

## ORIGINAL ARTICLE

# Comparison of wheat and rye flour solutions for skin prick testing: a multi-centre study (Stad 1)

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## Clinical & Experimental Allergy

### Summary

**Background** Skin prick testing (SPT) is the basic method for diagnosing IgE-mediated allergies. However, skin reactivity is related to the quality of allergen extracts, which are often poorly defined for occupational allergens.

**Objective** To compare wheat and rye flour SPT solutions from different producers.

**Materials and methods** Standardized SPTs were performed in seven allergy centres with wheat and rye flour solutions from four producers in 125 symptomatic bakers. Optimal cut-off levels for weal sizes were assessed with the Youden Index. Comparisons between SPT results of different solutions were made with flour-specific IgE (sIgE) as the gold standard. Sensitivities, specificities, positive and negative predictive values, and test efficiencies were calculated and compared with McNemar and  $\chi^2$ -tests. The influence of the choice of the gold standard (sIgE or challenge) test was examined for 95 subjects. Additionally, SPT solutions were analysed for protein and antigen content.

**Results** The optimal cut-off level for all SPT solutions was a weal size of  $\geq 1.5$  mm. While differences between wheat and rye flours were small, differences between producers were important. Variability of sensitivities (0.31–0.96), negative predictive values (0.42–0.91), and test efficiencies (0.54–0.90) were higher than variations of specificities (0.74–1.00) and positive predictive values (0.88–1.00). Similar results were obtained when using challenge test results as the gold standard. Variability could be explained by the different antigen contents of the SPT solutions.

**Conclusion** There is a wide variability of SPT solutions for wheat and rye flour from different producers, mainly with respect to sensitivities, negative predictive values, and test efficiencies. Improvement and standardization of SPT solutions used for the diagnosis of baker's asthma are highly recommended.

**Keywords** baker's asthma, occupational allergy, skin prick test

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### Introduction

Skin prick tests (SPTs) are recommended as a first-line method to detect IgE-mediated hypersensitivity to high-molecular-weight inhalant allergens. SPT also remains a very important step in the diagnosis of occupational allergies, e.g. baker's asthma, which is one of the most frequent forms of occupational asthma. In 2006, 58% of

589 confirmed cases of occupational asthma in Germany were caused by bakery-derived allergens [1]. Because SPT solutions are often poorly standardized for occupational allergens, it was the aim of our multi-centre study Stad 1 (standard allergy diagnosis – type 1) to compare wheat and rye flour SPT solutions from different producers.

It is known that significant differences exist between SPT procedures [2, 3]. The performance of SPT often

differs due to the used allergen solutions, lancets or needles, the retention time of SPT solutions on the skin, or the method of measuring the test results. Thus, a consistent SPT protocol with a harmonized technical procedure and identical scoring of the results for all participating allergy centres was generated. According to this protocol, wheat and rye flour SPT solutions from four different producers were evaluated.

A few years ago, a monocentre study with wheat and rye flour SPT in symptomatic bakers gave evidence for insufficient concordance of the results with bronchial challenge tests [4]. In the present multi-centre study, further commercially available SPT solutions were tested in a larger number of bakers according to a standardized protocol, and in addition, wheat and rye flour SPT solutions produced at BGFA were included.

## Materials and methods

### *Study design*

This multi-centre study included 125 bakers (74% males). All subjects answered a questionnaire, and SPTs and specific IgE (sIgE) determinations were performed. Ninety-five bakers (76%) underwent challenges with rye flours. While SPTs and challenge tests were performed in different centres, sIgE measurements were performed centrally in one facility (BGFA). All participants of the study gave written informed consent before examination. The study was approved by the ethics committee of the Ruhr University, Bochum, and was conducted in accordance with the Declaration of Helsinki.

### *Specific IgE*

sIgE antibodies to wheat and rye flour were measured in all 125 sera by ImmunoCAP (Phadia, Uppsala, Sweden) according to the manufacturer's recommendations. CAP values  $\geq 0.35$  kU/L were considered positive.

### *Skin prick test*

SPTs were performed between February 2003 and July 2005 with identical batches of wheat and rye flour solutions of Allergopharma (Reinbek, Germany), Bencard (Munich, Germany), and Hal (Duesseldorf, Germany) and with wheat and rye flour SPT solutions produced by BGFA mixing water/salt soluble with ethanol soluble seed proteins in 50% glycerol in a 7 : 1 ratio. Cereal proteins were extracted, dialysed, and lyophilized as described previously [5]. In addition, a panel of common inhalant allergens, including grass pollen, birch pollen, house dust mite (HDM) (*Dermatophagoides pteronyssinus*), and cat dander (all Allergopharma), was tested. Atopy was defined as a mean weal diameter  $\geq 3$  mm to at least one of these

common aeroallergens. Histamine (10 mg/mL) and saline were used as positive and negative controls, respectively.

In each centre, identical SPT solutions were pricked twice considering the corresponding European position papers [6, 7] according to a standardized procedure. SPTs were performed in a predetermined order on the untreated skin on both volar forearms in the opposite direction. In all centres, for each allergen, a new commercial steel lancet (ALK-Abelló, Madrid, Spain) was used. After 15 min, test solutions were wiped off with alcohol, and contours of weals were drawn with a ballpoint pen. The contours were transferred to a blank sheet of paper by a transparent tape. Assessment of SPT results was carried out centrally by a single person. Weal sizes (mean value of the largest diameter and the diameter at the midpoint, at a right angle) were recorded in millimetres. For all analyses, the means of both SPT determinations were used.

The optimal cut-off level for evaluation was chosen by the Youden Index (see statistical analysis).

### *Challenge tests*

Depending on anamnestic data and on the decision of the examiner in each centre, in 95 symptomatic bakers, challenge tests were performed with rye flour. Bronchial challenge tests were performed with nebulized aqueous flour solutions in 24 cases, with native flours simulating the situation at the workplace in 63 cases, and nasal challenges in eight cases.

A nasal challenge test was considered positive if nasal symptoms were followed by a decrease of nasal flow by at least 40% from baseline. Allergen-induced airway responsiveness was measured in all except one centre by body plethysmography. A positive test result was assumed if specific airway resistance doubled and increased to  $\geq 2.0$  kPa s, or if the decline in forced expiratory volume in 1 s was  $\geq 20\%$ . Overall decisions on whether the challenge was positive or negative were provided by the respective examiner.

### *Measurement of proteins and antigens in the different flour skin prick test solutions*

Protein concentrations in SPT solutions were measured by the Bradford assay [8] with bovine serum albumin as the standard. The amount of wheat antigens in wheat SPT solutions and the amount of rye antigens in the rye SPT solutions were measured by rabbit IgG sandwich ELISA. While for wheat flour this method was described earlier [9], the ELISA for rye flour was newly developed. Protein G affinity-purified rabbit antibodies to rye flour were coated to microtitre plates overnight at 4 °C. The wells were blocked with casein buffer (RDI, Flanders, NJ, USA) and washed three times. Eight standards with concentrations between 0.15 and 20 ng/mL rye flour protein were

incubated for 1 h at 37 °C. After washing, biotinylated antigen affinity-purified anti-rye rabbit antibodies were added. Ninety minutes later, all wells were washed and then incubated for 1 h with 1 : 10 000 diluted horseradish-peroxidase/streptavidin conjugates (poly-HRP80-Streptavidin, RDI) and developed as described earlier [10]. Sample concentrations were read by interpolation of optical density values (OD 414 nm) on a four-parameter fit of the standards, using Softmax Pro 4.7.1 from Molecular Devices (Sunnyvale, CA, USA). One unit per millilitre (U/mL) is equivalent to the OD obtained with 1 µg standard flour protein/mL.

#### *Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)*

Ten microlitres of each wheat and rye flour solution were separated electrophoretically in a pre-cast NuPAGE® Bis-Tris gel (Invitrogen, Groningen, the Netherlands), and silver stained.

#### *Statistical analysis*

Correlations between SPT results from the right and the left arm were analysed with Pearson's correlations, followed by analyses of SPT performances of solutions by different producers and with varying gold standards using the means of SPT determinations on the right and the left arm. For evaluation of SPT solutions, the results of sIgE determinations were taken as the gold standard because specific challenge tests were not performed in all cases. True positives (tp) were bakers with a positive gold standard and a positive SPT; true negatives (tn) were bakers with a negative gold standard and a negative SPT; false positives (fp) were bakers with a negative gold standard and a positive SPT; and false negatives (fn) were bakers with a positive gold standard and a negative SPT. Two-by-two tables were used to calculate sensitivities  $[tp/(tp+fn)]$ , specificities  $[tn/(tn+fp)]$ , positive predictive value (PPV)  $[tp/(tp+fp)]$ , and negative  $[tn/(tn+fn)]$  predictive value (NPV) as well as test efficiencies  $[(tp+tn)/(tp+fp+tn+fn)]$ . For the evaluation of SPT results, the weal size that leads to the maximum Youden Index (sensitivity+specificity-1) was chosen as the optimal cut-off level [11].

For sensitivity, specificity, PPV, NPV, and test efficiency, the 95% confidence intervals (95% CI) were calculated with binomial distributions. The sensitivity, specificity, and test efficiency of solutions from different producers were compared with McNemar tests. Differences in PPV and NPV were analysed using  $\chi^2$  tests according to Leisenring et al. [12]. *P*-values below 0.05 were considered significant.

To construct receiver operating characteristic (ROC) curves, sensitivity was plotted vs. 1-specificity over all measured weal sizes. The area under the curve (AUC), ranging between 0 and 1, with 95% CI, was calculated to evaluate and compare the AUC of SPT solutions [13]. In a

second step, challenge tests with rye flour, which were performed in 95 bakers, were used as the gold standard.  $\chi^2$ -tests were used to compare the resulting contingency tables for rye solutions based on gold standard challenge test and sIgE.

All statistical analyses were performed using SAS/STAT software, version 9.2 (2005; SAS, Cary, NC, USA).

## Results

### *Subjects*

All bakers suffered from work-related symptoms of rhinitis, conjunctivitis, cough, chest tightness, shortness of breath, or wheezing. Eighty-eight subjects (70%) complained of asthma and out of these 80 also reported rhinitis. Thirty-two bakers (26%) reported rhinitis, but no symptoms of asthma. One hundred and twelve bakers (90%) reported improvement during holidays or weekends. The mean age was  $41 \pm 13$  years, and 44 (35%) were current smokers, and 20 (16%) ex-smokers. Sixty-one subjects (49%) were atopic according to SPT results with common aeroallergens. Forty-four bakers (35%) were still working as bakers at the time of the study. The mean time since leaving the job was  $4.3 \pm 3.8$  years. All bakers were examined within the scope of claims for compensation due to occupational asthma in seven allergy centres, six localized in Germany and one in Poland.

### *Specific immunoglobulin E*

Out of 125 bakers, 85 (68%) showed sIgE to wheat flour (range 0.35 to  $> 100$  kU/L, median 2.31 kU/L) and 83 (66%) sIgE to rye flour (range 0.35 to  $> 100$  kU/L, median 3.24 kU/L).

### *Skin prick test results*

The comparison of weal sizes on the right arm with those obtained on the left arm showed high and significant correlations for all SPT solutions (Table 1). There were no significant differences between SPT results obtained with the same allergen at different prick positions (near the wrist or the antecubital fossa) (data not shown). Thus, we performed no separate calculations for SPT of the right and the left arm, but used the means of both SPT determinations for further analysis.

For all SPT solutions, the maximum Youden Index was reached with a weal size of  $\geq 1.5$  mm (Table 1). More detailed calculations were performed with this 'optimal cut-off level'.

As shown in the two-by-two tables, depending on the SPT solution, between 30% and 69% of bakers showed a positive SPT result to wheat flour while between 21% and

**Table 1.** Pearson correlation coefficients of skin prick test (SPT) results obtained with different wheat flour (Wf) and rye flour (Rf) SPT solutions on the right and left arms of 125 bakers

|                  | Correlation coefficient | <i>P</i> -value | Youden Index at the cut-off level (mm) |      |       |      |
|------------------|-------------------------|-----------------|----------------------------------------|------|-------|------|
|                  |                         |                 | ≥ 1.5                                  | ≥ 2  | ≥ 2.5 | ≥ 3  |
| Wf Hal           | 0.67                    | 0.0023          | <b>0.34</b>                            | 0.31 | 0.29  | 0.29 |
| Wf Allergopharma | 0.78                    | <0.0001         | <b>0.52</b>                            | 0.45 | 0.42  | 0.36 |
| Wf Bencard       | 0.80                    | <0.0001         | <b>0.63</b>                            | 0.60 | 0.53  | 0.49 |
| Wf BGFA          | 0.78                    | <0.0001         | <b>0.75</b>                            | 0.71 | 0.66  | 0.58 |
| Rf Hal           | 0.77                    | 0.0001          | <b>0.31</b>                            | 0.29 | 0.28  | 0.19 |
| Rf Allergopharma | 0.67                    | 0.0018          | <b>0.57</b>                            | 0.51 | 0.45  | 0.37 |
| Rf Bencard       | 0.81                    | <0.0001         | <b>0.60</b>                            | 0.53 | 0.51  | 0.49 |
| Rf BGFA          | 0.82                    | <0.0001         | <b>0.70</b>                            | 0.73 | 0.70  | 0.66 |

Youden Index at different cut-off levels was calculated with the mean weal size according to the gold standard specific IgE. The highest values are typed in bold.

73% of bakers reacted positively in SPT with rye flour (Table 2).

### Evaluation of skin prick test results

The differences between rye and wheat flours were small (Table 2). The differences between producers were much higher with respect to sensitivities (31–96%) and NPV (42–91%) than with respect to specificities (74–100%) and PPV (88–100%). Consequently, the variability of test efficiencies between producers was also high (Table 2).

For all SPT solutions, the differences in the sensitivities, test efficiencies, and, with one exception, NPV were highly significant. Differences in specificity were significant ( $P < 0.01$ ) only when comparing the BGFA rye flour SPT solution with the rye flour solutions of Hal or Allergopharma (Table 2).

ROC plots were produced for all wheat (Fig. 1a) and rye flour (Fig. 1b) SPT solutions. The area under the curve (AUC) was between 0.73 and 0.89 (wheat flour) and between 0.71 and 0.92 (rye flour). For both wheat and rye flour, AUC was maximal with the non-commercial (BGFA) SPT solutions (Figs 1a and b).

In a second step, challenge tests with rye flour, which were performed in 95 bakers [63 (66%) positive], were used as the gold standard. The comparison of the resulting two-by-two tables for rye flour SPT solutions based on the gold standards rye flour-sIgE or a specific challenge with rye flour showed only small differences (Table 3), which were not significant ( $P$ -values: Hal 0.735, Allergopharma 0.583, Bencard 0.863, BGFA 0.189).

The evaluation of rye flour-sIgE related to the gold standard-specific challenge test with rye flour showed a sensitivity of 87%, a specificity of 63%, a PPV of 82%, an NPV of 71%, and a test efficiency of 79%.

**Table 2.** Evaluation of SPT solutions for wheat flour (Wf) and rye flour (Rf) in 125 bakers based on the gold standard flour-sIgE

| SPT solution no.   | sIgE positive |              |              |              | sIgE negative          |              |                      |              | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI)           | NPV (95% CI) | Test efficiency (95% CI) |
|--------------------|---------------|--------------|--------------|--------------|------------------------|--------------|----------------------|--------------|----------------------|----------------------|------------------------|--------------|--------------------------|
|                    | SPT pos. (n)  | SPT neg. (n) | SPT pos. (n) | SPT neg. (n) | SPT pos. (n)           | SPT neg. (n) | SPT pos. (n)         | SPT neg. (n) |                      |                      |                        |              |                          |
| 1 Wf Hal           | 35            | 50           | 3            | 37           | 0.41 <sup>2,3,4*</sup> | 0.31–0.52    | 0.93 <sup>NS</sup>   | 0.80–0.98    | 0.92 <sup>NS</sup>   | 0.79–0.98            | 0.43 <sup>2,3,4*</sup> | 0.32–0.54    | 0.58 <sup>2,3,4*</sup>   |
| 2 Wf Allergopharma | 53            | 32           | 4            | 36           | 0.62 <sup>1,3,4*</sup> | 0.51–0.73    | 0.90 <sup>NS</sup>   | 0.76–0.97    | 0.93 <sup>NS</sup>   | 0.83–0.98            | 0.53 <sup>1,3,4*</sup> | 0.40–0.65    | 0.71 <sup>1,3,4*</sup>   |
| 3 Wf Bencard       | 66            | 19           | 6            | 34           | 0.78 <sup>1,2,4*</sup> | 0.67–0.86    | 0.85 <sup>NS</sup>   | 0.70–0.94    | 0.92 <sup>NS</sup>   | 0.83–0.97            | 0.64 <sup>1,2,4*</sup> | 0.50–0.77    | 0.80 <sup>1,2,4*</sup>   |
| 4 Wf BGFA          | 79            | 6            | 7            | 33           | 0.93 <sup>1,2,3*</sup> | 0.85–0.97    | 0.83 <sup>NS</sup>   | 0.67–0.93    | 0.92 <sup>NS</sup>   | 0.84–0.97            | 0.85 <sup>1,2,3*</sup> | 0.69–0.94    | 0.90 <sup>1,2,3*</sup>   |
| 5 Rf Hal           | 26            | 57           | 0            | 42           | 0.31 <sup>6,7,8*</sup> | 0.22–0.42    | 1.00 <sup>8†</sup>   | 0.91–1.00    | 1.00 <sup>7,8†</sup> | 0.87–1.00            | 0.42 <sup>6,7,8*</sup> | 0.33–0.53    | 0.54 <sup>6,7,8*</sup>   |
| 6 Rf Allergopharma | 53            | 30           | 3            | 39           | 0.64 <sup>5,8*</sup>   | 0.53–0.74    | 0.93 <sup>8†</sup>   | 0.81–0.99    | 0.95 <sup>8†</sup>   | 0.85–0.99            | 0.57 <sup>5,8*</sup>   | 0.44–0.68    | 0.74 <sup>5,7,8*</sup>   |
| 7 Rf Bencard       | 62            | 21           | 6            | 36           | 0.75 <sup>5,8*</sup>   | 0.64–0.84    | 0.86 <sup>NS</sup>   | 0.71–0.95    | 0.91 <sup>5†</sup>   | 0.82–0.97            | 0.63 <sup>5,8*</sup>   | 0.49–0.76    | 0.78 <sup>5,6,8*</sup>   |
| 8 Rf BGFA          | 80            | 3            | 11           | 31           | 0.96 <sup>5,6,7*</sup> | 0.90–0.99    | 0.74 <sup>5,6†</sup> | 0.58–0.86    | 0.88 <sup>5,6†</sup> | 0.79–0.94            | 0.91 <sup>5,6,7*</sup> | 0.76–0.98    | 0.89 <sup>5,6,7*</sup>   |

A weal size of ≥ 1.5 mm was defined as positive.

Significantly different in comparison to the SPT solutions with the mentioned SPT solution number (see first column),

\* $P < 0.0001$ , <sup>†</sup> $P < 0.01$ , <sup>§</sup> $P < 0.05$ .

NS, not significant; PPV, positive predictive value; NPV, negative predictive value; pos., positive; neg., negative; SPT, skin prick test; CI, confidence interval.

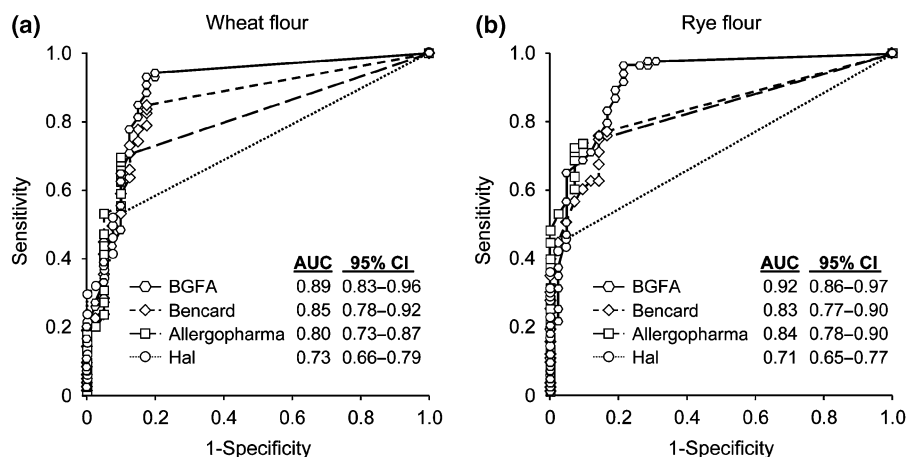


Fig. 1. Receiver-operating characteristic curves for the four wheat flour (a) and rye flour (b) skin prick testing solutions. Presented are results of 125 bakers with the gold standard flour-sIgE including area under the curve (AUC) and 95% confidence interval (95% CI).

Table 3. Comparative evaluation of rye flour (Rf) SPT solutions (cut-off level  $\geq 1.5$  mm) in a subgroup of 95 bakers with gold standard rye flour-sIgE and specific challenge with rye flour

| SPT solution                       | Gold standard positive |              | Gold standard negative |              | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI)     | NPV (95% CI)     | Test efficiency (95% CI) |
|------------------------------------|------------------------|--------------|------------------------|--------------|----------------------|----------------------|------------------|------------------|--------------------------|
|                                    | SPT pos. (n)           | SPT neg. (n) | SPT pos. (n)           | SPT neg. (n) |                      |                      |                  |                  |                          |
|                                    |                        |              |                        |              |                      |                      |                  |                  |                          |
| Gold standard 'rye flour-sIgE'     |                        |              |                        |              |                      |                      |                  |                  |                          |
| Rf Hal                             | 17                     | 50           | 0                      | 28           | 0.25 (0.16–0.37)     | 1.00 (0.88–1.00)     | 1.00 (0.80–1.00) | 0.36 (0.25–0.48) | 0.47 (0.37–0.57)         |
| Rf Allergopharma                   | 42                     | 25           | 1                      | 27           | 0.63 (0.50–0.74)     | 0.96 (0.82–1.00)     | 0.98 (0.88–1.00) | 0.52 (0.38–0.66) | 0.73 (0.64–0.82)         |
| Rf Bencard                         | 50                     | 17           | 3                      | 25           | 0.75 (0.63–0.84)     | 0.89 (0.72–0.98)     | 0.94 (0.84–0.99) | 0.60 (0.43–0.74) | 0.78 (0.71–0.87)         |
| Rf BGFA                            | 65                     | 2            | 5                      | 23           | 0.97 (0.90–1.00)     | 0.82 (0.63–0.94)     | 0.93 (0.84–0.98) | 0.92 (0.74–0.99) | 0.93 (0.87–0.98)         |
| Gold standard 'specific challenge' |                        |              |                        |              |                      |                      |                  |                  |                          |
| Rf Hal                             | 16                     | 47           | 1                      | 31           | 0.25 (0.15–0.38)     | 0.97 (0.84–1.00)     | 0.94 (0.71–1.00) | 0.40 (0.29–0.51) | 0.49 (0.39–0.60)         |
| Rf Allergopharma                   | 39                     | 24           | 4                      | 28           | 0.62 (0.49–0.74)     | 0.88 (0.71–0.96)     | 0.91 (0.78–0.97) | 0.54 (0.39–0.68) | 0.71 (0.61–0.80)         |
| Rf Bencard                         | 48                     | 15           | 5                      | 27           | 0.76 (0.64–0.86)     | 0.84 (0.67–0.95)     | 0.91 (0.79–0.97) | 0.64 (0.48–0.78) | 0.79 (0.71–0.87)         |
| Rf BGFA                            | 58                     | 5            | 12                     | 20           | 0.92 (0.82–0.97)     | 0.63 (0.44–0.79)     | 0.83 (0.72–0.91) | 0.80 (0.59–0.93) | 0.82 (0.74–0.90)         |

SPT, skin prick test; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; pos., positive; neg., negative.

### Protein and antigen analysis of the skin prick test solutions

SPT solutions were analysed for protein and antigen contents (Table 4) and by SDS-PAGE (Fig. 2). The protein patterns and antigen contents of the SPT solutions showed considerable differences; antigen concentrations ranged from 49 to 945 U/mL for wheat flour and from 11 to 4766 U/mL for rye flour. Sensitivities, NPV, and test efficiencies were positively associated with antigen content, whereas weak negative associations were demonstrated for specificities and PPV (Tables 2 and 4).

### Discussion

Skin testing remains the central test to confirm an allergic response. It is minimally invasive, the results are easily quantifiable and correlate well with organ

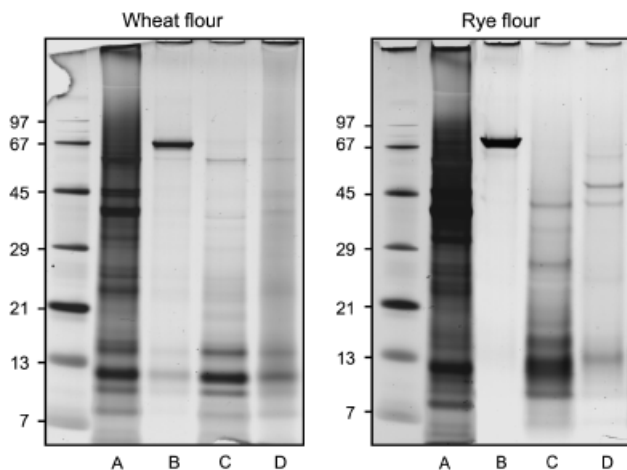
challenges [14]. Additionally, SPT is less expensive than other tests in allergy diagnosis [15]. SPT is also the basic method for diagnosing sensitization to occupational allergens, and often its outcome is relevant for compensation and socio-economic consequences. Therefore, this diagnostic test should not be limited by SPT solutions of low quality.

Comparative studies of SPT solutions from different producers have rarely been performed, especially with occupational allergens. Evaluation of latex SPT solutions was performed in a study with 46 paediatric patients with suspected latex allergy. Sensitivities of three commercial latex SPT solutions varied from 65% to 96% and specificities from 88% to 94% [16].

In an earlier study, two commercial wheat flour and rye flour SPT solutions (ALK-Abelló and Bencard) had been

**Table 4.** Protein and antigen concentrations in wheat and rye flour skin prick test (SPT) solutions

| SPT solution  | Wheat flour                  |                | Rye flour                    |                |
|---------------|------------------------------|----------------|------------------------------|----------------|
|               | Protein ( $\mu\text{g/mL}$ ) | Antigen (U/mL) | Protein ( $\mu\text{g/mL}$ ) | Antigen (U/mL) |
| Hal           | 237                          | 49             | 223                          | 11             |
| Allergopharma | 191                          | 158            | 183                          | 23             |
| Bencard       | 451                          | 501            | 402                          | 248            |
| BGFA          | 1827                         | 945            | 2176                         | 4766           |

**Fig. 2.** Equal volumes of skin prick testing solutions were separated in Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and silver stained. Lane A, BGFA; lane B, Hal; lane C, Bencard; lane D, Allergopharma.

assessed in 51 (wheat) and 69 (rye) symptomatic bakers in relation to specific bronchial challenge tests as the gold standard, although all solutions were not tested in each baker [4]. Sensitivities of SPT obtained in that study with a cut-off level of at least 50% of the histamine control were 45% (ALK-Abelló), and 67% (Bencard) for wheat flour, and 40% (ALK-Abelló) and 50% (Bencard) for rye flour. Specificities between producers varied from 86% to 100% (both flours).

In the present multi-centre study, we increased the number of subjects tested and the number of test solutions: SPTs with four different wheat and rye flour SPT solutions were performed in 125 symptomatic bakers using a standardized SPT protocol, which makes the results of the seven participating centres comparable. By testing the solutions according to a precisely defined protocol, we were able to exclude that the arm (right/left) or the test position on the arm affected the outcome.

The quality of the SPT solutions differed considerably. Interestingly, variations of specificities (74–100%) and PPV (88–100%) were considerably smaller than those of sensitivities (31–96%), NPV (42–91%), and test efficiencies (54–89%). This is in accordance with the previous

literature data. The SPT solutions from Hal reached a sensitivity of only 41% for wheat flour and 31% for rye flour, while a sensitivity of 78% (wheat) and 75% (rye) could be obtained with the best commercial SPT solutions (Bencard). With the non-commercial SPT solutions (BGFA), sensitivities of 93% (wheat) and 96% (rye) could be achieved. In addition, the highest test efficiencies and AUCs were obtained with the non-commercial SPT solutions (BGFA).

Evaluation of SPT results with rye flour in a subgroup of 95 bakers in relation to both gold standards, sIgE and challenge tests, resulted in comparable values. It is often believed that IgE-mediated sensitization to occupational allergens may occur without disease, whereas challenge tests are considered as the gold standard for the diagnosis of occupational asthma. Our findings demonstrate a very close association between sensitization and challenge results in bakers if the test materials are well chosen. This is an important finding of the present study, as it questions the existence of IgE-mediated sensitization to occupational antigens without clinical disease (although this may occur in individual cases). It is of minor importance as to how sensitization is assessed: on comparing the diagnostic value of sIgE and SPT (with the best commercial test solution) with challenge tests as the gold standard, sIgE showed somewhat higher sensitivity, but lower specificity. Overall, sIgE and SPT performed equally well, and thus we recommend to include both tools in the initial diagnostic evaluation in baker's allergy.

The present study was performed in subjects with suspected occupational disease (suspected cases). Whereas predictive values depend largely on the prevalence rates of cases under study, sensitivity and specificity are presumably inherent properties of the test [17]. Thus, we used the Youden Index, considering sensitivity and specificity equally, to define the optimal positivity criterion (cut-off level), because this index should be independent of selection bias.

Large differences (546–8270 ng/mL) of the single mouse allergen Mus m 1 in three commercially available mouse extracts by ELISA were reported recently by others [18]. In that study, a more detailed analysis of the diagnostic efficiency of the different SPT solutions was not performed. In the present study, we also performed *in vitro* analysis of the flour SPT solutions, showing striking differences in the antigen content and the pattern of protein bands in SDS-PAGE. As a rule, solutions with a higher antigen content and more bands in SDS-PAGE showed higher sensitivities, NPV, and test efficiencies. While in our study the SPT solutions of Hal showed a moderate protein content and intensity of protein bands in SDS-PAGE, the antigen content was very low. This explains sensitivities below 40%. Taking into account the molecular weight of the protein band in SDS-PAGE and the manufacturer's information, most of the protein in the flour SPT solutions of Hal seems to be human serum albumin (67 kDa). Thus, the

amount of antigen correlates better with the quality of an SPT solution than the amount of total protein.

The highest sensitivities, NPV, and test efficiencies were obtained with the non-commercial solutions (BGFA). Besides the overall higher amount of antigen, the content of ethanol-soluble wheat or rye proteins in these solutions may have contributed to positive results. It is also known that ethanol-soluble cereal proteins are relevant allergens for baker's asthma [19, 20]. Thus, increasing the antigen content of flour SPT solutions is recommended to those manufacturers whose solutions show low test efficiencies.

It could be shown that for wheat and rye flour, the highest Youden Index was obtained already with small weal diameters ( $\geq 1.5$  mm). Although such small weal sizes are unusual in clinical practise, other studies confirm our findings. Based on a study involving > 11 000 subjects tested with extracts of HDM, cat, timothy grass, and *Cladosporium*, Bousquet et al. [21] stated that a cut-off level of over 0 mm is the most appropriate definition of positive SPT to assess allergic sensitization in epidemiological studies.

Based on the fact that positive SPT results in symptomatic bakers are good predictors for a positive challenge test, and in such cases specific challenges may even be dispensable [22], standardized extracts of high quality are required. The present study suggests that by increasing the antigen concentration of flour SPT solutions, it is possible to increase sensitivity without substantial loss of specificity.

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