

Original article

Allergen content of grass pollen preparations for skin prick testing and sublingual immunotherapy

Background: The allergen content of diagnostics and immunotherapeutics is crucial for effective diagnosis and treatment. The aim of this study was to quantify and compare the allergen content of different grass pollen preparations for skin prick testing and sublingual immunotherapy (SLIT).

Methods: Five skin prick test (SPT) solutions and 10 sublingual immunotherapeutics were analysed for protein and allergen concentration by Bradford assay, inhibition of IgE-binding to *Phleum pratense* ImmunoCAPs and content of the main allergen Phl p 5 by two-site enzyme immunoassay. In addition, the grass pollen preparations were compared by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting analyses.

Results: Protein concentrations of SPT solutions ranged from 15 to 427 µg/ml, and Phl p 5 concentrations ranged from 0.15 to 18.3 µg/ml. The ranking of SPT solutions concerning Phl p 5 content and IgE inhibition capacity was the same, and the ranking of protein and allergen content was closely correlated ($r = 0.9$). Protein content of the maintenance doses of the immunotherapeutics ranged from 5 to 153 µg, Phl p 5 content ranged from 0.2 to 21.6 µg. IgE inhibition capacity of the maintenance doses was closely correlated to their Phl p 5 and protein content. SDS-PAGE and immunoblots confirmed the differences in protein and allergen content.

Conclusions: Grass pollen preparations for SPT and SLIT varied greatly concerning protein and allergen content. Whereas this result corresponds to previous analyses results of SPT solutions, it was the first comparison of grass pollen immunotherapeutics. For diagnosis and therapy, these differences should be taken into account.

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Allergen extracts used for the diagnosis and treatment of allergy are currently based on extracts prepared from natural allergen sources and supplied by different manufacturers in Europe, who usually use their own in-house reference materials and their own proprietary units to express potencies. Recently, a review (1) summarized the information obtained by European manufacturers of sublingual immunotherapy (SLIT) allergen extracts on unit definition and dosage of allergens. In conclusion, the monthly maintenance dose the manufacturers recommended for SLIT was 5–45 times higher than the dose for subcutaneous immunotherapy, but no comparison was possible between the manufacturers. Even if the amount of major allergens was given, differences in the quantification technique, the reference extracts and antibodies used can influence the outcome.

Thus, for comparison of diagnostics and immunotherapeutics from different manufacturers, the same analytical methods and materials should be used. For some major allergens, enzyme-linked-immunosorbent assays (ELISAs) and recombinant reference allergens suited for

standardization exist, according to the criteria of the 'CREATE' project (development of certified reference materials for allergenic products and validation of methods for their quantification) (2). One of these major allergens is Phl p 5 from *Phleum pratense*, which belongs to the most relevant grass pollen allergens according to prevalence and potency. To quantify the amount of this major allergen in skin prick test (SPT) solutions and SLIT preparations for grass pollen allergy in our study, one of the validated ELISA protocols based on monoclonal antibodies to Phl p 5 (3) was used. Most grass pollen preparations are mixtures of different grass pollen species. However, based on the close molecular relationship between pollen allergens of different species of the Pooideae subfamily, high cross-reactivity was observed, allowing for the measure of group 5 allergens of various Pooideae species even with an ELISA based on monoclonal antibodies to Phl p 5 (4). In addition, most other groups of Pooideae allergens, especially groups 1, 2, 3, 4, 7 (calcium binding protein) and 12 (profilin), are highly cross-reactive, with the latter two named here being

panallergens of plant pollen. Therefore, the allergenic potencies of grass pollen extracts are not mainly derived from the number of species used for the mixtures, but from the doses of major and, with less impact, minor allergens (4–6). At the moment, validated assays for all grass pollen allergens are not available. Therefore, in addition to the Phl p 5 content measured with the ELISA based on monoclonal antibodies to Phl p 5, we used protein concentration and IgE inhibition to compare a panel of SPT solutions and immunotherapeutics for grass pollen allergy. No comparison of the allergen content of grass pollen immunotherapeutics has been performed before.

Methods

Grass pollen preparations for SPT and SLIT

Ten grass pollen immunotherapeutics (Slitone® B204 five grass pollen mix solution from ALK-Abelló, Madrid, Spain; Grazax® tablets with *P. pratense* pollen from ALK-Abelló, Hørsholm, Denmark; Allerbio sublingual 704 with five grass pollen mix solution from Allerbio, Varennes-en-Argonne, France; Allerslit® forte with six grass pollen mix solution from Allergopharma, Reinbek, Germany; Oralvac® plus B2 twelve grass pollen mix solution from Allergy Therapeutics, Worthing, UK; Igevac® grass pollen solution from ARTU Biologicals Europe B.V., Lelystad, The Netherlands; Sublivac® grass pollen solution from HAL Allergy B.V., Haarlem, The Netherlands; TOL SL grass pollen from Laboratorios LETI S.L., Tres Cantos-Madrid, Spain; Sulgen® MG01 six grass pollen mix solution from Immunotek, Madrid, Spain and Staloral® 688 five grass pollen mix solution from Stallergenes, Antony Cedex, France) and five SPT solutions (five grass pollen mix solution Alyostal 688 from Stallergenes, 12 grass pollen mix solution B2 from Allergy Therapeutics, six grass pollen mix solution 006 from Allergopharma, 10 grass pollen mix solution 07-30 from HAL and five grass pollen mix solution 299 from ALK-Abelló, Spain) were selected for the analysis. All grass pollen batches were obtained during the spring of 2007. In addition, Grazax® placebo tablets from ALK-Abelló, without an active component, were used as the control.

Patients' sera and specific IgE to grass pollen allergens

Two different pool sera (Pool A and Pool B) of 10 patients each, all suffering from hay fever and with specific IgE of CAP-classes ≥ 3 to *P. pratense* ImmunoCAP g6 measured with CAP-Fluorescent enzyme immunoassay (CAP-FEIA, ImmunoCAP 250, Phadia, Uppsala, Sweden), were characterized concerning IgE-binding to the following eight single *P. pratense* pollen allergens rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5b, rPhl p 6, rPhl p 7, rPhl p 11 and rPhl p 12. Results are shown in Table 1.

Protein determination

The protein concentration of the grass pollen solutions and a *P. pratense* pollen preparation obtained from Allergon, Ängelholm, Sweden, were measured by Bradford assay with bovine serum albumin used as the standard. The volumes of the recommended maintenance dosages (drops per day) were determined by weighing,

and the protein content per dosage was calculated. The Grazax® tablets were dissolved in 1 ml water before analysis.

Quantification of grass pollen allergens

To measure the major allergen Phl p 5, a monoclonal antibody-based ELISA distributed by Indoor Biotechnologies, Manchester, UK was used. This assay had been validated in the CREATE project using recombinant Phl p 5a (Allergopharma, Reinbek, Germany) as the reference, and was performed as previously described (3). Each grass pollen solution was serially diluted to fit the measuring range of the assay and, as a rule, the ELISA values of four dilutions in range measured twice on different days were used to calculate the Phl p 5 content with a four-parameter fit (Softmax Pro 4.7.1, Molecular Devices, Sunnyvale, CA, USA). The mean coefficient of variation (CV) of the dilution series was 13.3%, and the mean interassay CV was 11.2%.

To quantify the total content of IgE-binding grass pollen allergens, inhibition of specific IgE-binding of the pool sera to *P. pratense* ImmunoCAPs was measured with the ImmunoCAP system. Fifty microliters of diluted pool sera (Pool A: 1 : 15 and Pool B: 1 : 10) were mixed with 10 μ l of diluted grass pollen solution, and then incubated with a *P. pratense* ImmunoCAP. Bound IgE was measured as described by the manufacturer. With the *P. pratense* preparation from Allergon, a reference curve for inhibition was recorded with each pool serum. Using a four-parameter fit calculated with Softmax Pro, the allergen content of each grass pollen solution (serially diluted to fit the measuring range) was estimated in comparison to the *P. pratense* reference. All dilutions with values in range were used to calculate the allergen content of each grass pollen solution. An inhibition unit (IU_{Pool A} and IU_{Pool B}) of one is equivalent to the inhibition of IgE-binding obtained with 1 μ g of reference protein.

The Grazax® tablets were dissolved as described above.

Electrophoresis and immunoblotting

For analytical sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), 10 μ l of SPT solutions, or 1/100 of the peak dose of immunotherapeutics, were mixed with Laemmli (7) sample buffer and separated electrophoretically in a pre-cast NuPAGE® Bis-Tris gel (Invitrogen, Groningen, the Netherlands). The analytical gels were silver stained according to the method of Blum et al. (8). For immunoblots, SPT solutions and immunotherapeutics were diluted and concentrated with Centricon centrifugal filter units (3000 MWCO, Millipore, MA, USA). For

Table 1. Specific IgE to *Phleum pratense* allergens of pooled sera

	Pool A	Pool B
	slgE [kU/l]	slgE [kU/l]
<i>P. pratense</i> (g6)	56.90	45.80
rPhl p 1	35.20	31.60
rPhl p 2	28.20	5.95
nPhl p 4	28.80	23.40
rPhl p 5b	56.60	29.00
rPhl p 6	10.00	12.00
rPhl p 7	3.20	1.20
rPhl p 11	32.00	1.60
rPhl p 12	0.41	1.40

electrophoresis, 10 µl of 10 : 1 concentrated SPT solution or 1/10 of the peak dose of immunotherapeutics were separated. The separated proteins were transferred by semi-dry blotting to polyvinylidene fluoride (PVDF) membrane (Millipore) (9). Blotted membranes were stained by Coomassie brilliant blue R-250, or blocked with 1% BSA and 1% polyvinylpyrrolidone-40 in TBS buffer (15 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 7.8) over night for immunostaining.

The blots were incubated overnight with patients' sera at room temperature and developed as described previously (9).

Statistical analysis

Spearman and Pearson correlation and two-tailed *P*-values of data obtained with the different methods of allergen quantification were calculated with the software GRAPHPAD PRISM (Graphpad Software Inc., La Jolla, CA, USA).

Results

Five SPT solutions of grass pollen mixtures were analysed for protein, allergen and Phl p 5 concentrations (Table 2). The grass pollen allergen concentration of SPT solutions reflects their capacity to inhibit IgE-binding of pooled sera to *P. pratense* ImmunoCAPs. Two different serum pools with slightly different reactivity to single *P. pratense* allergens (Table 1) were used for the inhibition experiments. The values obtained with serum Pool A were higher than with serum Pool B, reflecting relatively higher inhibition by SPT solutions in comparison to the grass pollen preparation used as the standard.

All methods show considerable differences between SPT preparations from the different manufacturers. However, the ranking according to the values obtained by the different methods basically remained the same, e.g. with the highest concentrations in the HAL SPT solution, and lowest concentrations in the solution from Allergy Therapeutics. The values obtained with the different methods are significantly correlated (Table 3).

Sublingual immunotherapeutics are applied in most cases, starting with a low dosage that is gradually increased until a maximum dosage (maintenance dose) is reached. Protein content of the starting solutions from Slitone[®], Oralvac[®] plus, Tol SL, Staloral[®] 300 and Sulgen[®] A was below the detection limit of the protein assay. The same was found for the Phl p 5 content of the

starting solutions from Oralvac[®] plus and Tol SL. The starting doses of Slitone[®], Sulgen[®] A and Staloral[®] contained a few nanograms of Phl p 5 only, while the lowest dose of Igevac[®], Sublivac[®], Allerslit[®] forte and Grazax[®] contained 390, 720, 4700 and 4950 ng of Phl p 5, respectively. The protein and allergen content of the maintenance doses of immunotherapeutics are shown in Table 4. Substantial differences in the content of protein, allergen (IgE-inhibition) and the major allergen Phl p 5 were observed between the different immunotherapeutics, up to factors of 30, 60 and 100, respectively. However, the values and ranking of the immunotherapeutics obtained with the different measurements are closely correlated (Table 5).

In addition, the SPT solutions and immunotherapeutics were analysed using SDS-PAGE and by immunoblot with the two pool sera (Figs 1 and 2). While the band pattern of all grass pollen solutions was similar, the density of protein bands differed. Immunotherapeutics have been sorted on the gels according to the protein content of the peak doses during maintenance treatment. Silver and Coomassie staining of the gels and the immunoblots confirmed differences in protein and allergen content of the preparations from the different manufacturers, with the exception of the Grazax[®] tablet (Fig. 2, lane 9) which could not be separated in preparative SDS-PAGE (Fig. 2B–D). Grazax[®] tablets contain fish gelatin, which interfered with separation and staining of the grass pollen proteins in cases of high load. The immunoblots obtained with the two different pool sera were very similar (Figs 1C,D and 2C,D).

Discussion

The comparison of the SPT solutions from the different manufacturers revealed great variation in protein, allergen and Phl p 5 contents, with a significant correlation between the results of these methods. The differences between the solutions with the highest (HAL) and lowest (Allergy Therapeutics) values amounted to a factor of 28.5 for protein, and 21.5–53 for allergen, depending on the IgE-pool used, and even a factor of 122 for Phl p 5 concentration. This is consistent with the results of Meno

Table 2. Protein, allergen and Phl p 5 concentration of SPT solutions

Manufacturer	Conc.	Protein (µg/ml)	Allergen (IU _{Pool A} /ml)	Allergen (IU _{Pool B} /ml)	Phl p 5 (µg/ml)
Allergopharma	50 000 SBE/ml	77	502	137	8.75
ALK-Abelló	10 HEP	84	315	122	4.19
Allergy Therapeutics	10 000 DU/ml	15	59	15	0.15
HAL Allergy	10 000 AU/ml	427	3125	323	18.30
Stallergenes	100 RI	30	151	104	2.70

SBE, standardized biological units; HEP, histamine equivalent prick; DU, diagnostic units; AU, allergen units; RI, Reaktionsindex. IU: IgE Inhibition units, IgE of Pool A and Pool B to *Phleum pratense* allergens are shown in Table 1.

Table 3. Correlation of the values (Pearson coefficient r) and rank (Spearman coefficient r , in brackets) between the different methods (see Table 2)

	Protein	Allergen _{Pool A}	Allergen _{Pool B}	Phl p 5
Protein	1			
Allergen _{Pool A}	0.996*** (0.900)	1		
Allergen _{Pool B}	0.954* (0.900)	0.945* (1.000)	1	
Phl p 5	0.946* (0.900)	0.947* (1.000)	0.971** (1.000)	1

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

et al. (6), who found that the grass pollen SPT solution from Allergy Therapeutics contained nearly no group 5 allergen, and that the solution from HAL contained considerably more groups 1 and 5 allergens when compared with the other products. A SPT study with 28 clinically *P. pratense*-sensitized subjects and 25 controls without grass pollen sensitization revealed (wheal sizes ≥ 3 mm considered positive) 100% sensitivity and specificity with the solution with the highest *P. pratense* concentration, and a loss of only 3.6% or 7.1% sensitivity if this solution was diluted by a factor of 9 or 27, respectively (10). Thus, the useful diagnostic range of grass pollen SPT solutions may be rather broad. In a recent study, with *P. pratense* SPT solutions from four manufacturers, a great variation between the content of single allergens was also found, although all gave a positive reaction in SPTs in 10 grass pollen allergic patients (11). However, it is not possible from the *in vitro* data of our study to conclude which of the tested grass pollen solutions yields the optimal compromise concerning sensitivity and specificity for grass pollen diagnostics. Nevertheless, it is helpful to have units of allergen content of SPT solutions, e.g. the content of the major allergen Phl p 5 quantified with a standard method, instead of manufacturer-dependant units only, to interpret and

Table 5. Correlation of the values (Pearson coefficient r) and rank (Spearman coefficient r , in brackets) between the different methods (see Table 4).

	Protein	Allergen _{Pool A}	Allergen _{Pool B}	Phl p 5
Protein	1			
Allergen _{Pool A}	0.967*** (0.976***)	1		
Allergen _{Pool B}	0.882*** (0.924***)	0.928*** (0.912***)	1	
Phl p 5	0.804** (0.961***)	0.913*** (0.948***)	0.857** (0.963***)	1

** $P < 0.01$, *** $P < 0.001$.

transfer the results of *in vivo* studies with grass pollen SPTs.

The same is true, and probably even more important, for interpretation of the results of *in vivo* studies with grass pollen immunotherapy. Especially for SLIT, the content of allergens seems to be important for the success of treatment. The differences between the immunotherapeutics with the lowest vs highest maintenance dose amounted to a factor of 31 for protein, 26–60 for allergen, depending on the used serum pool, and to 108 for the daily Phl p 5 dosages. Similarly, low protein, allergen and Phl p 5 daily maintenance dosages were shown for the Slitone[®] and TOL SL grass pollen therapeutics, from ALK and LETI, respectively, while the highest daily dosage was obtained with Allerslit[®] forte from Allergopharma with each method. High dosages of protein, allergen and Phl p 5 also yielded the sublingual grass pollen immunotherapeutics Staloral[®] 300, Igevac[®] and Grazax[®], from Stallergenes, ARTU and ALK, respectively. In some cases, information by the manufacturer regarding Phl p 5 content in grass pollen immunotherapeutics was available (1, 12). According to these concentrations, the daily maintenance dose of the major allergen Phl p 5 should be about 1 μ g in Leti Tol SL, 9 μ g in HAL Sublivac[®], 15 μ g in ALK Grazax[®], and 20 μ g in

Table 4. Protein, allergen and Phl p 5 content of the daily peak dose during maintenance treatment of immunotherapeutics

Manufacturer	Brand (conc.)	Protein (μ g)	Allergen (IU _{PoolA})	Allergen (IU _{PoolB})	Phl p 5 (μ g)
Laboratorios LETI	Tol SL (100 HEPL/ml)	5	17	11	0.2
ALK-Abelló	SLIT one (1000 STU/ml)	6	15	11	0.2
Allergy Therapeutics	Oralvac plus (768 000 TU/ml)	14	40	33	0.6
Immunotek	Sulgen (30 000 TU/ml)	23	129	104	0.9
Allerbio	Sublingual (100 RE/ml)	46	106	119	1.7
HAL Allergy	Sublivac (10 000 AU/ml)	56	226	88	3.6
ARTU Biologicals	Igevac (9500 BE/ml)	102	511	216	7.8
Stallergenes	Staloral 300 (300 IR/ml)	107	564	252	8.4
ALK-Abelló	Grazax (75 000 SQ-T)	150*	596	158	5.0
Allergopharma	Allerslit forte (715 000 SE/ml)	153	894	285	21.6

HEPL, histamine equivalent prick leti; STU, standard treatment units; TU, therapeutic units; RE, biologische Einheiten; AU, allergen units; BE, biologische Einheiten; IR, index of reactivity; SQ-T, standardized quality units tablet; SE, sublinguale Einheiten.

IU: IgE Inhibition units, IgE of Pool A and Pool B to *Phleum pratense* allergens are shown in Table 1.

*The Grazax tablet contains fish gelatin. The protein content of a placebo tablet without active component was determined to 117 μ g in Bradford assay.

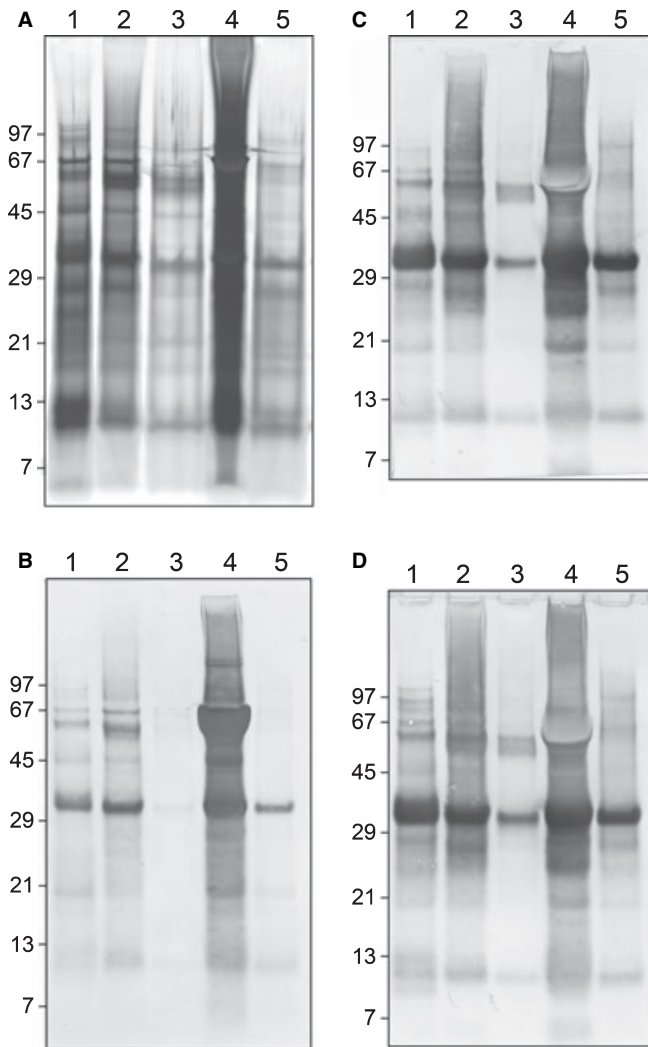


Figure 1. SPT solutions (1 Allergopharma, 2 ALK-Abello, 3 Allergy Therapeutics, 4 HAL, 5 Stallergenes) were separated by SDS-PAGE, silver stained (A), blotted and stained with Coomassie (B), or blotted and IgE-immunostained with Pool A (C) or Pool B (D).

Stallergenes Staloral[®], when compared with 0.2 µg (Tol SL), 3.6 µg (Sublivac[®]), 5 µg (Grazax[®]) and 8.4 µg (Staloral[®]) Phl p 5 measured in our study. While the rankings remain the same, the measured values are approximately a factor of 2.4–5 lower than expected. This deviation is most probably due to the different assays and/or standards used. During the CREATE project, four Phl p 5 assays were performed in five different laboratories, and the results obtained with different standards, natural Phl p 5 and recombinant isoforms rPhl p 5a and rPhl p 5b, were compared. Two assays were found to detect all Phl p 5 isoforms and to work properly in all laboratories. The assay now distributed by Indoor Biotechnologies, and with rPhl p 5a as the standard, which had been used for our measurements,

usually gave the lowest values in the CREATE study (3). If the manufacturers use another assay or natural Phl p 5 as standard to quantify the Phl p 5 content in their product, higher values than in our measurements would be expected. However, the manufacturers' methods for quantification of Phl p 5 are not given, although it is likely that, for quantification of Phl p 5 in Grazax[®], the assay from ALK (4) was used, which was the other validated Phl p 5 assay in the CREATE project.

Although several other methods to quantify single grass pollen allergens exist (5, 11), these are not yet validated by different laboratories and are not commercially available. Therefore, in addition to the major allergen content of Phl p 5, we analysed relative IgE-inhibition capacity of the grass pollen preparations. Being aware that the sensitization pattern of sera influences the outcome of IgE-inhibition experiments, we used two pool sera of 10 different patients each, and characterized the pool sera concerning binding to single *P. pratense* allergens. Both pools had similarly high IgE-titers to rPhl p 1, rPhl p 5b and nPhl p 4, but differed in their titers to Phl p 2 and Phl p 11, which were lower in Pool B than in Pool A. All of these allergens are detected with high frequency in grass pollen sensitized patients, while IgE-titers are found to be the highest with group 5 allergens, followed by group 1 and group 4 allergens, and lower to group 2 allergens and group 11 allergens (13–15). The sum of IgE reactivities to single allergens was much higher than the value obtained with the *P. pratense* extract CAP gx6. This phenomenon had been observed previously by Mari (15). He found a two to eight times higher sum of IgE values to single *P. pratense* allergens than to the extract CAP, even if sIgE to rPhl p 6 was skipped for calculation of the sum to avoid double detections of binding to same epitopes as on rPhl p 5. His explanation was a limiting factor represented by the amount of allergenic and non-allergenic components in an extract. It is an advantage of the analysis with single allergen-CAPs that quantitative limitations of the allergen on the solid phase can be avoided. Overall, the two pool sera showed typical binding patterns. Relative inhibition capacities of grass pollen extracts obtained with serum Pool A were systematically higher as with Pool B. This may be due to a higher content of group 2 and group 11 grass pollen allergens in the diagnostic and immunotherapeutic grass pollen preparations in comparison to the *P. pratense* standard used. However, the ranking of SPT solutions was the same with both pools and only slightly different for immunotherapeutics; for example, the ALK Grazax[®] and the HAL Sublivac[®] immunotherapeutics had slightly higher ranks with Pool A than with Pool B. As additional methods for the comparison of the extracts SDS-PAGE and Western blots were used. Both, Coomassie staining of the separated protein bands in gels and IgE-dependant staining of immunoblots are

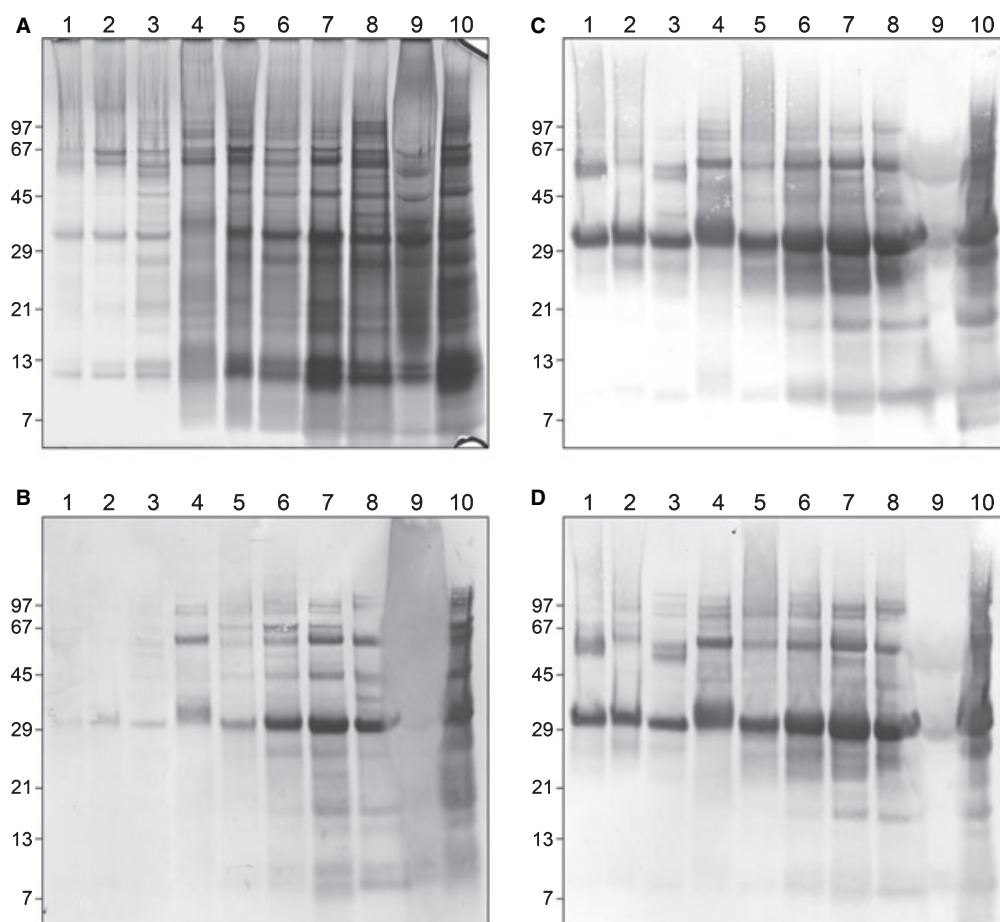


Figure 2. Immunotherapeutics (1 Laboratorios LETI Tol SL, 2 ALK-Abelló SLITone, 3 Allergy Therapeutics Oralvac plus, 4 Immunotek Sulgen, 5 Allerbio Sublingual, 6 HAL Allergy Sublivac, 7 ARTU Biologicals Igevac, 8 Stallergenes Staloral 300, 9 ALK-Abelló Grazax, 10 Allergopharma Allerslit forte) were separated by SDS-PAGE and silver stained (A), blotted and stained with Coomassie (B), or blotted and IgE-immunostained with Pool A (C) or Pool B (D). For each lane, either 1% (A) or 10% (B, C, D) of the peak doses during maintenance treatment were loaded.

well-established semi-quantitative visualization methods for proteins and allergens, respectively. The protein bands after Coomassie staining and immunodetection with both pools gave very similar patterns, although the difference between grass pollen preparations with low or high protein/allergen content seemed less remarkable in the immunostainings. Even a very low content of group 5 or group 1 allergen not visible by Coomassie staining at molecular weights of 30–35 kDa was well detected by IgE-staining. Overall, the staining patterns confirmed the data of the protein and allergen analyses with the exception of the Grazax[®] tablet, which was not separated and stained in preparative SDS-PAGE and immunoblots because of its content of fish gelatin. The gelatin is also measured in the Bradford protein assay (a placebo tablet contained about 117 µg protein), contributing to the measured protein content in the Grazax[®] tablet. However, there is no indication that this component influences IgE-inhibition or Phl p 5 quantification

by ELISA, because high dilutions (1 tablet in 10–400 ml) could be used for CAP-Inhibition and ELISA, and the binding curves were parallel to the standard curves.

Overall, there was a high correlation between Phl p 5 content, IgE-inhibition capacity and protein content of the grass pollen preparations, indicating that the proportion of group 5 allergens in natural grass pollen extracts does not significantly differ. Therefore, the standardized quantification of Phl p 5 content is a valuable tool for indicating allergen potency in grass pollen diagnostics and therapeutics.

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