1.5 mm.

As shown in Table 3 for some occupational allergens, for example, flour and cow hair/dander, maximum Youden Index, which was used to determine the optimal cut-point, was reached at a wheal diameter ≥1.5 mm. Although such small wheal sizes are unusual, it was confirmed by other studies. Based on a study involving more than 11 000 subjects tested with extracts of house dust mite, cat, timothy grass, and *Cladosporium*, Bousquet et al. (15) stated that a cut-off level of over 0 mm is the most appropriate definition of positive SPTs to assess allergic sensitization in epidemiological studies. In clinical practice, wheal diameters ≥3 mm are usually considered positive. However, in occupational medicine, especially in the case of claims, a very sensitive diagnosis seems to be important. In cases of small wheal sizes, additional replicates of the test supported by positive testing with other preparations and by serological IgE test should be considered as positive.

Evaluation of SPT solutions

Evaluation of sensitivity, specificity, test efficiency, PPV, and NPV was performed using the optimal cut-point of each SPT solution. Additionally, area under curve (AUC) values

Table 4 Evaluation results (min. – max.) obtained with SPT solutions for different occupational allergens based on the gold standard sIgE

Allergen (number of tested SPT solutions)	Sensitivity* (%)	Specificity* (%)	Test efficiency* (%)	PPV* (%)	NPV* (%)	AUC†
Wheat flour ($n = 4$)	38–58	89–93	60–71	88–92	49–58	0.67–0.77
Rye flour ($n = 4$)	21–81	88–98	47–84	89–97	40–72	0.61–0.87
Soy ($n = 3$)	33–44	82–85	71–76	36–48	80–84	0.61–0.64
Cow ($n = 5$)	50–92	90–97	83–93	77–89	83–97	0.77–0.94
Tp ($n = 3$)	45–89	80–100	82–88	62–100	83–96	0.69–0.92
Ld ($n = 3$)	78–100	80–100	85–94	64–100	93–100	0.88–0.97
As ($n = 3$)	50–88	85–100	83–88	63–100	87–96	0.73–0.90
NRL ($n = 5$)	67–89	92–96	85–94	75–89	88–96	0.80–0.93

Tp, *Tyrophagus putrescentiae*; Ld, *Lepidoglyphus destructor*; As, *Acarus siro*; PPV, positive predicted value; NPV, negative predicted value; AUC, area under curve; NRL, natural rubber latex.

*Evaluation was performed using the optimal cut-point of each SPT solution.

†Data were obtained by ROC (receiver-operating characteristic) curves.

obtained by receiver-operating characteristic (ROC) curves were calculated (Table 4).

While for all SPT solutions, specificity was between 80% and 100%, sensitivities, test efficiencies, PPVs, NPVs, and AUCs showed partially extreme discrepancies between SPT solutions. Skin prick testing solutions for some allergens like wheat flour and soy reached overall low sensitivities (all SPT solutions <59%). In contrast, sensitivities of SPT solutions for rye flour, cow, and storage mites were extremely variable depending on the manufacturer; values ranged, for example, from 21% to 81% (rye flour) and from 45% to 89% (*Tyrophagus putrescentiae*), respectively. With one exception, NRL SPT solutions reached a comparably high-quality independent of the manufacturer.

As a rule, solutions with both higher protein and higher antigen content showed higher sensitivity and test efficiency. However, special extraction conditions should be considered as described, for example, for flour. It is also known that ethanol-soluble cereal proteins are relevant allergens for baker's asthma (16, 17). Therefore, it is highly recommended that manufacturers, whose solutions showed low sensitivities, increase the antigen content of SPT solutions.

Evaluation of SPT results with flour in a subgroup of 70 (wheat flour) and 54 (rye flour) bakers, in relation to the gold standards, sIgE and challenge tests, resulted in comparable values (data not shown, but confirmed by a former study (3)). The data demonstrated that the great differences between the tested flour SPT solutions were independent of the gold standard used.

Conclusion

Skin testing is a cheap and effective way to determine the presence of sIgE, and together with a consistent history enables a confident diagnosis of IgE-mediated allergic disease. However, the quality of the results is based essentially on the quality of the SPT solution.

In vitro and *in vivo* results demonstrated considerable variability in the quality of commercial SPT solutions for selected occupational allergens, mainly depending on the protein and especially on the antigen content of the solutions. If the quality of SPT solutions used for the diagnosis of asthma in bakers and farmers, the most frequent types of occupational asthma in most European countries, is partially insufficient, it is highly likely that the quality of test solutions for other rare occupational and environmental allergens is also inadequate. This emphasizes the importance of allergen standardization and improvement of SPT solutions for all, but especially for occupational allergens. In the meantime, all users should not take their SPT solutions for granted.

Key message box

Skin prick testing (SPT) in combination with the clinical history of the patient is one important step in the diagnosis of occupational IgE-mediated allergies. The following points must be considered for the practical and effective use of SPT in the diagnostic work-up of occupational allergies:

- A high variability in protein and even higher variability in antigen content were detected in all SPT solutions for all tested occupational allergens from different manufacturers.
- Although SPT with higher protein content seems to have a higher potency *in vivo*, determination of protein content alone is not a feasible predictive marker for the quality of a SPT solution.
- Increasing the antigen content of SPT solutions is recommended to those manufacturers whose solutions showed low sensitivities.
- SPTs should always be performed with a metal lancet and, if practicable, in duplicate.
- Depending on the case, small wheal sizes could be relevant. After additional replication, they should be supported by serological IgE test.
- Until further notice, it is highly recommended to use SPT solutions from different manufacturers in parallel.
- Standardization of SPT for occupational allergens is highly recommended.

The presented data are useful when selecting a suitable SPT solution for the diagnosis of selected occupational allergies. Unfortunately, it was not possible to analyse all potential occupational allergens from all potential manufacturers, and so the presented tables are incomplete. Additionally, it is not possible to exclude batch-to-batch variability of the quality of SPT solutions or changes in the production process.

Based on the current situation with the diversity and variability of the antigen content of the SPT solutions, it is highly recommended to use SPT solutions from different manufacturers in parallel to avoid false-negative results. Overall, standardization of SPT for occupational allergens, including studies like those presented here, together with monitoring the progress of standardization, is highly recommended.

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Conflict of interest

All authors have no conflict of interest to declare.

Author contributions

This Position Paper is the result of the collaboration of a panel of experts who contributed to the document according to their different experiences and competences, coordinated by Prof. Dr. Monika Raulf-Heimsoth.

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