
European allergen extract units and potency: review of available information

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Background: There is considerable variability in how allergen extract potency is measured and reported worldwide. In Europe, where many sublingual immunotherapy studies have been conducted, manufacturers report allergen extract potency as units based on an in-house reference, making it difficult to understand the exact doses used and to compare studies.

Objectives: To describe the various methods of expressing extract potency that European allergen extract manufacturers use and to gather reports on the micrograms of major allergen of the in-house units of European allergen extract manufacturers.

Methods: Information was derived from 3 sources: data on extract potency in micrograms of major allergen in articles on sublingual immunotherapy found by PubMed (references through October 2005) and in reference articles, brochures on allergen extracts from the manufacturers, and information provided by structured questionnaires e-mailed to the manufacturers.

Results: All but 1 of the European allergen extract manufacturers use in-house reference standards that are based on titrated skin prick testing of allergic patients. Subsequently, in vitro tests compare the potency of commercial batches with the in-house reference and potency is assigned as arbitrary units. Most manufacturers measure major allergen content of their standardized products but do not release this information with the package insert. Diversity in major allergen content was found.

Conclusions: Micrograms of major allergens given in articles on sublingual immunotherapy to express the dose administered cannot be used to translate the dose to US extracts. Extract potency can only be compared if uniform test methods and reference extracts are used.

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INTRODUCTION

In the past 2 decades there has been an increasing use of sublingual immunotherapy (SLIT) in Europe and a growing interest in this treatment modality worldwide, as shown by studies conducted recently in the American and Asian continents.^{1–3} Furthermore, meta-analyses,^{4,5} reviews,⁶ and guidelines⁷ for SLIT have been published. In response to this interest, a Joint Task Force of the American College of Allergy, Asthma and Immunology and the American Academy of Allergy, Asthma and Immunology's Immunotherapy and Allergy Diagnostic Committees performed a comprehensive review of all SLIT articles published up to October 2005 and prepared a report. One of the crucial points they came across was the dosing, which was difficult to understand because units between manufacturers and even within 1 manufacturer vary over time. Since 2005, a wealth of SLIT studies have been published, including some dosing studies.^{8–10} However, considerable variability exists in how allergen extract potency is measured and reported worldwide. In Europe, manufacturers report allergen extract potency as units based on an in-house reference standard (IHRs), making it difficult to understand the exact doses used. Therefore, we decided to perform a review on the extract potency of allergens produced by European manufacturers.

Measurements of Allergen Extract Potency

Allergen extracts are complex mixes of biological material. There are various ways in which to express the potency of an allergen extract. The in vivo methods are all based on a reference derived from titrated skin tests of allergic patients with either the percutaneous or intradermal techniques. The in vitro techniques include protein separation methods, allergen detection methods, and allergen quantification methods. The best known example of the last one is the measurement of the micrograms of major allergen in an extract.

An estimated biological potency should be based on a combination of major allergen content and protein characterization. Internationally, micrograms of major allergen is the most recognized way to express allergen extract strength because it appears to correlate well with overall biological potency of the extract.^{11–13} The Joint Task Force on Practice Parameters on Allergen Immunotherapy,¹⁴ a World Health Organization position paper on allergen immunotherapy,¹⁵ and the first World Health Organization-supported Allergic Rhinitis and its Impact on Asthma document¹⁶ all provide recommendations for maintenance subcutaneous immunotherapy (SCIT) dose based on micrograms of major allergen. An excellent review on the subject was published recently.¹⁷ However, the measurement of micrograms of major allergen content of an extract has some pitfalls¹⁸ that might make comparison of results among different manufacturers practically impossible, as will be discussed in the final part of this article.

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Table 1. Extract Potency Definitions as Stipulated by CBER of the FDA and Most European Allergen Extract Manufacturers

Manufacturer	Products	Potency expression	Units Based on	Parameters
CBER	Allergens	(B)AU	Intradermal test in highly allergic patients. No control.	Erythema size, 50 mm total of 2 diameters, arithmetical mean.
ALK	Allergens for SLIT and depot for SCIT	BU, HEP, STU, and SQ-U	BU: SPT in 30 allergic patients. No control. HEP: same with histamine control of 10 mg/mL. STU and SQ-U: based on therapeutic response.	100 BU = produces a wheal with a mean average size of 70 mm ² . HEP = concentration that gives same reaction as histamine control.
Allerbio	Allergens	IR	SPT with 3 serial 1/10 dilutions in allergic patients. 9% codeine phosphate is control (6 mm).	100 IR = concentration that gives geometrical mean diameter of wheal same as control (6 mm).
Allergopharma	Allergens, SCIT: Allergoids and depot	SBU	SPT in allergic patients 0.1% histamine control.	1,000 BU = concentration that gives the same wheal as control.
Allergy Therapeutics	Allergens, allergoids	DU/TU and SU for allergoids	SPT in 25 allergic, 15 atopic and 10 healthy people.	10,000 DU is value with lowest false-positive and false-negative results. Fixed DU/TU relation for each allergen.
HAL Allergy	Allergens, SCIT: Allergoids and depot	(B)AU	Intradermal test in 15 highly allergic patients, chosen from a pool of patients. No control.	Erythema size total of 2 diameters, arithmetical mean of 50 mm = D ₅₀ .
IPI-ASAC	Allergens, SCIT: Polymerized and depot	UBE	Duplicate SPT with 3 serial 1/10 dilutions in 20–30 allergic patients, negative and histamine, 0.10-mg/mL, control.	Mean concentration provoking a wheal equivalent to histamine control × 10,000 = UBE.
Leti	Allergens, SCIT: Allergoids and depot	HEP _L	Duplicate SPT in at least 20 allergic patients, 10-mg/mL histamine control.	10 HEP _L = concentration that gives a wheal equivalent to 10 mg/mL of histamine.
Stallergènes	Allergens, SCIT: depot	IR	Quadruple SPT with 3 serial 1/10 dilutions in 30 allergic patients. Control is 9% codeine phosphate (7 mm).	100 IR = concentration with a geometrical mean diameter of wheal size same as control (7 mm).

Abbreviations: (B)AU, bioequivalent allergy units; BU, Brighton or biological units; CBER, Center for Biological Evaluation and Research; DU/TU, diagnostic/therapeutic units; FDA, Food and Drug Administration; HEP, histamine equivalent prick; HEP_L, histamine equivalent prick_{Leti}; IR, index of reactivity; SBU, standard biological units; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; SPT, skin prick test; SQ-U, standardized quality units; STU, standard treatment units; UBE, unidades biológicas equivalentes.

In-house Reference Standard

In the United States, the Food and Drug Administration (FDA), through its Center for Biologics Evaluation and Research, has established for certain allergens (called standardized allergens) a standard potency test and reference extracts to which the allergen manufacturers have to compare their batches of allergen extracts. The situation in Europe is different. In 1989, the Nordic guidelines provided recommendations for the standardization of allergen extracts¹⁹ via skin prick tests, which have partially been adapted by many of the European manufacturers. This method measures the extract's

overall biological activity, and so the IHRS for the allergen in question is established through in vivo testing of allergic patients. Commercial extracts are analyzed with a predefined set of in vitro tests and compared against the reference extract with an in vitro assay (eg, enzyme-linked immunosorbent assay [ELISA] inhibition) and then assigned potency units. Each European manufacturer defines its own standardization method. The objective of this article is to describe per European manufacturer the current methods for determining their allergen extract potency and, if the information is available, translate the manufacturer's IHRS into micrograms of major allergen.

METHODS

Three different sets of source data were used for this study. First, micrograms of major allergen data were subtracted from articles on SLIT from a reference list compiled with data from articles known to the Joint Task Force members, reference articles on SLIT, and PubMed (referenced through October 2005 using the keywords *sublingual immunotherapy*, *allergen extract*, *extract potency*, and *allergen immunotherapy*). Some studies reviewed provided detailed information about the allergen extract, including the micrograms of major allergen per milliliter of extract. Second, the allergen extract manufacturers' brochures and product information sheets served as source material. Third, a standardized questionnaire was sent by e-mail to the medical and/or marketing department of the allergen manufacturers with the following questions: (1) How do you express the potency of your allergen extracts? (2) What is the definition of that potency expression? (3) How many micrograms of major allergen, with the tests you use, have your standardized SLIT extracts (an Excel file was added to be filled in)? (4) What dose (in milliliters) and frequency do you recommend for SCIT maintenance treatment and for SLIT maintenance.

In a second e-mail, they were asked for the *in vitro* tests they use for potency control of the commercial batches. We confirmed, when possible, information that appeared to be conflicting. From the start all parties knew data were asked with the intention to publish. All companies that added specific information not found in brochures and literature gave verbal or written permission for publication of the data given (see "Acknowledgments" section).

RESULTS

Table 1 gives the potency expression each manufacturer uses and its definition. We review per allergen extract manufacturer the replies we received on the standardized questionnaire and the micrograms of major allergen of their standardized products (if available). All European Allergen Manufacturers Group members with nonmodified aller-

gens in their product line were contacted. However, some manufacturers did not provide information because they are of the opinion that giving micrograms of major allergen data on their products does not add to overall understanding until uniform test methods and reference extracts are available.

ALK-Abelló (Denmark, Spain)

Treatment potency expression. In Northern European countries standardized quality units (SQ-U) are used. In Spain, standard treatment units (STU) are used. The diagnostic skin test potency unit is called histamine equivalent prick (HEP) and Brighton or biological units (BU). BU is a unit used in some of the first SLIT publications.

Definition. Standardized quality units (SQ-U) and STU are applied to subcutaneous products and are based on clinical efficacy trials not on skin prick tests. As a result of these trials, the optimal dose for maintenance treatment is defined as 100,000 SQ-U or 1,000 STU. The older units, HEP and BU, however, were based on skin prick tests (Table 1). Their relationship to the newer clinically defined units varies per individual allergen. As an example, the STU/BU relation for house dust mite (100 STU = 1 BU) varies from that for *Lolium* (40 STU = 1 BU).

Micrograms of major allergen. The micrograms of SLIT products as published in the literature and as given by the company are specified in Table 2. Furthermore, the company provided data in Table 3 on the micrograms of major allergen content of 2 alum depot preparations, Pangramin (Spain) and Alutard (Denmark), for subcutaneous use. The same reagents and tests have been used over time and at various ALK-Abelló manufacturing locations, what improves data consistency.

Recommended doses. For SCIT maintenance, the recommended dose is 100,000 SQ-U or 0.8 mL of 1,000-STU/mL alum depots every 4 to 6 weeks. For SLIT maintenance, the recommended dose is 5 drops (approximately 0.2 mL) of 1,000 STU/mL extract 3 times a week. Recent clinical trials

Table 2. Micrograms of Major Allergen Data for ALK-Abelló SLIT Products^a

Allergen	Year(s) of publication	Potency	Major allergen	Micrograms per milliliter of major allergen	
				Published	Pangramin SLIT
Mites	2000, 2003	1,000 STU = 10 BU	Der p + f 1/Der p + f 2	4/2	4/2
Cat	2003	10 BU	Fel d 1	2	2
Birch	2001	10 BU	Bet v 1	6.6	22
Olive	2000, 2003	10 BU	Ole e 1	15	15
<i>Phleum pratense</i>	1998	SQ	Phl p 5	125	Spain: 9
5 Grasses	1992, 1999, 2004/2000, 2003	10 BU	Group 5 allergens	1/2.5	Orchard: 7.5
<i>Parietaria</i>	1994, 1995, 2000	10 BU	Par j 1	0.6	Italy: 0.6
Mugwort	2003	10 BU	Art v 1	18	1.8
<i>Alternaria</i>	2003	10 BU	Alt a 1	2.5	0.6

Abbreviations: BU, biological units; SLIT, sublingual immunotherapy; SQU, standardized quality units; STU, standard treatment units;

^a Numbers are only of value for the specific products indicated and in the time frame given.

Table 3. Micrograms of Major Allergen Data on Some ALK Subcutaneous Products as Provided by the Company^a

Genus species	Major allergen	SQ products, $\mu\text{g}/100,000$ SQ-U ^b	UM products, $\mu\text{g}/1,000$ STU ^b
<i>Dermatophagoides pteronyssinus</i>	Der p 1	9.8	4.0
	Der p 2	ND	2.0
<i>Dermatophagoides farinae</i>	Der f 1	13.8	4.0
	Der f 2	ND	2.0
<i>Betula verrucosa</i>	Bet v 1	12.3	22
<i>Olea europea</i>	Ole e 1	ND	15
<i>Phleum pratense</i>	Phl p 5	20.2	4.0
<i>Dactylis glomerata</i>	Dac g 1	12.0	2.5
<i>Lolium perenne</i>	Lol p 5	12.5	1.5
<i>Poa pratense</i>	Poa p 5	15.2	2.5
<i>Festuca pratense</i>	Fes p 5	18.6	2.5
<i>Artemisia vulgaris</i>	Art v 1	ND	1.8
<i>Parietaria judaica</i>	Par j 1	5.9	Spain, 0.06; Italy, 0.6
<i>Ambrosia</i>	Amb a 1	?	9
<i>Plantago</i>	Pla l 1	?	7
<i>Felis domesticus</i>	Fel d 1	14.6	2.0
<i>Canis familiaris</i>	Can d 1	8.0	ND
<i>Alternaria alternate</i>	Alt a 1	?	0.3

Abbreviations: ND, not determined; SQ, standardized quality; STU, standard treatment units; UM, Unidades de Masa (mass units).

^a This table was provided on request for this article in 2006: Alutard (SQ products) and Pangramin Depot (UM products). In many countries, it is not permitted to market the major allergen content as indicated in the table. This information should therefore be regarded as scientific information only. Moreover numbers are only of value for the specific products indicated and in the time frame given.

^b Adapted from Product Literature, ALK-Abelló, Spain and Italy (does not apply to ALK US products).

have been performed with a lyophilized sublingual grass tablet of 75,000 standardized quality tablet daily containing 15 μg of Phl p 5.²⁰

Control of commercial batches. The control of commercial batches consists of the evaluation of the composition of the extract, estimate of major allergen content, and total biological activity. When quantifying the major allergen content, the SQ procedure uses rocket immunoelectrophoresis or radio immunodiffusion, whereas the Unidades de Masa procedure (STU), used in Spain, uses ELISA.

Allerbio (France)

Potency expression. Index of reactivity (IR) is used to express potency.

Definition. The geometric mean diameter of the wheals generated is calculated for each dilution and plotted as shown in Figure 1. A regression line drawn helps to find the dilution that produces a wheal with a mean diameter of 6 mm. The IR was first defined by Dr Bernard Guerin in 1983 at Stallergènes. When he started Allerbio, the same units were conserved.²¹ However, Stallergènes later changed their definition of 100 IR to that concentration that causes a wheal with a mean diameter of 7 mm. Because concentrations are plotted logarithmically, a 7-mm diameter corresponds to a more concentrated extract.

Micrograms of major allergen. Data on micrograms of major allergen are available for in-house use only.

Maintenance doses. For SLIT, the maintenance dose is 20 drops of 100 to 300 IR/mL.

Control of commercial batches. The control of commercial batches is with isoelectric focusing and sodium dodecyl sul-

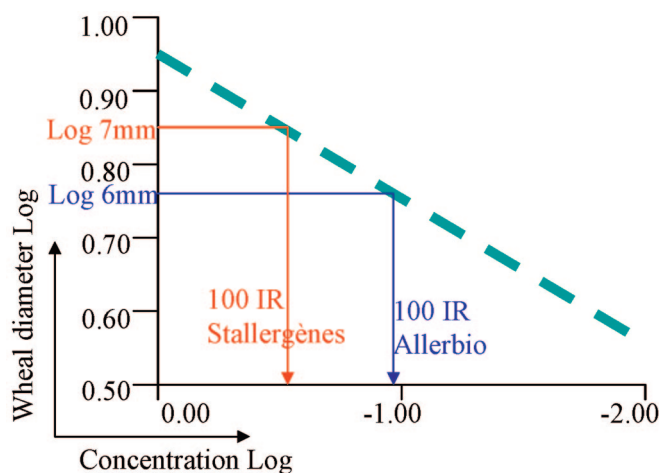


Figure 1. Logarithmic plot of wheal diameter against skin prick test solution concentration: determination of index of reactivity (IR) by Allerbio (reference, 6 mm) and Stallergènes (reference, 7 mm). Given the fact that Allerbio uses a smaller mean wheal diameter as reference, the 100 IR of this manufacturer corresponds to a higher dilution than the 100 IR of Stallergènes, where 100 IR refers to the concentration that gives a mean wheal of 7 mm. As the concentrations are plotted logarithmically (higher dilutions to the right), this leads almost to a 50% difference in concentration between the 100 IR Allerbio and 100 IR Stallergènes extracts.

fate–polyacrylamide gel electrophoresis (SDS-PAGE) for qualitative analysis. ELISAs for the in vitro quantification and radioallergosorbent testing (RAST) inhibition with pooled serum samples are used to compare the extract against

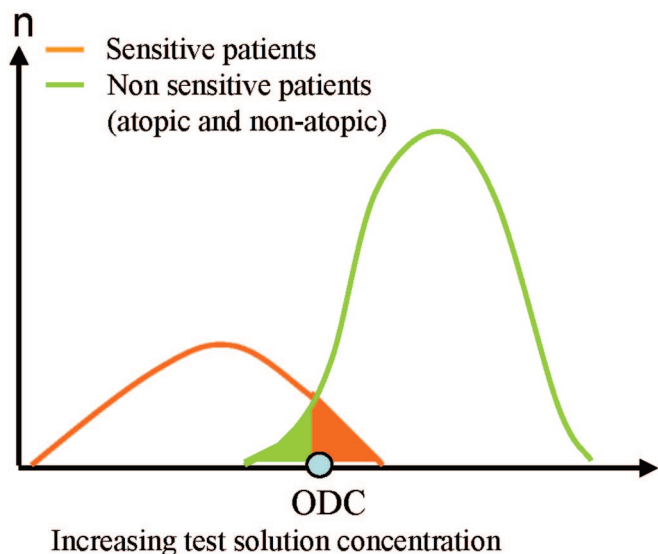


Figure 2. Selection of the optimal diagnostic concentration (ODC): number of persons with positive skin test results (n) is shown on the y-axis and the skin prick test solution concentration is shown on the x-axis. At the point where the curves of sensitive and nonsensitive people cross, the number of errors is lowest. Green indicates false-positive results in nonsensitive people; orange, false-negative results in sensitive people.

a reference extract for determination of total IgE-binding capacity.

Allergopharma (Germany)

Potency expression. Potency is expressed as standard biological units (SBU) for preparations used for diagnosis and therapeutic units (TU) for extracts used for treatment.

Potency measure definition. Allergopharma has designed its extract preparation such that its allergen extracts contain as many proteins (recognized as allergens) as possible from an allergen source, adjusting the type of raw material and extraction times to optimize the total IgE-binding capacity of the extract, as confirmed by allergograms of allergic patients (Table 1).

Micrograms of major allergen. Data on micrograms of major allergen are not given. In 1997, one concentration was published in an article on SLIT (1,000 SBU = 0.54 μg of Der p 1).²²

Control of commercial batches. The control of commercial batches is performed with SDS-PAGE for protein profile, Western blot for allergen profile, and RAST inhibition tests for specific IgE-binding capacity. Major allergens are quantified with ELISA with monoclonal antibodies.

Allergy Therapeutics (United Kingdom, now merged with Bencard in Germany)

Potency expression. In the articles published on SLIT using Allergy Therapeutics' extracts, dilutions of 10,000 SOU/mL (standard oral units) are used. However, the potency of the actually available Oralvac tablets is expressed in TU, al-

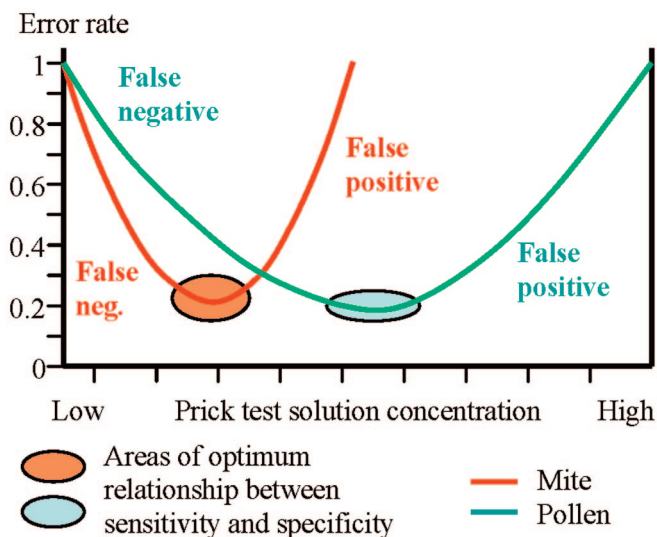


Figure 3. Selection of the optimal diagnostic concentration (ODC). An ODC for mite will be selected at a skin prick test solution concentration for which the error rate, plotted vertically, is lowest (red shaded area). A lower skin prick test solution concentration will lead to too many false-negative test results and a higher test concentration will lead to too many false-positive test results. For a weaker allergen, pollen, this ODC will be found at a higher concentration, more to the right on the horizontal axis (blue shaded area).

though the product is the same. Extracts for SPT are in diagnostic units (DU).

Potency measure definition. Allergy Therapeutics has determined a method to emphasize the issue of false-negative or false-positive results by testing with SPT 3 groups of people (Fig 2, Table 1). A dose of 10,000 DU is now the concentration at which the percentage of false-positive results and false-negative results is lowest. For house dust mite, a more potent allergen, this is at a lower concentration than for pollen (Fig 3). The TU are directly related to DU, with 10,000 DU of house dust mite corresponding to 16,666 TU and 10,000 DU of pollen corresponding to 80,000 TU.

Micrograms of major allergen. A publication from 2001²³ used grass drops with 10,000 SOU (equal to 220 μg of Phl p 1). Recently, the company provided the following data per milliliter of the maintenance vials (Nr.3) of Oralvac: *Alternaria alternata* (Alt a 1), 0.7 $\mu\text{g}/\text{mL}$; *Dermatophagoides farinae* (Der f 1), 140 $\mu\text{g}/\text{mL}$ (questionable data because reference extract is of doubtful quality, per the company); grass (Phl p 1), 45 $\mu\text{g}/\text{mL}$; and birch (Bet v 1), 35.9 $\mu\text{g}/\text{mL}$.

Control of commercial batches. The control of commercial batches is based on biological potency estimates with sandwich ELISAs, IgE reactivity ELISAs, and electrophoretic techniques.

HAL Allergy (The Netherlands)

Potency expression. Potency is expressed in allergy units (AU).

Table 4. Micrograms of Major Allergen Data for UBE Standardized Extracts of IPI-ASAC Provided by the Company^a

Genus species	Major allergen	Micrograms of major allergen in 1,000 UBE	Commercial extract, UBE/mL	Commercial extract, µg/mL
<i>Dermatophagoides pteronyssinus</i>	Der p 1	0.53	1,755	0.93
	Der p 2	0.22		0.39
<i>Dermatophagoides farinae</i>	Der f 1	3.85	130	0.5
	Der f 2	X		X
<i>Phleum pratense</i>	Phl p 1	24.1	159	3.8
	Phl p 5	9.5		1.5
<i>Dactylis glomerata</i>	Dac g 1	11.5	240	2.8
	Dac g 5	2.1		0.5
<i>Lolium perenne</i>	Lol p 1	16.5	176	2.9
	Lol p 5	5.6		1.0
<i>Poa pratensis</i>	Poa p 1	66	265	17.5
	Poa p 5	X		X
<i>Cynodon dactylon</i>	Cyn d 1	X	2,920	X
<i>Felis domesticus</i>	Fel d 1	0.04	18,900	0.76
<i>Alternaria</i>	Alt a 1	0.1	5,088	0.5

Abbreviation: UBE, unidades biológicas equivalentes.

^a Numbers are only of value for the specific products indicated and in the time frame given. Quantity of UBE per milliliter varies per allergen. See data in last column.

Potency measure definition. HAL Allergy adapted the AU system, developed by P.C. Turkeltaub²⁴ and adopted by the FDA Laboratory of Allergenic Products and referred to as the intradermal dilution for 50 mm sum of erythema (ID₅₀EAL) method. The ID₅₀EAL method entails preparing a series of 3-fold dilutions of a reference extract and injecting 0.05 mL intradermally to 15 to 20 highly sensitive allergic patients. The dilution that results in an erythema with the sum of the longest diameter and mid-point (orthogonal) diameter equaling 50 mm is considered the end point (D₅₀). The mean D₅₀ is calculated, and the potency of the extract is reported in bioequivalent allergy units (BAU). This method differs from the Nordic method, which uses the prick-percutaneous technique and compares the wheal size with a histamine dose-response curve, whereas the ID₅₀EAL method uses the intradermal technique and then measures the erythema response. The steep

dose-effect curve of intracutaneous tests allows a more precise definition of the ID₅₀ value (Table 1).

Micrograms of major allergens. The only data available come from a publication in 2004 on SLIT (1,000 AU = 3.7 µg of Phl p 5).²⁵

Recommended maintenance dose. The recommended maintenance dose is 10,000 AU of aluminum hydroxide adsorbed depot extract each 4 to 6 weeks for SCIT and 2,500 AU each day for SLIT.

Control of commercial batches. The control of commercial batches is performed with SDS-PAGE, enzyme allergosorbent test inhibition, immunoblotting, and, where possible, major allergen content.

IPI-ASAC (Spain)

Potency expression. Potency is expressed in equivalent biological units (unidades biológicas equivalentes; UBE).

Table 5. Micrograms of Major Allergen Data of Leti Retard Provided by the Company^a

Genus species	Major allergen(s)	Concentration of commercially available extract, HEP _L /mL	Major allergen, µg/mL
<i>Dermatophagoides pteronyssinus</i>	Der p 1, Der p 2	10	3, 2
<i>Dermatophagoides farinae</i>	Der f 1, Der f 2	10	1, 1
<i>Betula verrucosa</i>	Bet v 1	100	35
<i>Phleum pratense</i>	Phl p 1, Phl p 5	100	5, 10
<i>Dactylis glomerata</i>	Dac g 1, Dac g 5	100	7, 28
<i>Lolium perenne</i>	Lol p 1, Lol p 5	100	16, 40
<i>Poa pratensis</i>	Poa p 1, Poa p 5	100	4, 5
<i>Cynodon dactylon</i>	Cyn d 1	100	72
<i>Felis domesticus</i>	Fel d 1	10	3
<i>Canis familiaris</i>	Can f 1	10	5
<i>Alternaria</i>	Alt a 1	10	0.05

Abbreviation: HEP_L, histamine equivalent prick_{Leti}.

^a Monthly dose was 0.5 mL subcutaneously. Numbers are only of value for the specific products indicated and in the time frame given.

Potency measure definition. To derive an IHRS, IPI-ASAC uses an adaptation of the method recommended by the Nordic guidelines (Table 1).

Micrograms of major allergens. See Table 4 for the micrograms of major allergens.

Recommended maintenance dose. The recommended maintenance dose is 0.8 mL once or twice monthly for SCIT and 0.6 mL twice weekly for SLIT for the products listed in Table 4.

Control of commercial batches. The control of commercial batches is performed with IgE inhibition assay. When possible, major allergens are measured with a capture ELISA using monoclonal antibodies of commercial kits or in-house produced ones.

Leti (Spain)

Potency expression. Potency is expressed in histamine equivalent prick_{Leti} (HEP_L).

Potency measure definition. Leti bases its HEP unit on the recommendations of the Nordic guidelines (Table 1).

Micrograms of major allergens. See Table 5 for the micrograms in major allergens.

Recommended maintenance dose. The recommended maintenance dose is 0.5 mL monthly for SCIT and 2 drops (0.09 mL) daily of 100 HEP_L/mL for pollens and of 10 HEP_L/mL for mites, molds, and epithelia for SLIT.

Tests performed on batches released. New allergen extract preparations are compared with these internal reference extracts by means of bioequivalence in vitro with REINA Competition.²⁶ A concentration of 10 HEP_L produces in vitro 50% of inhibition.

Stallergènes (France)

Potency expression. Potency is expressed in IR and index of concentration (IC).

Potency measure definition. The method (Table 1) is almost the same as the one used by Allerbio, differing only in the diameter of the reference value (see explanation in the "Allerbio" section and Fig 1). A total of 100 IR of Stallergènes extract as a consequence has a higher concentration than 100 IR of Allerbio extract. An unstandardized extract is said to have 100 IC/mL when its manufacturing parameters lead to the same dilution ratio as that of a standardized reference extract at 100 IR/mL from the same genealogic family, taken as a reference. The same unit of potency is used for immunotherapy extracts and for extracts for diagnostic procedures.

Micrograms of major allergen. Stallergènes has been measuring major allergen content in their extracts for more than 10 years. The assay's technology has evolved over time from RAST inhibition with polyclonal antibodies to sandwich ELISA with monoclonal antibodies. This might explain the discrepancy between the micrograms of major allergen data published in the literature on SLIT and the current company data on their package insert (Table 6). Furthermore, not all of

the cited trials were sponsored by Stallergènes, so different tests may still have been used.

Recommended maintenance dose. The recommended maintenance dose is 0.8 mL of 10 IR/mL for the depot product. In SLIT for mites, 5 grasses, or birch, the recommended maintenance dose is 240 IR on alternate days or 120 IR daily. For cat and *Alternaria*, it is just 80 IR on alternate days or 40 IR daily.

Tests performed on batches released. Tests performed were RAST or ELISA inhibition, isoelectric focusing or SDS-PAGE, and major allergen measurements for clinically important allergens such as mites, grass pollen, cat, dog, tree pollen, ragweed, and *Alternaria*.

DISCUSSION

Although the different companies all have their own way of measuring extract potency, some general principles are identified. An IHRS is created that is tested in vivo on allergic patients and in vitro qualitatively and quantitatively. The commercial batches are then compared against the IHRS in vitro only to document their equipotency. For the standardized extracts, one of these in vitro tests is the quantification of micrograms of major allergen(s).

If we try to translate into micrograms of major allergen per month the data presented herein for recommended SLIT maintenance doses, we can see results fall in a wide range (Table 7). At least 2 different explanations could be given for this observation. First, a difference in dosing seems to exist, because manufacturers' recommendations for monthly maintenance SLIT dose diverge between 5 and 45 times the dose they recommend for SCIT maintenance treatment with their products. A second explanation has to be sought in the pitfalls that exist in the measurement of micrograms of major allergen as a sole indicator of potency. Apart from micrograms of major allergen, the quality of allergen extracts depend on antigen composition, allergen extract purification, stability, and overall potency. The first only focuses on one allergen (eg, Der p 1). For complex allergens, the measurement of the total IgE-binding capacity might be important to give additional potency information, because it also measures the presence of various minor allergens.

Furthermore, the measurement of micrograms of major allergen as such is not a standardized procedure worldwide. The outcome depends on the technique, the reference extract, and the antibodies used.¹⁸ Monoclonal antibodies may not bind to all relevant isoallergens and may give different values from rabbit antibody assays.²⁷ In addition, patients in different parts of the world may react differently because of variation in natural exposure.²⁸ As a consequence, the comparison of allergen extracts of different companies is of limited use, unless the same analytic methods and materials will be used (eg, monoclonal antibody and reference standards). This is the aim of the Certified References for Allergens and Test Evaluation (CREATE) program, sponsored by the European Union.²⁹

Table 6. Micrograms of Major Allergen Data for Stallergènes' Products of 100 IR as Given in the Literature⁶ vs Data Released by the Company in 2006^a

Genus species	Major allergen	Year(s) of publication	Concentration Corresponding to 100 IR, µg/mL	
			Published	Given by company
<i>Dermatophagoides pteronyssinus</i>	Der p 1	1997, 2000 1999, 2001, 2004 2002 2004 (tablet)	2.4 8 4.8 2.7	20
<i>Dermatophagoides farinae</i>	Der f 1	1997, 2000 1999, 2001, 2004 2002 2004 (tablet)	1.9 14 3.7 3.1	50
<i>Betula verrucosa</i>	Bet v 1			50
<i>Oliva</i>	Ole e 1	1998	13.5	10
<i>Juniperus ashei</i>	Jun a 1			100
<i>Phleum pratense</i>	Phl p 5	1994 1999	6.4 8.5	7
<i>Lolium perenne</i>	Lol p 1	2002	3.92	
5 Grasses	Dac g 5	2004	14	
	Lol p 1		24	
<i>Ambrosia</i>	Amb a 1	2003 2003 2004	70 160 116	100
<i>Parietaria</i>	Par j 1	1999	70	
<i>Felis domesticus</i>	Fel d 1			45
<i>Canis</i>	Can f 1			20
<i>Alternaria</i>	Alt a 1			6

Abbreviation: IR, index of reactivity.

^a Numbers are only of value for the specific products indicated and in the time frame given.

Table 7. Monthly Micrograms of Major Allergen Dose for SLIT and for SCIT^a

Manufacturer	Monthly SLIT dose, µg		Monthly SCIT dose, µg	SLIT × SCIT
	<i>Dermatophagoides pteronyssinus</i> (Dpt)	<i>Phleum pratense</i>		
ALK	10.8	450	Dpt: 3.2; grass: 20.2	Dpt: 3.4; grass: 22.3
Allergopharma	48 ^b	No data	No data	No data
HAL Allergy	2,500 AU/d	266 ^c	10,000 AU	7.5
IPI-ASAC	5.0 (5.4 mL)	8.1	0.8 mL	6.7
Leti	8.1 (2.7 mL)	13.5	0.5 mL	5.4
Stallergènes	720 (120 IR/d)	252	8 IR	450

Abbreviations: AU, allergy units; IR, index of reactivity; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.

^a Numbers are only of value for the specific products indicated and in the time frame given.

^b See Hirsch et al.²²

^c See Bufe et al.²⁵

Hence, the tables that report major allergen content of the different European extract manufacturers, included in this article, should not be used as the only tool to compare potency. In addition, equipotent doses for US licensed allergen extracts should not be calculated based on the micrograms of major allergens in these tables. American physicians who are prescribing SLIT must inform their patients that no FDA-approved sublingual extracts currently exist. Furthermore, they must be aware of the danger of applying nonsci-

entifically supported doses to patients in an off-label prescription, as shown by a report of anaphylactic reaction to SLIT in a US patient.³⁰

The true potency of an extract depends not only on the microgram of major allergen, as already discussed in detail herein, but also on the overall biological activity. The best comparison among extracts from different manufacturers is in clinical trials, as currently being performed for SLIT in the United States. A surrogate measure could be comparison of

the total biological activity found with in vivo potency tests (eg, titrated skin testing in the same allergic patients), in combination with in vitro qualitative assays and single component estimates, but always using identical tests and reagents.

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REFERENCES

- Bowen T, Greenbaum J, Charbonneau, et al. Canadian trial of sublingual swallow immunotherapy for ragweed rhinoconjunctivitis. *Ann Allergy Asthma Immunol.* 2004;93:425–430.
- Gomez Vera J, Flores Sandoval G, Orea Solano M, Lopez Tiro J, Jimenez Saab N. Safety and efficacy of specific sublingual immunotherapy in patients with asthma and allergy to *Dermatophagoides pteronyssinus*. *Rev Alerg Mex.* 2005;52:231–236.
- Niu C, Wang J, Huang J, Lue K, Chen W. Efficacy and safety of sublingual immunotherapy with high dose house dust mite extract in asthmatic children: a multicenter randomized double-blind, and placebo-controlled study. *J Allergy Clin Immunol.* 2004;113(suppl 2):S108.
- Penagos M, Compalati E, Tarantini F, et al. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3 to 18 years of age: a meta-analysis of randomized, placebo-controlled, double-blind trials. *Ann Allergy Asthma Immunol.* 2006;97:141–148.
- Wilson DR, Torres Lima M, Durham SR. Sublingual immunotherapy for allergic rhinitis [review]. *Cochrane Database Syst Rev.* 2003;(2):CD002893.
- Cox LS, Linnemann D, Nolte H, Weldon D, Finegold I, Nelson H. Sublingual immunotherapy: a comprehensive review. *J Allergy Clin Immunol.* 2006;117:1021–1035.
- Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E. Standards for practical allergen-specific immunotherapy: non-injective immunotherapy. *Allergy.* 2006;82(suppl):S14–S19.
- Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol.* 2006;117:802–809.
- Malling HJ, Lund L, Ipsen H, Poulsen L. Safety and immunological changes during sublingual immunotherapy with standardized quality grass allergen tablets. *J Investig Allergol Clin Immunol.* 2006;16:162–168.
- Larsen TH, Poulsen LK, Melac M, Combebias A, Andre C, Malling HJ. Safety and tolerability of grass pollen tablets in sublingual immunotherapy: a phase-I study. *Allergy.* 2006;61:1173–1176.
- Dreborg S, Einarsson R. The major allergen content of allergenic preparations reflect their biological activity. *Allergy.* 1992;47(4 pt 2):418–23.
- Lombardero M, Quirce S, Duffort O, et al. Monoclonal antibodies against *Olea europaea* major allergen: allergenic activity of affinity-purified allergen and depleted extract and development of a radioimmunoassay for the quantization of the allergen. *J Allergy Clin Immunol.* 1992;89:884–894.
- Boluda L, Casanovas M, Prieto JL, Fernandez-Caldas E. Determinations of Par j 1 by a competitive enzyme immunoassay using human specific IgE and IgG: validation by skin prick testing. *J Investig Allergol Clin Immunol.* 1998;8:207–213.
- American Academy of Allergy, Asthma and Immunology and American College of Allergy, Asthma and Immunology. Allergen immunotherapy: a practice parameter. *Ann Allergy Asthma Immunol.* 2003;90(1 suppl 1):1–40.
- Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy.* 1998;53(44 Suppl):1–42.
- Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol.* 2001;108(5 suppl):S147–S334.
- van Ree R. Indoor allergens: relevance of major allergen measurements and standardization. *J Allergy Clin Immunol.* 2007;119:270–277.
- Grier TJ, Hazelhurst DM, Duncan EA, West TK, Esch RE. Major allergen measurements: sources of variability, validation, quality assurance, and utility for laboratories, manufacturers, and clinics. *Allergy Asthma Proc.* 2002;23:125–131.
- Nordic Council on Medicines. *Registration of Allergenic Preparations: Nordic Guidelines*. 2nd Ed. Uppsala, Sweden: NLN Publications; 1989: 1–34. Publication 23.
- Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol.* 2006;117:802–809.
- Guerin B. Purification and standardization of allergens. *Rev Med Suisse Romande.* 1994;114:251–254.
- Hirsch T, Sahn M, Leupold W. Double-blind placebo-controlled study of sublingual immunotherapy with house dust mite extract (D.pt.) in children. *Pediatr Allergy Immunol.* 1997;8:21–27.
- Drachenberg KJ, Pfeiffer P, Urban E. Sublingual immunotherapy: results from a multi-centre, randomised, double-blind, placebo-controlled study with a standardised birch and grass/rye pollen extract. *Allergologie.* 2001;24:525–534.
- Turkeltaub PC. Assignment of bioequivalent allergy units based on biological standardization methods. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M.* 1988;82:19–40.
- Bufe A, Ziegler-Kirbach E, Stoeckmann E, et al. Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: a double-blind placebo-controlled study. *Allergy.* 2004;59:498–504.
- Casanovas M, Marañón F, Bel I. Comparison of skin-prick test assay and reverse enzyme immunoassay competition (REINA-C) for biological activity of allergens. *Clin Exp Allergy.* 1994;24:134–139.
- Calabozo B, Duffort O, Carpizo JA, Barber D, Polo F. Monoclonal antibodies against the major allergen of *Plantago lanceolata* pollen, Pla 1 1: affinity chromatography purification of the allergen and development of an ELISA method for Pla 1 1 measurement. *Allergy.* 2001;56(5):429–35.
- Casas R, Ferrandiz R, Wihl JA, Fernandez B, Dreborg S. Biologic activity of *Dermatophagoides siboney* and *Blomia tropicalis* allergens in exposed and unexposed mite-allergic individuals: effect of patient selection on the biologic standardization of mite extracts. *Allergy.* 1999;54:392–396.
- van Ree R. The CREATE project: EU support for the improvement of allergen standardization in Europe. *Allergy.* 2004;59:571–574.
- Dunsky EH, Goldstein MF, Dvorin DJ, Belecanech GA. Anaphylaxis to sublingual immunotherapy. *Allergy.* 2006;61:1235.

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