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Variation in allergen content in Sublingual Allergen Immunotherapy (SLIT) with house dust mites (HDM)

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Conflict of interests

The authors had no conflict of interest.

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Key words: allergen content, efficacy, evidence based medicine, house dust mites' sublingual allergen immunotherapy, weekly dosage

Short title: Allergen content in SLIT-HDM

Abstract:

- **Background:** Allergen immunotherapy is a treatment modality which can be applied using different vaccines. The aim of this study was to quantify and compare the allergen content of different house dust mites' sublingual treatments and to review the evidence on their efficacy.

- **Methods:** Five sublingual allergen immunotherapy products were ordered and purchased at an ordinary pharmacy and masked for blinding before study was started. Detection of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* allergens Der p 1, Der f 1, Der p 2 and Der f 2 was carried out by immunoblotting and fluorescent multiplex. A literature search for meta-analyses and systematic reviews that included SLIT-HDM products was performed.

- **Results:** Der p 1 concentrations ranged from 0.6 to 14.5 µg/ml; similar figures were found for Der f 1 that ranged from 0.2 to 12.4 µg/ml. Der p 2 + Der f 2 ranged from 0.2 to 1.5 µg/ml. Data on efficacy are scarce for most of the five products.

- **Conclusions:** Substantial variations regarding allergen content were found among these five SLIT-HDM products. Therefore, it can be necessary to guarantee the quality of the SLIT-HDM products and to demonstrate their effectiveness before they are marketed. It seems necessary, for the moment, to take into account these characteristics of the products before prescribing.

Introduction

In the last decade, the gradual development of specific immunological and molecular biology techniques has allowed us to reach a more accurate diagnosis through the identification of allergenic components (molecular diagnosis) (1-4) and it has also helped to develop AIT treatments with a precise allergen content and, therefore, more effective and safer. Beyond the progress in diagnosis, the publication of European guidelines (5) defining the methodology for clinical trials has allowed investigators to better demonstrate the value of the AIT supported by evidence-based medicine (6). Currently, 20 meta-analyses assessing effectiveness of AIT in various indications (asthma and rhinitis), by different administration routes, and based on different allergens are available, but only a few products available for prescription are represented in existing meta-analyses (7). Then an allergist are not sure if the products available to prescribe are effective (8,9).

Regarding SLIT-HDM allergens, both the dosages recommended by the different manufacturers in Europe (10) and the composition and quantification of the allergens (11) are diverse. Extracts are complex mixtures of biological materials, and each manufacturer uses a different reference unit (in-house reference standard) to provide batch to batch consistency.

Knowing the allergen content of each therapeutic product is a critical point; however, a fully standardized assessment of allergens is not yet available in Europe despite the efforts of the CREATE project (12). Only some specific techniques and materials are recognized by such project as candidates to be used as standards (13).

We set out to 1) measure the protein composition and to quantify the major allergens in SLIT-HDM products available for prescription in Spain, using the same measurement methods; 2) identify, by means of a systematic review of the literature, the existing data on the allergen content and the biological activity of products included; and 3) review the scientific evidence on their effectiveness.

Methods

In vitro analysis

A comparative study of the five most relevant SLIT-HDM products in Spain (produced under Good Manufacturing Practices conditions) was carried out (in alphabetical order: Allergovac Sublingual Plus, Bial-Aristegui; SLITone ULTRA, ALK-Abello; Staloral 300 Rapid, Stallergenes; Sublivac, Hal Allergy; TOL Forte, Laboratorios Leti). All of them were ordered and purchased at an ordinary

pharmacy in Cádiz (Spain), and kept in refrigerator under the same conditions in which patients undergo treatment. Each extract was masked for blinding and a number (from 1 to 5) was randomly assigned to each one before study was started, so the investigators were not aware, at any time of the study, of which product they were testing. All analyses were performed at the Research and Development Unit, Lobaton Clinic, S.L.P., Cádiz, Spain).

Details on vaccines manufacturing, composition, and excipients are shown in Table 1. Excipient concentrations of each extract are not available. To remove all possible excipients, each of the extracts was treated with Vivaspin® 3.0K (Sartorius, Hannover, Germany). Initially the membranes were washed with deionized water (Milli-Q water, HPLC grade, 18 MΩ from a A10-Synthesis water polishing system, Millipore). Milli-Q water 15 ml was added to each 5 ml of extract, followed by a centrifugation according to the instructions of the manufacturer. In this way, glycerol and other salts were removed. Centrifugation time needed to reach initial sample volume was used. After treatment with Vivaspin 3.0K, total protein levels measured by BCA were consistent with those found in SDS-PAGE gels. The removal of glycerol and other excipients was needed to avoid interferences with the measurements when taking the same volume for different extracts, as it has been stated by other authors (14) and in the datasheet of the BCA method. However, an analysis was made with the same method without the use of Vivaspin® 3.0K in order to evaluate the homogeneity of the results without the removal of glycerol (see repository). Samples were homogenized by pipetting before sample loading to gel.

Two different batches of HDM-SLIT maintenance products, all of them consisting of a mix 1:1 *D. pteronyssinus* : *D. farinae*, were used (see Table 1).

SDS-PAGE and protein estimation

Equal volumes of each *D. pteronyssinus* : *D. farinae* extract (15 µl) after treatment with Vivaspin® 3.0K, were applied to 14% SDS-PAGE in reducing conditions and proteins were visualized by silver staining (15). Electrophoresis was running in a Mini-Protean System (Biorad) to 90 V during 1,5 hours. Protein content of the extracts was measured using Pierce® Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Scientific) (16).

Quantification of Der p 1, Der f 1 and Group 2 of Dermatophagoides by fluorescent multiplex array (Bioplex).

Equal volumes of each *D. pteronyssinus* / *D. farinae* extract were separated by 14% SDS-PAGE and blotted onto nitrocellulose (Protan® Whatman, Dassel, Germany). Aliquots from each extract were diluted 1/500 in assay buffer (PBS, 1% BSA, 0.02% Tween-20, pH 7.4) after treatment with Vivaspin® 3.0K, as reported for SDS-PAGE. Again, an analysis with the same methodology without the use of Vivaspin® 3.0K was made in order to evaluate the heterogeneity of the results (coefficient of variation) without the removal of glycerol (see repository). Quantification of the allergens was performed by quantitative MARIA® assay according to the manufacturer's instructions (Indoor Biotechnologies, Cardiff, UK). Results were expressed in µg/mL after taking into account the dilution factor. The 12-point standard curve ranges were: 125-0.06 ng/mL for Der p1 and Der f1 and 50-0.02 ng/mL for Mite Group 2 (Der p2 + Der f2). Standard curves were prepared according to manufacturer instructions using control standard vial provided with the kit.

ProteOn XPR36 (Plasmon Resonance Interaction System) of D. pteronyssinus / D. farinae extracts

Immobilization of selected capture agent, anti-mouse IgG whole molecule antibody, on Proteon XPR36 GLM chip.

The immobilization step was performed in the horizontal orientation of the ProteOn XPR36 system using a flow rate of 30 µl/min at 25°C on a GLM chip (see repository). HDM major allergens were screened for with Proteon XPR36.

All binding measurements were performed with PBST as the continuous running buffer at 25°C, following the directions of Bronner *et al* (17) with some modifications. In summary, in all experiments 50 µg of monoclonal antibody were diluted with PBST and injected in the vertical orientation. ProteOn XPR36 fluidics was used for 6 min (150 µl) at 25 µl/min, allowing the mAbs to be captured by the capture agent on the GLM chip (see repository).

Data processing and analysis

All binding sensograms were collected, processed, and analyzed using the integrated ProteOn Manager software (Bio-Rad Laboratories) using Langmuir with drift conditions.

Literature Review

The identification of the studies in which the content or the biological potency of the SLIT-HDM products was reported was based on a literature research in Pubmed, Web of Science, and The Cochrane Library databases looking for meta-analyses on SLIT, the search was limited to the English language but not by publication date. The basic search terms were (“systematic review” OR “meta-analysis”) AND (“sublingual AND immunotherapy”) with their corresponding synonyms, MeSH terms and truncations. All the reports which included systematic reviews were selected for full text reading in order to gather data on composition and biological potency.

Results

SDS-PAGE and BCA method

Clear-cut differences in the definition of the bands can be seen for the five extracts in SDS-PAGE gels (Figure 1). A higher intensity is observed in the band of molecular weight (MW) 25 and 14 KDa in the product 1 and 2 (products are listed herein in the order of blinding).

Total protein content measurement was based on the bicinchoninic acid (BCA) method (see repository).

Detection of HDM major allergens by immunoblotting

As it can be seen in Figure 2, after the development with anti-Der p 1 or anti-Der f 1, product 1 had the highest band intensity, followed by product 2; the other products had a very weak intensity with the volume used. Using a development with anti-Der p 2 + anti-Der f 2, a band can be seen at a MW of 25 KDa non-specifically recognized, corresponding to Der p 1. Moreover, product 2 showed the highest band intensity for group 2, followed by product 1. In the other products, the band intensity corresponding to 14 KDa was very weak.

MARIA® and ProteOn

As shown in Table 2, both quantitative methods expressing data in µg/ml showed the same tendency for the HDM major allergens; again, products 1 and 2 exhibited higher concentrations for each allergen, mainly in group 1, than the rest of products.

Amount of HDM major allergens in µg administered per day, week and month for each extract

Based on the daily maintenance dose recommended by each manufacturer in Spain, the amount of allergens administered was calculated in terms of daily, weekly and monthly doses.

Monthly dosage of Der p 1 was higher than 100 µg for products 1 and 2, whereas for products 4 and 5 it was not higher than 14 µg in any analysis, and product 3 did not reach 2 µg. Similar figures could be observed for Der f 1 and Der p 2 + Der f 2. (Table 3)

The affinity constant (KD) values for HDM major allergens in each extract are summarized in Table 4. A lower KD value indicates a higher affinity of the antibody for the epitope. Product 3 and product 4 showed a higher (and thus worse) affinity constant (KD) for Der p 1 and Der f 1 compared to products 1, 2 and 5, which are found in the same order observed for the control extracts. Regarding Der p 2 + Der f 2, similar KD values were found except for product 3 that had a higher (worse) KD.

Literature Review

The literature search identified 55 reviews/meta-analyses (see repository). The review of these articles led to the identification of 30 clinical studies in which SLIT-HDM was used. A table in the repository displays the differences concerning the allergen content of the HDM products used in each study. Substantial differences were found in the allergen content of the HDM products used in each study. In some studies allergen content (9/30) and/or biological units (3/30) are not recorded. Only 2 of the 5 products studied are referenced and some of the studies published data with product presentations and dosages that are not commercialized now in Spain. Among the articles presenting data on the allergen content (21/30), the cumulative weekly administered doses varied across trials, with the minimum value being 1 µg and the highest amount reaching 332 µg for Der p 1. In the majority of the studies (22/30), a fifty/fifty *D. pteronyssinus* and *D. farinae* mix was used. Reported data on efficacy were scarce for most of the five products. A detailed report of the literature review will be published separately.

Discussion

Our study was performed with the SLIT-HDM products from five of the most important manufacturers in Spain, and revealed large differences, not only for protein content but also for major allergens content. Results showed a very good internal consistency, and were in line with the reviewed literature.

In the Western blot studies, we could observe that monoclonal antibody (Der p2 + Der f2), employed by the CREATE project, recognizes a few amount of Group 1 allergens like is shown in Figure 2. Besides, the data obtained with the antibody Der p 2 + Der f 2 yielded results that were too low, far from the expected values in the controls used for the *MARIA*®. Different sequence polymorphisms of the major allergens can influence the quantification when using this *MARIA*® kit (18-20). This fact could be responsible for such a low recognition in our assays.

We should also consider that within the CREATE project (12) no consensus was achieved for any of the main allergens of HDM, even though Der p 2 reached a high reproducibility (21). Our native extract controls for group 1 showed a high reproducibility; however that was not true for group 2. The consistency in the comparison is better when using the quantitative values for the major allergens in each extract calculated with both methods (*MARIA*®/ProteOn), especially when the removal of excipients with Vivaspin® 3.0K was made, and a good correlation is also observed with previous qualitative and quantitative values. Again, a difference between two of the products (Staloral 300 Rapid and SLITone Ultra), with values above 10 µg/ml for Der p 1, and the rest of the extracts (none of them exceeding 3 µg/ml) is confirmed.

The data obtained for group 2 should be interpreted cautiously because the controls used do not validate the results. Proportionally, we observed more Der p 2 – f 2 in SLITone Ultra (in agreement with the blot). For products 3, 4, and 5 the amount of group 2 was at the limit of quantification. However the potency measurements in HDM extracts are based on the presence of the major allergen (in this case Der p 1 + Der f 1) because the role of group 2 is uncertain (11).

We have used the specific monoclonal antibody as a ligand to measure each one of the major allergens in HDM. There are very few studies that use this technique in allergy (22-26).

Larenas-Linnemann *et al* (10) compared the protein content and the relative potency (ELISA) of various masked allergen extracts (among them *D. pteronyssinus*) for SLIT from four European manufacturers. The allergenic content range was 2.8-24.0 µg/mL for Der p 1 and 0-7.7 µg/mL for Der p 2.

Mosges *et al* (27) compared the biologic activity of two SLIT preparations in order to establish a "conversion factor" between them. The biologic activity was 22 times superior for one of them.

In the present study, the differences between the solutions with the highest (Staloral 300 Rapid and SLITone ULTRA) and lowest values amounted to a factor of more than 24 for Der p 1, more than 62 for Der f 1 and more than 7 for Der p 2 + Der f 2.

Other authors have also reported differences among HDM diagnostic products (15, 28).

Our results, based on a consistent methodology, allow reliable comparisons to be performed for the main extracts for SLIT treatment with marketed HDM allergens, that we obtained in the same way our patients do. Only some of them showed a significant allergen content, which confirms the heterogeneity of the marketed products suggested in the literature. Besides, these are the only ones that are backed by publications reporting efficacy in clinical trials (29). This was also highlighted by Sander *et al* (30) for SLIT based on grass preparations. They highlighted the need to take into account these differences when designing the treatment for patients allergic to grass pollen. We emphasize the same need when prescribing SLIT HDM in patients allergic to mites

There are no universally accepted AIT dosage guidelines, except for grass pollen tablets, and the meagre reported data suggest that some dosages could have been insufficient (7,31). Our review confirms a high heterogeneity in allergen content when reported, with values ranging from 1 µg to 332 µg for Der p 1 in calculated weekly doses.

In the current study, we compared the composition and main allergens amount of most relevant SLIT-HDM products in Spain. The methods endorsed by CREATE have been applied in a critical manner to study the characteristics of such extracts, with advantages and disadvantages of each technique being evaluated.

In contrast to previous composition studies (10,15,28,30), products were purchased in a pharmacy so that they were provided in the same way patients receive them, and afterwards masking for blinding was applied. Our data indicates that two out of the five studied extracts (Staloral 300 Rapid and SLITone ULTRA) are different from the rest. Interestingly, according to reported data, the scientific evidence currently available for most of them is scarce (29).

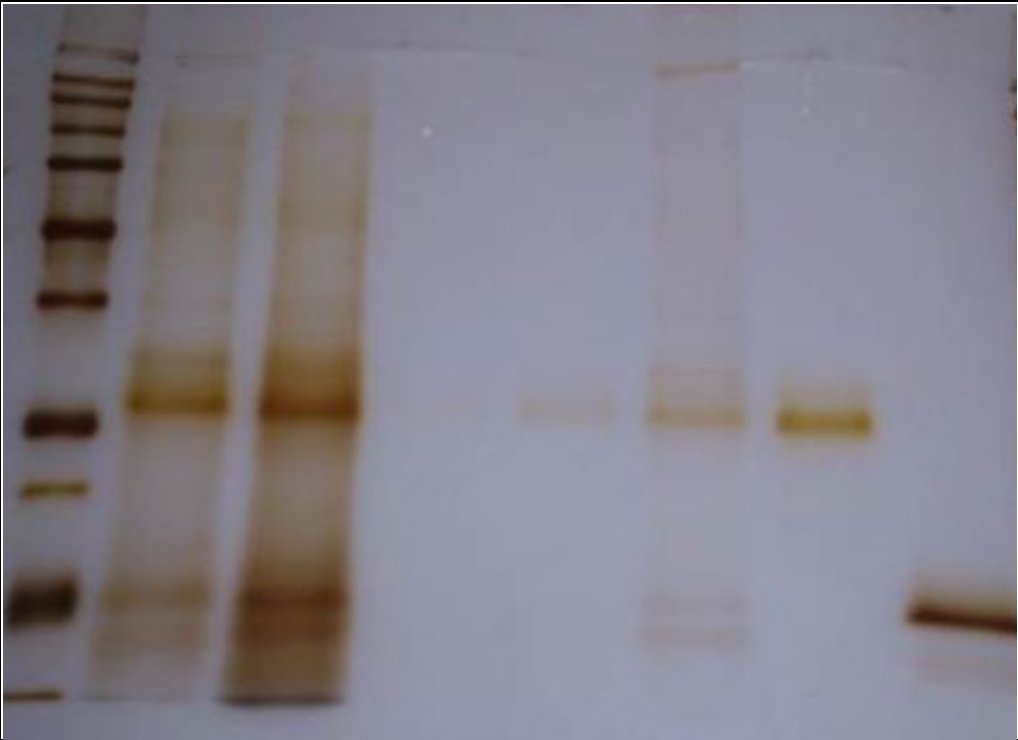
As we postulated and other authors have suggested, choice of a SLIT-HDM immunotherapy is now a difficult task. Clinicians need to rely on the evidence, and they have to know the quality assurance processes applied and the exact allergens content for each marketed product.

The data presented in this study highlight that not all SLIT products are equivalent, and for most of them there is a large scope for improvement. Thinking of our patients, we must keep moving toward that goal in the coming years.

Figure 1. SDS-PAGE (silver nitrate® staining is shown): Samples were loaded in sequence (blinded order), 1: Staloral Rapid 300; 2: SLITone ULTRA; 3: TOL Forte; 4: Sublingual Allergovac Plus; 5: Sublivac, with 6 and 7 being native controls for Der p 1 and Der p 2, respectively. M = marker for molecular weight between 250 - 10 kDa.

First lane corresponds to molecular weight marker, followed by extracts 1 to 5. Lanes 7 and 8 correspond to native Der p1 and Der p2 extracts. Bottom figures show BCA-measured protein levels for each extract (mg/ml)-

No significant differences were observed between the two studied batches of each extract in the SDS-PAGE gels. (Data not shown)

	Marker	Extract 1	Extract 2	Extract 3	Extract 4	Extract 5	nDer p1	nDer p2
250								
150								
100								
75								
50								
37								
25								
20								
15								
10								
	BCA (mg/ml)	305.16±13.47	770.24±16.83	61.11±3.93	276.58±17.96	197.22±4.49	26.98±1.62	13.13±0.56

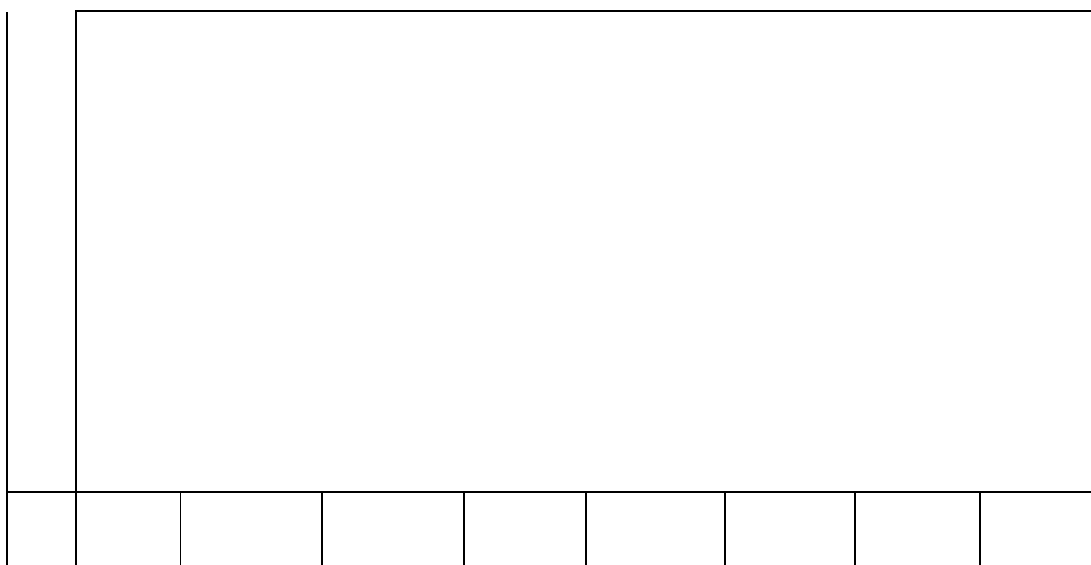


Figure 2. Immunoblotting. After transfer of proteins to PVDF membranes, they were faced against the following monoclonal antibodies, a) anti-Der p 1; b) anti-Der f 1; c) anti (Der p 2 + Der f 2). M = Marker MW. Blinded order: 1: Staloral 300 Rapid; 2: SLITone ULTRA; 3: TOL Forte; 4: Allergovac Sublingual Plus; 5: Sublivac 6: Control nDer p 1; 7: Control nDer f 1; 8: Control nDer p 2 + nDer f 2. On the top of gel, band intensity is shown for each sample present in gel, as measured in gels without signal saturation. Bands were quantified using Quantity One software.

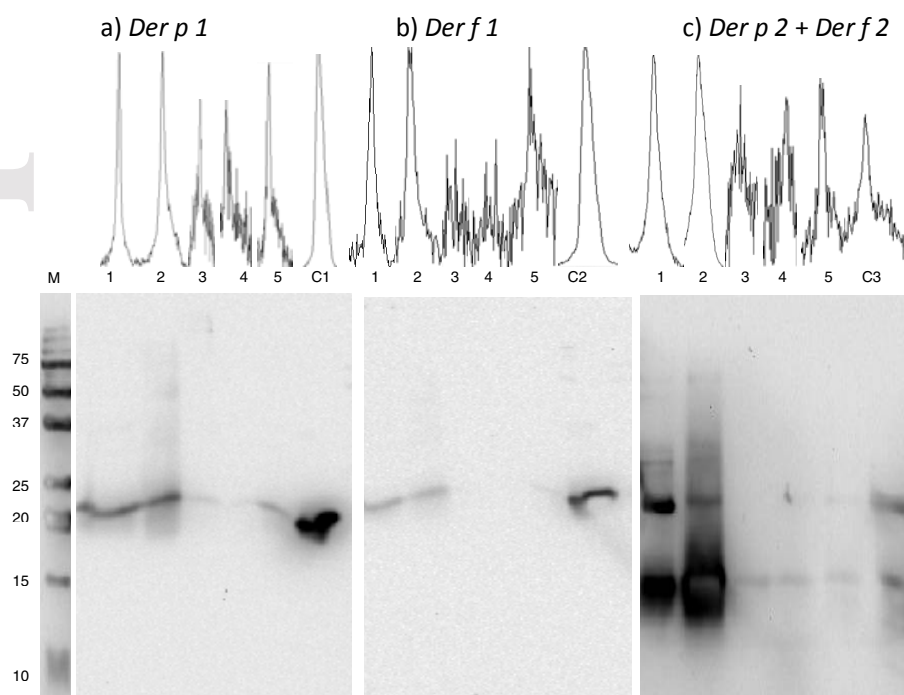


Table 1. Composition, additives, and manufacturing details of vaccines tested.

Extracts	Product name - Biological potency	Marketing lab	First Batch			Second Batch			Additives
			Reference	Manufacture re date	Expiration date	Reference	Manufacture re date	Expiration date	
1	Staloral 300 Rapid - 300 IR/mL	Stallergenes	00010850 47	02/2013	02/2014	00011115 23	07/2013	07/2014	Sodium chloride, glycerol, purified water
2	SLITone ULTRA - 300 SRU	Alk-Abello	02701625 1001	02/2013	09/2013	02719167 1001	09/2013	06/2014	Sodium chloride, glycerol, purified water, sodium bicarbonate
3	TOL Forte - 30 HEPL/mL	Laboratorios Leti	23690411 00	No data	11/2013	24810421 00	No data	02/2014	Sodium chloride, phenol, sodium phosphate monobasic dihydrate, sodium phosphate dibasic dodecahydrate, glycerol, purified water
4	Allergovac Sublingual Plus - 223432 SU/mL	Bial-Aristegui	ESX018107 4	02/2013	02/2014	ESB067368 2	09/2013	09/2014	Sodium chloride, glycerol, phenol, purified water
5	Sublivac - 10000 AU/mL	Hal Allergy	059779	02/2013	12/2013	064456	09/2013	08/2014	Sodium chloride, glycerol, phenol, purified water

Table 2. Measurements of allergen content with MARIA® and ProteOn

Content in µg/ml	MARIA	ProteOn
	mean ± SD	mean ± SD
Der p 1		
Staloral 300 Rapid	12.4 ± 1.6	11.1 ± 1.0
SLITone ULTRA	14.5 ± 1.0	12.8 ± 0.8
Tol forte	0.6 ± 0.3	0.5 ± 0.1
Allergovac Sublingual Plus	0.6 ± 0.1	2.5 ± 0.0
Sublivac	2.0 ± 1.4	1.1 ± 0.1
Der f 1		
Staloral 300 Rapid	12.4 ± 1.3	9.3 ± 0.7
SLITone ULTRA	6.2 ± 1.8	5.3 ± 1.9
Tol forte	0.3 ± 0.3	0.9 ± 0.1
Allergovac Sublingual Plus	0.2 ± 0.1	1.0 ± 0.3
Sublivac	2.8 ± 1.7	4.4 ± 1.9
Der p 2 + Der f 2		
Staloral 300 Rapid	0.6 ± 0.4	3.4 ± 0.3
SLITone ULTRA	1.5 ± 0.2	5.8 ± 1.6
Tol forte	0.1 ± 0.1	1.1 ± n/a
Allergovac Sublingual Plus	0.2 ± n/a	0.6 ± 0.8
Sublivac	0.2 ± 0.0	0.3 ± 0.3

n/a, not applicable; SD, standard deviation;

Products are displayed in blinded order (1: Staloral 300 Rapid; 2: SLITone Ultra; 3: Tol forte; 4: Allergovac Sublingual Plus, 5: Sublivac)
The data obtained with the antibody Der p 2 + Der f 2 supplied by Indoor Biotechnologies gave results which were too low, far from the expected values in the controls used for the MARIA assay (approximately 10 times lower than expected.)

Table 3. Calculation of daily, weekly and monthly ordinary dosages (µg) according to the measurements made with MARIA and ProteOn

Calculation of dosages according to determinations of MARIA															
	Daily dosage (µg/day)					Weekly dosage (µg/week)					Monthly* dosage (µg/month)				
	Staloral 300 Rapid	SLITone ULTRA	Tol forte	Allergovac Sublingual Plus	Sublivac	Staloral 300 Rapid	SLITone ULTRA	Tol forte	Allergovac Sublingual Plus	Sublivac	Staloral 300 Rapid	SLITone ULTRA	Tol forte	Allergovac Sublingual Plus	Sublivac
Der p 1 Mean ± SD	9.95 ± 1.26	7.23 ± 0.48	0.06 ± 0.03	0.15 ± 0.02	0.43 ± 0.3	29.84 ± 3.78	50.63 ± 3.34	0.40 ± 0.23	0.74 ± 0.11	3.01 ± 2.08	129.5 ± 16.4	219.7 ± 14.5	1.73 ± 1.0	3.2 ± 0.5	13.7 ± 9.0
Der f1 Mean ± SD	9.93 ± 1.06	3.11 ± 0.91	0.03 ± 0.03	0.05 ± 0.02	0.61 ± 0.36	29.78 ± 3.19	21.77 ± 6.38	0.23 ± 0.20	0.24 ± 0.08	4.27 ± 2.49	129.3 ± 13.9	94.5 ± 27.71	0.98 ± 0.8	1.0 ± 0.4	18.5 ± 10.79
Der p 2 +Der f 2 Mean ± SD	0.46 ± 0.32	0.76 ± 0.1	0.01 ± 0.01	0.04 ± n/a	0.04 ± 0.01	1.39 ± 0.95	5.34 ± 0.72	0.05 ± 0.04	0.18 ± n/a	0.31 ± 0.07	6.0 ± 4.1	23.2 ± 3.1	0.24 ± 0.2	0.78 ± n/a	1.36 ± 0.32
Calculation of the dosage according to determinations of ProteOn															
Der p 1 Mean ± SD	9.25 ± 0.27	6.42 ± 0.38	0.05 ± 0.01	0.6 ± 0.01	0.24 ± 0.02	26.59 ± 2.44	44.94 ± 2.67	0.32 ± 0.04	2.99 ± 0.05	1.67 ± 0.13	115.4 ± 10.6	195.0 ± 11.6	1.9 ± 0.5	12.97 ± 0.22	7.05 ± 0.28
Der f1 Mean ± SD	7.41 ± 0.54	2.67 ± 0.97	0.09 ± 0.01	0.23 ± 0.06	0.94 ± 0.4	22.23 ± 1.61	18.67 ± 6.76	0.63 ± 0.04	1.15 ± 0.30	6.57 ± 2.83	96.5 ± 7.0	81.0 ± 29.3	2.4 ± 0.2	4.97 ± 1.32	28.5 ± 12.3
Der p 2 +Der f 2 Mean ± SD	2.74 ± 0.20	2.91± 0.79	0.10 ± n/a	0.00 ± 0.20	0.07 ± 0.07	8.21 ± 0.61	11.97 ± 0.89	0.70 ± n/a	0.71 ± 0.98	0.47 ± 0.48	35.6 ± 2.6	88.4 ± 24.1	3.0 ± n/a	3.07 ± n/a	2.02 ± 2.12

* A month has been equalled to 4.34 weeks

n/a, not applicable; SD, standard deviation;

Administered volumes according to index record:

Extract 1: 800 µl/day, 2400 µl/week, 10416 µl/month

Extract 2: 500 µl/day, 3500 µl/week, 15190 µl/month

Extract 3: 95 µl/day, 665 µl/week, 2886.1 µl/month

Extract 4: 240 µl/day, 1200 µl/week, 5208 µl/month

Extract 5: 215 µl/day, 1505 µl/week, 6531.7 µl/month

Table 4. Affinity constants of *Der p 1*, *Der f 1* and *Der p 2 + Der f 2* for the different extracts

		<i>Der p 1</i>	<i>Der f 1</i>	<i>Der p 2+ Der f 2</i>
		KD (10^{-10} M)	KD (10^{-10} M)	KD (10^{-10} M)
Staloral 300 Rapid	Mean $\pm$ SD	2.86 $\pm$ 2.57	4.77 $\pm$ 4.16	40.1 $\pm$ n.a.
SLITone ULTRA	Mean $\pm$ SD	0.92 $\pm$ 0.31	5.52 $\pm$ 6.90	29.3 $\pm$ n.a.
Tol forte	Mean $\pm$ SD	37.5 $\pm$ 3.75	537 $\pm$ 753	3750 $\pm$ n.a.
Allergovac Sublingual Plus	Mean $\pm$ SD	13.0 $\pm$ 18.2	26.1 $\pm$ 32.5	47.3 $\pm$ n.a.
Sublivac	Mean $\pm$ SD	4.57 $\pm$ 4.60	36.1 $\pm$ 8.98	11.2 $\pm$ n.a.

SD, standard deviation.

Association (Ka) and disassociation (Kd) constants are shown in repository

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REPOSITORY

MATERIALS AND METHODS

Reference code of the different used batches.

	Product name	Marketing lab	First Batch	Second Batch
Product 1	Staloral 300 Rapid	Stallergenes	0001085047	0001111523
Product 2	SLITone ULTRA	Alk-Abello	027016251001	027191671001
Product 3	TOL Forte	Laboratorios Leti	2369041100	2481042100
Product 4	Allergovac Sublingual Plus	Bial-Aristegui	ESX0181074	ESB0673682
Product 5	Sublivac	Hal Allergy	059779	064456

Please notice the products are numbered in the blind labelling order instead of alphabetical.

Immobilization of selected capture agent, anti-mouse IgG whole molecule antibody, on Proteon XPR36 GLM chip.

The immobilization step was performed in the horizontal orientation of the ProteOn XPR36 system using a flow rate of 30 µl/min at 25°C on a GLM chip. All six channels were activated for 5 min (150 µl) using a mixture of 0.2 M EDC and 0.05 M sulfo-NHS. This was followed by an immediate

injection of 150 µl of 100 µg/ml anti-mouse IgG whole molecule (M-7023, Sigma) in 10 mM acetate buffer (pH 4.5). Finally, 150 µl of 1 M ethanolamine-HCl (pH 8.5) was injected to deactivate any remaining activated carboxyl groups.

Der p 1, Der f 1, Der p 2 and Der p 2 + Der f 2 screening with Proteon XPR36.

All binding measurements were performed with PBST as the continuous running buffer at 25°C, following the directions of Bronner *et al* (1), with some modifications. In summary, in all experiments 50 µg monoclonal antibody were diluted with PBST and injected in the vertical orientation. ProteOn XPR36 fluidics was used for 6 min (150 µl) at 25 µl/min, allowing the mAbs to be captured by the capture agent on the GLM chip.

In the first set of experiments, a mix of standard of *Der p 1, Der p 2, Der f 1* and *Der f 2* was injected in the horizontal orientation of the ProteOn XPR36 fluidics using a flow rate of 75 µl/min for 130 seconds (165 µl). Five different concentrations (400, 200, 100, 25, 12.5 nM) were injected in channels 1 to 5, respectively, and running buffer was injected simultaneously in the sixth channel to correct for loss of the captured supernatant antibodies from the chip sensor surface during the experiment.

In the second set of experiments, the different extracts were injected in the horizontal orientation of the ProteOn XPR36 fluidics using the same conditions of the standard curves.

The same chip can be reused after removal of the supernatant antibodies and antigen from the capture agent, which was performed by injecting 30 µl of 0.85% phosphoric acid (flow rate= 100 µl/min) in all channels in the vertical orientation of the ProteOn CPR36 fluidics.

SDS-PAGE total protein content of the five products studied, measured with bicinchoninic acid (BCA). Values of the first batch analysed are presented with their standard deviation (SD) with and without the removal of glycerol with *Vivaspin® 3.0K*.

BCA	Mean $\pm$ SD ($\mu\text{g/ml}$)	
	With glycerol	without glycerol
Extract 1	559,76 $\pm$ 6,90	493,90 $\pm$ 86,23
Extract 2	891,46 $\pm$ 51,74	662,20 $\pm$ 100,03
Extract 3	420,73 $\pm$ 20,70	24,39 $\pm$ 18,97
Extract 4	428,05 $\pm$ 34,49	360,98 $\pm$ 91,41
Extract 5	447,56 $\pm$ 31,04	235,37 $\pm$ 44,84

Total protein content of the five products studied, measured with bicinchoninic acid (BCA). The average values of the two batches are presented with their standard deviation (SD).

SDS-PAGE and assessment of the total protein content

BCA	Mean $\pm$ SD ($\mu\text{g/ml}$)
<i>Staloral 300 Rapid</i>	429.2 $\pm$ 97.7
<i>SLITone ULTRA</i>	727.7 $\pm$ 60.1
<i>Tol forte</i>	108.9 $\pm$ 67.6
<i>Allergovac Sublingual Plus</i>	318.4 $\pm$ 59.1
<i>Sublivac</i>	218.6 $\pm$ 30.3
<i>nDer p 1 Control</i>	4.74 $\pm$ 0.59
<i>nDer p 2 Control</i>	1.70 $\pm$ 0.51
<i>nDer f 1 Control</i>	4.56 $\pm$ 0.51
<i>nDer f 2 Control</i>	2.06 $\pm$ 0.34

Measurements of allergen content with MARIA®. Values of the first batch analysed are presented with their standard deviation (SD) with and without the removal of glycerol with *Vivaspin® 3.0K*.

	Content in µg/ml	Der p1		Der f1	
		mean±s.d.	C.V.	mean±s.d.	CV
Extract1	With Glycerol	1.84±0.62	13.99	1.3±0.13	6.03
	Without Glycerol	10.84±1.26	1.2	9.18±1.06	1.1
Extract 2	With Glycerol	1.38±1.2	16.99	1.63±0.3	7.09
	Without Glycerol	7.57±0.47	1.6	2.46±0.91	2.9
Extract 3	With Glycerol	0.12±0.01	1.02	0.05±0.02	5.55
	Without Glycerol	0.03±0.03	0.52	0.01±0.02	3.6
Extract 4	With Glycerol	0.16±0.03	5.2	0.15±0.05	7.23
	Without Glycerol	0.17±0.02	1.5	0.04±0.01	3.5
Extract 5	With Glycerol	0.59±0.09	6.3	0.32±0.08	7.26
	Without Glycerol	0.64±0.29	3.4	0.862±0.35	5.8

C.V. Coefficient of variation

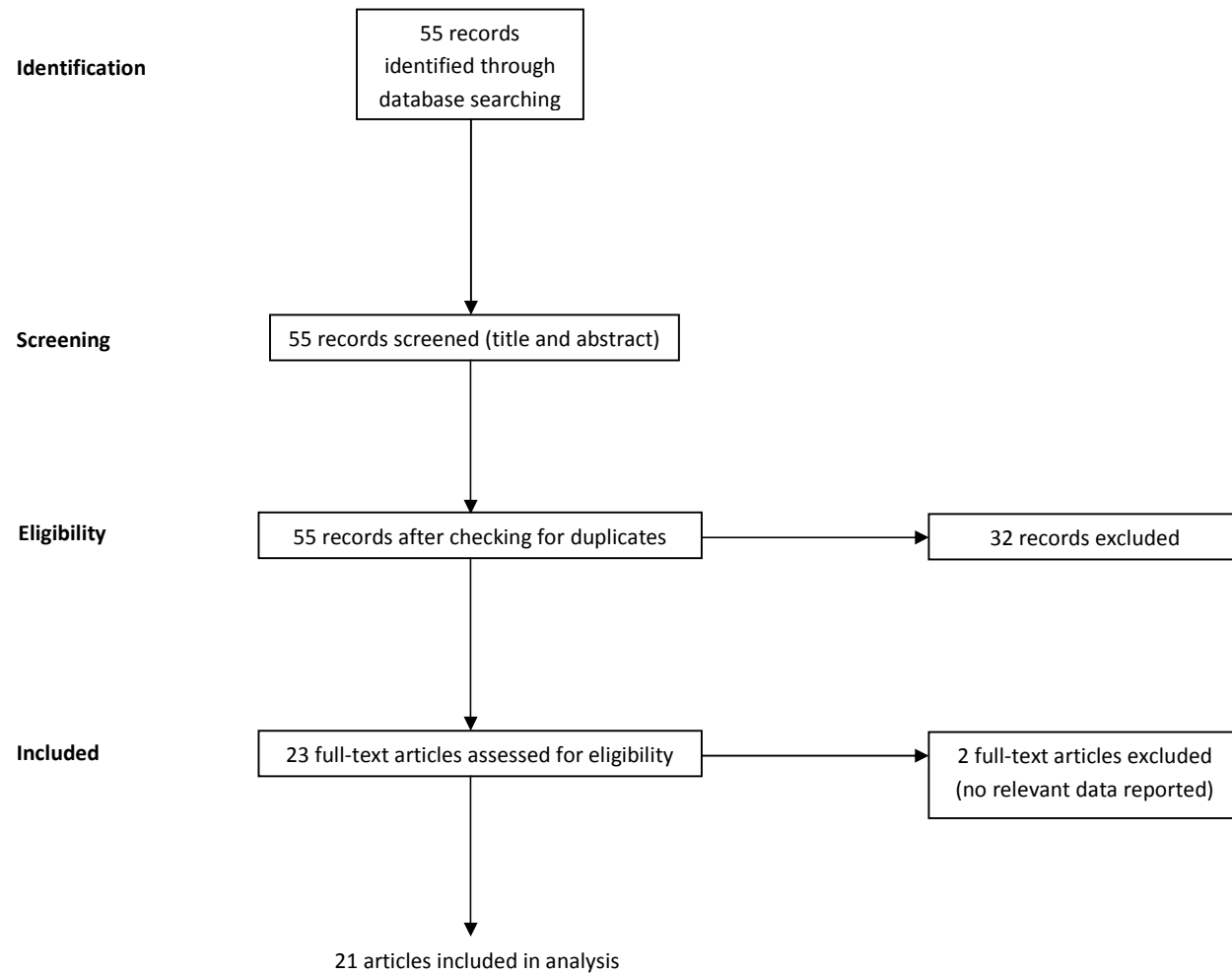
Association and disassociation constants of *Der p 1* and *Der f 1* in the different extracts

		<i>Der p 1</i>	<i>Der p 1</i>	<i>Der p 1</i>	<i>Der f 1</i>	<i>Der f 1</i>	<i>Der f 1</i>	<i>Der p 2 + Der f 2</i>	<i>Der p 2+ Der f 2</i>	<i>Der p 2+ Der f 2</i>
		Ka (1/Ms)	Kd (1/s)	KD (M)	Ka (1/Ms)	Kd (1/s)	KD (M)	Ka (1/Ms)	Kd (1/s)	KD (M)
Staloral 300 Rapid	Mean ± SD	$1.21 \times 10^6 \pm 1.34 \times 10^5$	$3.27 \times 10^{-4} \pm 2.69 \times 10^{-4}$	$2.86 \times 10^{-10} \pm 2.57 \times 10^{-10}$	$5.14 \times 10^7 \pm 6.87 \times 10^7$	$1.08 \times 10^{-3} \pm 1.50 \times 10^{-3}$	$4.77 \times 10^{-10} \pm 4.16 \times 10^{-10}$	$12.07 \times 10^6 \pm \text{n.a.}$	$8.36 \times 10^{-3} \pm \text{n.a.}$	$4.01^{-9} \pm \text{n.a.}$
SLITone ULTRA	Mean ± SD	$1.66 \times 10^6 \pm 9.42 \times 10^5$	$1.40 \times 10^{-4} \pm 3.61 \times 10^{-5}$	$9.24 \times 10^{-11} \pm 3.06 \times 10^{-11}$	$1.64 \times 10^6 \pm 1.35 \times 10^6$	$4.05 \times 10^{-4} \pm 3.39 \times 10^{-4}$	$5.52 \times 10^{-10} \pm 6.90 \times 10^{-10}$	$6.65 \times 10^5 \pm \text{n.a.}$	$1.95 \times 10^{-8} \pm \text{n.a.}$	$2.93 \times 10^{-9} \pm \text{n.a.}$
Tol forte	Mean ± SD	$8.15 \times 10^4 \pm 1.15 \times 10^5$	$3.27 \times 10^{-4} \pm 4.62 \times 10^{-4}$	$3.75 \times 10^{-9} \pm 3.75 \times 10^{-10}$	$1.69 \times 10^6 \pm 2.38 \times 10^6$	$8.26 \times 10^{-4} \pm 9.96 \times 10^{-4}$	$5.37 \times 10^{-8} \pm 7.53 \times 10^{-8}$	$5.50 \times 10^{-2} \pm \text{n.a.}$	$2.06 \times 10^2 \pm \text{n.a.}$	$3.75 \times 10^{-3} \pm \text{n.a.}$
Allergovac Sublingual Plus	Mean ± SD	$4.19 \times 10^6 \pm 5.92 \times 10^6$	$4.83 \times 10^{-5} \pm 6.82 \times 10^{-5}$	$1.30 \times 10^{-9} \pm 1.82 \times 10^{-9}$	$4.32 \times 10^6 \pm 6.10 \times 10^6$	$1.36 \times 10^{-3} \pm 1.92 \times 10^{-3}$	$2.61 \times 10^{-9} \pm 3.25 \times 10^{-9}$	$2.24 \pm \text{n.a.}$	$1.06 \times 10^{-8} \pm \text{n.a.}$	$4.73 \times 10^{-9} \pm \text{n.a.}$
Sublivac	Mean ± SD	$1.90 \times 10^5 \pm 6.58 \times 10^4$	$7.15 \times 10^{-5} \pm 5.73 \times 10^{-5}$	$4.57 \times 10^{-10} \pm 4.60 \times 10^{-10}$	$2.18 \times 10^5 \pm 1.30 \times 10^5$	$8.48 \times 10^{-4} \pm 6.68 \times 10^{-4}$	$3.61 \times 10^{-9} \pm 8.98 \times 10^{-10}$	$5.42 \times 10^5 \pm \text{n.a.}$	$6.06 \times 10^{-4} \pm \text{n.a.}$	$1.12 \times 10^{-9} \pm \text{n.a.}$

SD, standard deviation.

Literature Review

. PRISMA flow chart for the search strategy.



Systematic reviews and meta-analyses of SLIT.

Author, year	Population	Condition	Treatment	Allergens	Number of studies	Number of patients	Main results
Systematic reviews and/or meta-analyses							
Sopo <i>et al</i> , 2004 (2)	Children	Rhinitis Asthma Conjunctivitis	SLIT vs placebo	Several	8 (HDM 5)	372 (HDM 221)	Statistically significant Low or moderate clinical efficiency in asthma due to HDM
Wilson <i>et al</i> , 2005 (3)	Adults Children	Rhinitis Asthma ±Conjunctivitis	SLIT vs placebo	Several	22 (HDM 6)	979 (HDM 229)	Significant reduction of symptoms (SMD - 0.42 p = 0.002) Significant reduction of use of medication (SMD -0.43, p = 0.00003) (SMD -0.42, p = 0.002) A greater length of treatment does not clearly increase the efficacy The total dosage of the allergen can be important
Olaguibel <i>et al</i> , 2005 (4)	Children	Rhinitis Asthma ±Conjunctivitis	SLIT vs placebo	Several	7 (HDM 4)	256 (HDM 127)	Reduction of rhinitis symptoms (SMD - 0.44, ns) Reduction of asthma symptoms (SMD - 1.42, p = 0.01)
Penagos <i>et al</i> , 2006 (5)	Children	Rhinitis	SLIT vs placebo	Several	10 (HDM 4)	484 (HDM 126)	Significant reduction of symptoms (SMD - 0.56, p = 0.02) Significant reduction of use of medication (SMD -0.76, p = 0.03)
Calamita <i>et al</i> , 2006 (6)	Adults Children	Asthma	SLIT vs placebo	Several	25 (HDM 8)	1706 (HDM 354)	Reduction of asthma symptoms (SMD - 0.38, ns). Significant reduction of allergenic symptoms in general (SMD -1.18)
Cox, 2007 (7)	Children	Rhinitis Asthma	SLIT vs placebo	Several	19	1208	The SLIT is well tolerated in young children. Many studies show effectiveness. The optimum adherence dosages are not clear.

Penagos <i>et al</i> , 2008 (8)	Children	Asthma	SLIT vs placebo	Several	9 (HDM 6)	441	Significant reduction of symptoms (SMD - 1.14, p = 0.02) Significant reduction of use of medication (SMD -1.63, p = 0.007)
Röder <i>et al</i> , 2008 (9)	Children	Rhinitis ±Conjunctivitis	SLIT vs placebo SCIT vs placebo LNIT vs placebo OIT vs placebo	Several	SLIT 11 (HDM 4) SCIT 6 LNIT 4 OIT 7	1619 (SLIT 744 (HDM 197))	Insufficient evidence of effectiveness
Compalati <i>et al</i> , 2009 (10)	Adults Children	Rhinitis Asthma	SLIT vs placebo	HDM	Rinitis 8 Asthma 9	Rinitis 382 Asma 452	Significant reduction of symptoms (SMD - 0.95, p = 0.02); significant reduction of use of medication (SMD -1.88, p =0.04) Asthma: significant reduction of symptoms (SMD -0.95, p = 0.02); significant reduction of use of medication (SMD -1.48, p = 0.02)
Di Bona <i>et al</i> , 2010 (11)	Adults Children	Rhinitis	SLIT vs placebo	Pollen	19	2971	Significant reduction of symptoms (SMD - 0.32, p < 0.0001) Significant reduction of use of medication (SMD -0.76, p = 0.03)
Calderon <i>et al</i> , 2011 (12, 13)	Adults Children	Conjunctivitis ±Rhinitis	SLIT vs placebo	Several	42	3958	SLIT induces a significant reduction of: total score of ocular symptoms (SMD - 0.41, p < 0.00001); ocular reddening (SMD -0.33, p < 0.0001); ocular itching (SMD -0.31, p < 0.00001); tearing (SMD -0.23, p < 0.0001). No reduction in the use of eye drops was observed
Meadows <i>et al</i> , 2013 (14)	Adults Children	Rhinitis	SLIT vs placebo SCIT vs placebo SLIT vs SCIT	Several	SLIT 11 SCIT 17 SLIT vs SCIT 1	4744 (SLIT 2139) (SLIT vs SCIT 71)	The SLIT significantly reduces the symptoms, the use of medication and improves the quality of life. The effect is moderate and shows a great variability

Dretzke <i>et al</i> , 2013 (15)	Adults Children	Rhinitis	SLIT vs placebo SCIT vs placebo SLIT vs SCIT		SLIT 11 SCIT 17 SLIT vs SCIT 1		The SLIT significantly reduces the symptoms (SMD -0.33, $p < 0.00001$); the use of medication (SMD -0.27, $p < 0.00001$); and improves the quality of life (SMD -0.37, $p < 0.00001$). The direct comparison of SLIT and SCIT and the indirect comparisons are not conclusive.
Lin <i>et al</i> , 2013 (16)	Adults Children	Rhinitis Asthma ±Conjunctivitis	SLIT vs placebo SCIT vs placebo SLIT vs SCIT	Several	SLIT 60 (HDM 14) SCIT 74 (HDM 21) SLIT vs SCIT 8 (HDM 6)		There is sufficient evidence to back up the global effectiveness and the security both of SCIT and of SLIT There is not sufficient evidence to determine if SLIT or SCIT is superior.
Calderon <i>et al</i> , 2013 (17)	Adults Children	Asthma Rhinitis	SLIT vs placebo SCIT vs placebo	HDM	SLIT in asthma 14 SLIT in rhinitis 15 SLIT in asthma 19 SLIT in rhinitis 7	SLIT in asthma 1110 SLIT in rhinitis 958 SLIT in asthma 987 SLIT in rhinitis 397	Heterogeneity of basic treatment parameters (such as dosages, length) Significant effectiveness vs placebo regarding symptom score. The SLIT tests could have used insufficient dosages.
Kim <i>et al</i> , 2013 (18)	Children	Asthma Rhinitis ±Conjunctivitis	SLIT vs placebo SCIT vs placebo SLIT vs SCIT	Several	SLIT 18 SCIT 13 SLIT vs SCIT 3	SLIT 1583 SCIT 920 SLIT vs SCIT 135	High level of evidence that SLIT improves asthma symptoms; moderate that it improves rhinitis symptoms and conjunctivitis; and moderate that it reduces the use of medication. Low evidence that the SCIT is superior to SLIT.
Chelladurai <i>et al</i> , 2013 (19)	Adults Children	Asthma Rhinitis ±Conjunctivitis	SLIT vs SCIT	Several	8 (HDM 6)	555 (HDM 389)	SCIT and SLIT are effective to reduce the symptoms. Low evidence that SCIT is superior to SLIT for symptoms of asthma and moderate for symptoms of rhinoconjunctivitis.
Radulovic <i>et al</i> , 2010, 2011 (20, 21)	Adults Children	Rhinitis	SLIT vs placebo	Several	49 (HDM 9)	4589 (HDM 464)	The SLIT significantly reduces the symptoms (SMD -0.49, $p < 0.00001$ [HDM -0.97, $p = 0.02$]) and the use of medication (SMD -0.32, $p < 0.00001$ [HDM -0.52, $p = 0.07$])

Larenas-Linnermann <i>et al</i> , 2013 (22)	Children	Asthma Rhinitis	SLIT	Several	29	2469	New robust evidence of effectiveness of SLIT in rhinitis and scarce in asthma. HDM in asthma: evidence of reduction of the use of medication; HDM in rhinitis: moderate-low evidence of effectiveness.
Nelson <i>et al</i> , 2014 (23)	Adults Children	Rhinitis Asthma ±Conjunctivitis	SLIT vs SCIT	Several	11 (HDM 7)	534 (HDM 392)	Both SLIT and SCIT are effective vs a placebo. The evidence shows that a clinical advantage of SCIT is not well grounded.
Meta-analyses reviews							
Nieto <i>et al</i> , 2009 (24)	Adults Children	Rhinitis Asthma	SLIT	-	5	Studies 43	The evidence of the meta-analysis is not solid enough to back up the habitual use of SLIT
Incorvaia <i>et al</i> , 2011 (25)	Adults Children	Rhinitis	SLIT	Several	5 (HDM 1)	Patients 5072 (HDM 382)	The SLIT is effective and safe SMD in between -0.32 and -0.95
de Bot <i>et al</i> , 2011 (26)	Children	Rhinitis	SLIT	-	10	Studies 171	Promising SLIT results. Quality of tests limited
Incorvaia <i>et al</i> , 2012 (27)	Adults Children	Rhinitis	SLIT	Several	6 (HDM 1)	Patients 9661 (HDM 382)	The SLIT is effective and safe SMD in between -0.32 and -0.95

HDM SLIT studies identified in the meta-analyses (this table appears in the article, but the complete references are included here)

Author, year	Age range	Number of patients treated with SLIT/placebo (or control)	Length	Biological potency	Dosage in µg/ml (as described in the article)	Weekly dosage (the ones calculated* from the original data appear shadowed)	Condition
Pajno <i>et al</i> , 2000 (28)	8-15	12/12	24 months	BU	2.4 µg <i>Der p 1</i> / week 1.2 µg <i>Der p 2</i> / week	2.4 µg <i>Der p 1</i> 1.2 µg <i>Der p 2</i>	Asthma
Guez <i>et al</i> , 2000 (29)	6-51	25/14	24 months	IR	2.2 mg <i>Der p 1</i> 1.7 mg <i>Der f 1</i> (both values shown in cumulative doses over 24 months of study)	21 µg <i>Der p 1</i> 16 µg <i>Der f 1</i>	Rhinitis
Bahceciler <i>et al</i> , 2001 (30)	7-15	8/7	6 months	IR	0.56 mg <i>Der p</i> 0.98 mg <i>Der f</i> (both values shown in cumulative doses over 6 months of study)	21.5 µg <i>Der p</i> 37.6 µg <i>Der f</i>	Rhinitis Asthma
Mortemousque <i>et al</i> , 2003 (31)	6-60	26/19	24 months	IR	2.2 mg <i>Der p 1</i> 1.7 mg <i>Der f 1</i> (both values shown in cumulative doses over 24 months of study)	21 µg <i>Der p 1</i> 16 µg <i>Der f 1</i>	Conjunctivitis
Ippoliti <i>et al</i> , 2003 (32)	5-12	47/39	6 months	BU	2.4 µg <i>Der p 1</i> / week 1.2 µg <i>Der p 2</i> / week	2.4 µg <i>Der p 1</i> 1.2 µg <i>Der p 2</i>	Asthma
Tonnel <i>et al</i> , 2004 (33)	7-45	15/17	24 months	IR	1.28 mg <i>Der p 1</i> 1.47 mg <i>Der f 1</i> (both values shown in cumulative doses over 24 months of study)	12.3 µg <i>Der p 1</i> 14.1 µg <i>Der f 1</i>	Rhinitis

Author, year	Age range	Number of patients treated with SLIT/placebo (or control)	Length	Biological potency	Dosage in µg/ml (as described in the article)	Weekly dosage (the ones calculated* from the original data appear shadowed)	Condition
Niu <i>et al</i> , 2006 (34)	6-12	49/48	6 months	IR	1.7 mg <i>Der p</i> 3 mg <i>Der f</i> (both values shown in cumulative doses over 6 months of study)	70.8 µg <i>Der p</i> 125 µg <i>Der f</i>	Asthma
Passalacqua <i>et al</i> , 2006 (35)	18-50	28/28	24 months	AU	N/A	N/A	Rhinitis
Hirsch <i>et al</i> , 1997 (36)	6-15	15/15	12 months	N/A	570 µg <i>Der p 1</i> (cumulative dose over 12 months of study)	10.9 µg <i>Der p 1</i>	Rhinitis Asthma Conjunctivitis
Marcucci <i>et al</i> , 2003 (37) and 2005 (38)	4-16	13/11	12 months (double blind) + 12 months (open)	STU	110 µg group 1 55 µg group 2 (both values shown in cumulative doses over 12 months of study)	2.1 µg group 1 1.1 µg group 2	Rhinitis Asthma
Passalacqua <i>et al</i> , 1998 (39)	15-46	10/9	24 months	AU	N/A	N/A	Rhinitis Conjunctivitis
Tari <i>et al</i> , 1990 (40)	< 12	30/28	18 months	STU	N/A	N/A	Rhinitis Asthma
Giovane <i>et al</i> , 1994 (41)	< 12	10/8	3 years	TU	N/A	N/A	Asthma

Author, year	Age range	Number of patients treated with SLIT/placebo (or control)	Length	Biological potency	Dosage in µg/ml (as described in the article)	Weekly dosage (the ones calculated* from the original data appear shadowed)	Condition
Bousquet <i>et al</i> , 1999 (42)	7-42	42/43	24 months	IR	4.2 mg <i>Der p 1</i> 7.3 mg <i>Der f 1</i> (both values shown in cumulative doses over 24 months of study)	40.3 µg <i>Der p 1</i> 70.1 µg <i>Der f 1</i>	Asthma
Mungan <i>et al</i> , 1999 (43)	18-46	15/(11)	12 months	IR	N/A	N/A	Rhinitis Asthma
Lue <i>et al</i> , 2006 (44)	6-12	10/10	6 months	IR	1.7 mg <i>Der p</i> 3.0 mg <i>Der f</i> (both values shown in cumulative doses over 6 months of study)	70.8 µg <i>Der p</i> 125 µg <i>Der f</i>	Asthma
Pham-Thi <i>et al</i> , 2007 (45)	5-15	54/55	18 months	IR	6,9 mg <i>Der p 1</i> 14,7 mg <i>Der f 1</i> (both values shown in cumulative doses over 18 months of study)	88.3 µg <i>Der p 1</i> 188.2 µg <i>Der f 1</i>	Rhinitis Asthma
Tseng <i>et al</i> , 2008 (46)	6-18	30/33	6 months	IR	1.56 mg <i>Der p</i> 2.71 mg <i>Der f</i> (both values shown in cumulative doses over 6 months of study)	59.9 µg <i>Der p</i> 104.1 µg <i>Der f</i>	Rhinitis
Bush <i>et al</i> , 2011 (47)	18-50	10/11	12-18 months	AU	high dosage: 70 µg <i>Der f 1</i> / day low dosage: 1 µg <i>Der f 1</i> / day	high dosage: 490 µg <i>Der f 1</i> low dosage: 7 µg <i>Der f 1</i>	Rhinitis Asthma

Author, year	Age range	Number of patients treated with SLIT/placebo (or control)	Length	Biological potency	Dosage in µg/ml (as described in the article)	Weekly dosage (the ones calculated* from the original data appear shadowed)	Condition
O'Hehir <i>et al</i> , 2009 (48)	18-65	15/15	12-24 months	IR	17.1 mg <i>Der p 1</i> and 3.4 mg <i>Der p 2</i> cumulative over the first year 34.6 mg <i>Der p 1</i> ; 6.9 mg <i>Der p 2</i> cumulative over the second year	328.3 µg <i>Der p 1</i> and 65.3 µg <i>Der p 2</i> first year of treatment 332.2 µg <i>Der p 1</i> ; 66.2 mg <i>Der p 2</i> second year of treatment	Rhinitis
Yukselen <i>et al</i> , 2012 (49)	< 12	11/10	12 months	TU	N/A	N/A	Rhinitis Asthma
Bozek <i>et al</i> , 2013 (50)	60-75	51/57	28 months	IR	N/A	N/A	Rhinitis
Piazza and Bizzaro, 1993 (51)		14/14	24 months	STU	1 µg <i>Der p 1</i> three times a week	3 µg <i>Der p 1</i>	Rhinitis
Tahamiler <i>et al</i> , 2008 (52)	12-51	97/(96)	36 months	STU	N/A	N/A	Rhinitis
Eifan <i>et al</i> , 2010 (53)	5-10	16/(16)	12 months	STU	295.5 µg <i>Der p 1</i> 295.5 µg <i>Der f 1</i> (both values shown in cumulative doses over 12 months of study)	5.7 µg <i>Der p 1</i> 5.7 µg <i>Der f 1</i>	Rhinitis Asthma
Keles <i>et al</i> , 2011 (54)	5-12	13/12	12 months	N/A	54.3 µg <i>Der p 1</i> 54.3 µg <i>Der f 1</i> (both values shown in cumulative doses over 12 months of study)	1 µg <i>Der p 1</i> 1 µg <i>Der f 1</i>	Asthma
de Bot <i>et al</i> , 2012 (55)	6-18	125/126	24 months	BU	435 µg <i>Der p 1</i> No detectable <i>Der f 1</i> (both values shown in cumulative doses over 2 years)	4.2 µg <i>Der p 1</i> No detectable µg <i>Der f 1</i>	Rhinitis

Author, year	Age range	Number of patients treated with SLIT/placebo (or control)	Length	Biological potency	Dosage in µg/ml (as described in the article)	Weekly dosage (the ones calculated* from the original data appear shadowed)	Condition
Yonekura <i>et al</i> , 2010 (56)	7-15	20/11	12 months	N/A	5 µg <i>Der f 1</i> (weekly)	5 µg <i>Der f 1</i>	Rhinitis
Lee <i>et al</i> , 2011 (57)	4-53	134	12 months	STU	N/A	N/A	Rhinitis

AU, allergenic units; BU biologic units; IR index of reactivity; N/A, not available; STU**, skin test units; STU*, standard treatment units; TU, therapeutic units

N/A, not available

* A month has been equalled to 4.34 weeks

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