Major Allergens and Allergenic Extracts

ALK TECHNICAL MEMO
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Allergenic Extract Complexity

ALK has been in the allergenic extract market for more than 95 years. Since entering the US market in 1985, ALK has grown to be one of the top US extract suppliers, providing consistent, quality products to numerous markets offering allergy testing and treatment. This technical memo serves as a reference and overview of the concept of major allergens in allergenic extracts. Additionally, the Medical Scientific Affairs team is available to answer any questions you may have.

Allergenic extracts are complex biological materials. Each preparation contains non-allergenic materials along with a wide range of proteins and glycoproteins, some of which can be allergens in atopic patients. Numerous studies have demonstrated that most extracts contain a few allergens that induce specific IgE in the majority of patients who exhibit sensitivity to the extract. A *major allergen* is one that induces IgE in at least 50% of sensitive patients in a population and exhibit strong IgE binding[1,2].

When allergenic extracts are manufactured, they are labeled with various units that denote potency or concentration. Worldwide, many different types of units are used in conjunction with allergenic extracts. In the US, extracts may be labeled as weight-to-volume (w/v) which reflects the ratio of source material, such as pollen, to the volume of extracting fluid. Other US extracts are labeled in protein nitrogen units (PNU), which is an indication of the protein content of the extract. The FDA has established potency specifications for a limited number of allergenic extracts. Allergy Units (AU) and Bioequivalent Allergy Units (BAU) are applied to dust mite extracts (AU) and grass and cat extracts (BAU). Short Ragweed extracts are labeled in w/v or PNU, but also describe the quantity of the major allergen in Antigen E Units (also known as Amb a 1) [3].

In Europe, the situation is even more complex since each manufacturer may develop their own unit measurements based on in-house references. Thus, products may be labeled in SQ Units, IR Units, HEP, UM, BAU, and other units. As a result, virtually all publications relating to allergy immunotherapy clinical trials report dosing in terms of local units and the amount of particular major allergen. This allows physicians and researchers to compare the doses used in different clinical studies with those used in clinical practice as well as in other research studies[1,2]. The range of units applied to allergenic extracts makes it difficult for physicians and researchers to compare the results of clinical studies in which the treatment doses were described in different units. As a result, an international effort was established to implement a common language for expressing immunotherapy dosing[1,2].

Major allergen content has been accepted as a convenient, objective marker for dosing[1,2]. This concept has been incorporated into the US Immunotherapy Practice Parameters (ITPP) in the 2011 update[3]. The ITPP suggests that physicians contact their extract suppliers to obtain major allergen values for key extracts they use. ALK can provide major allergen data for standardized products.

The Usefulness of Major Allergen Values

Many of the studies that have demonstrated the efficacy of allergy immunotherapy were conducted in Europe using products labeled in a variety of units. For example, in a number of widely cited immunotherapy studies including some for grass, mite, cat, and birch, the dosing was expressed in SQ Units (ALK-Abelló A/S, Horsholm, Denmark). When these studies were published, the dosing was also expressed in terms of the major allergen content of the maintenance doses delivered[4,5]. Today, virtually all clinical immunotherapy trials report doses in the labeled units and in terms of one or more major allergens. Published US immunotherapy studies have also reported dosing in terms of major allergen[6,7]. Allergy researchers around the world are now reporting immunotherapy dosing in terms of the quantity of major allergen in each maintenance dose. ALK US endorses this approach and several of our scientists have participated in developing and validating methods for quantifying major allergen levels in allergen extracts[1,2].

ALK routinely measures major allergen contents in standardized products as well as in a number of non-standardized products. ALK R&D laboratories in Denmark and Spain have published a number of scientific papers on these analytical methods[8-19].

Allergists in clinical practice have become aware of the trend toward establishing dosing based on major allergen content. As this awareness has grown, many allergists have requested major allergen content data from manufacturers as recommended by the ITPP. This information is reported to be useful to allergists in allowing them to compare their treatment protocols with those reported in the literature and suggested in the ITPP[3].

Summary

Analytical results indicated considerable differences in major allergen content of non-standardized pollen extracts. Many of those differences can be attributed to variations in the allergen content of the source pollen materials. Standardized extracts exhibit less variability between lots as would be expected. However, standardized products vary in their major allergen content among U.S. manufacturers.

The following tables give an overview of the major allergen content of a range of ALK allergenic extracts. In addition, the table for standardized extracts indicates the range of major allergen values found in samples from across the US industry.

- ALK has developed and validated a number of immunochemical methods for determining the major allergen content of allergenic extracts.
- Allergy research laboratories worldwide are expressing immunotherapy dosing in terms of micrograms of major allergen.
- Allergists should read and follow the package inserts that accompany the allergenic extracts used in their practices. Broad guidelines suggesting target immunotherapy doses were provided in the Immunotherapy Practice Parameters issued by the ACAAI and AAAAI in 2011.
- Lot-specific Certificates of Analysis are available upon request for more than 20 important allergenic extracts. Physicians may refer to the average major allergen values presented in the table below or may use the specific values provided on each Certificate of Analysis.
- The major allergen values presented in the following tables were obtained at or near the time those products were released. Preliminary stability data have been described elsewhere[24].

Table 1. Standardized Extracts

ALK Standardized Extracts			
Product	**Probable Therapeutic Dose from Literature***	**ALK-Abelló Major Allergen Content Mean µg/mL ****	
Mite: D. pteronyssinus – 10,000 AU n = 53	7-15 µg Der p 1	Der p 1 + Der p 2	120
U.S. Extract Industry Range: Der p 1 & Der p 2: 8 – 538			
Mite: D. farinae – 10,000 AU n = 52	7-15 µg Der f 1	Der f 1 + Der f 2	160
U.S. Extract Industry Range: Der f 1 & Der f 2: 48-216			
Cat Hair/Epithelial – 10,000 BAU n = 62	10-15 µg Fel d 1	Fel d 1	40
U.S. Extract Industry Range: 26 – 44			
Bermuda – 10,000 BAU/ml: n = 33	Not yet reported	Cyn d 1	200
U.S. Extract Industry Range: 125-449			
Orchard Grass – 100,000 BAU/ml: n = 22	10-20 µg Dac g 5	Dac g 5:	760
U.S. Extract Industry Range: 294 – 940			
Meadow Fescue – 100,000 BAU/ml: n = 16	10-20 µg Fes e 5	Fes e 5:	150
U.S. Extract Industry Range: 75 – 190			
Perennial Rye – 100,000 BAU/ml: n = 22	10-20 µg Lol p 5	Lol p 5:	470
U.S. Extract Industry Range: 200 – 436			
June/Kentucky Bluegrass – 100,000 BAU/ml: n = 14	10-20 µg Poa p 5	Poa p 5:	320
U.S. Extract Industry Range: 118 – 421			
Timothy Grass – 100,000 BAU/ml: n = 26	10-20 µg Phl p 5	Phl p 5:	620
U.S. Extract Industry Range: 354 - 1,336			

*Immunotherapy maintenance dose or dose range reported in scientific literature from controlled trials.

**Major allergen average values determined by ALK using immunochemical (ELISA) methods and in-house reference analytical standards. Data on file.

Table 2. Center-AL Extracts

Center-Al, Alum-Precipitated Extracts		
Extract Product Type	**Major Allergen**	**ALK Major Allergen Content Mean µg/mL**
Kentucky Blue/June 20k PNU/mL N= 13	Gr 5	45
Meadow Fescue 20k PNU/mL N= 9		145
Orchard 20k PNU/mL N=12		205
Perennial Rye 20k PNU/mL N= 11		95
Timothy 20k PNU/mL N=13		110
Timothy 10k PNU/mL N=13		40
Brome 20k PNU/mL N=9		15
Bermuda 10k PNU/mL N=17	Cyn d 1	25
Bermuda 20k PNU/mL N=11		65
Short Ragweed 20k PNU/mL N=7	Amb a 1	110
Short Ragweed 10k PNU/mL N=7		55
Olive (pollen) 10k PNU/mL N=14	Ole e 1	370
Birch 10k PNU/mL N=13	Bet v 1	55
Alder 10k PNU/mL N=10		20
English Plantain 10k PNU/mL N=16	Pla l 1	10

Short Ragweed Extracts (Antigen E Content)

All products that contain Short Ragweed extracts must be analyzed for major allergen content by a method prescribed by the FDA. The results of this analysis are confirmed by the FDA. The Antigen E, or Amb a 1, value is printed on each label. The mean value for aqueous 1:10 w/v short ragweed is 500 µg/mL and glycerinated 1:20 w/v is 250 µg/mL. Effective maintenance doses for Short Ragweed were reported to be in range of 6-24µg Antigen E Units (micrograms) per injection.

Non-Standardized Extracts

In addition to the standardized products listed in Table 1, ALK has developed the capability to analyze the major allergen content in a number of non-standardized products. The major allergen data provided are representative of assays performed in the ALK Allergy Lab. This data is intended to be informational only, and does not confer or imply that all lots manufactured will conform to these concentrations that are noted in Tables 2 and 3.

ALK has been using the same methods to determine major allergen content of source materials and production lots for more than 12 years and has data on major allergen content for many of the most widely prescribed allergenic extracts[20-23].

Table 3. Non-Standardized Extracts

Non-Standardized Extracts		
Extract Product Type	**Major Allergen**	**ALK Major Allergen Content Mean µg/mL**
Birch* 1:10 w/v aqueous N=32	Betula Group 1	420 µg/mL
Birch* 1:20 w/v glycerinated N=39		240 µg/mL
English Plantain 1:10 w/v aqueous N=17	Pla l 1	35 µg/mL
English Plantain 1:20 w/v glycerinated N=21		15 µg/mL
Olive (Pollen) 1:10 w/v aqueous N=7	Ole e 1	> 350 µg/mL
Olive (Pollen) 1:20 w/v glycerinated N=15		> 590 µg/ml
Mugwort/Sage/Wormwood 1:10 w/v aqueous N=25	Art v 1	3000 µg/mL
Mugwort/Sage/Wormwood 1:20 w/v glycerinated N=14		1500 µg/mL
Brome 1:10 w/v aqueous N=9	Gr 5	135 µg/mL
Brome 1:20 w/v glycerinated N=4		85 µg/mL
Dog Hair 1:10 w/v glycerinated N=6	Can f 1	<5 µg/mL
Alternaria 1:20 w/v glycerinated N=22	Alt a 1	<1 µg/mL

*B. nigra, B. verrucosa

ALK Commitment

ALK is committed to helping Allergy Specialists maintain uniformity of care for their patients. Please do not hesitate to contact your Allergy Consultant, Customer Service (800.325.7354) or Medical Scientific Affairs (855.782.9323, science@alk.net, or submit your scientific questions to our 24/7 online helpdesk in a support ticket at:

https://alkinc.freshdesk.com) should you have additional questions or concerns regarding ALK products.

The information provided in this memo is not sufficient for proper diagnosis and treatment of patients. A thorough evaluation by their healthcare provider is required prior to any changes to immunotherapy. All decisions regarding potential patient care are solely at the discretion of the treating physician. Please see full prescribing information.

References:

1. Van Ree R. Indoor Allergens: Relevance of Major Allergen Measurements and Standardization. *J. Allergy Clin Immunol*. 2007;119(2):270-277.
2. Van Ree R, Dorpema JW, Vieths S. Allergy vaccines: a need for standardization in mass units of major allergen. *Pharmeuropa Bio*. 2005;2005(1):27-30.
3. Cox L, Nelson H, Lockey R, Calabria C, Chacko T, Finegold I et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol*. 2011;127(1 Suppl):S1-55.
4. Niggemann B, Jacobsen L, Dreborg S, Ferdousi HA, Halken S, Host A et al. Five-year follow-up on the PAT study: specific immunotherapy and long-term prevention of asthma in children. *Allergy*. 2006;61(7):855-859.
5. Möller C, Dreborg S, Ferdousi HA, Halken S, Høst A, Jacobsen L et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol*. 2002;109(2):251-256.
6. Nanda A, O'connor M, Anand M, Dreskin SC, Zhang L, Hines B et al. Dose dependence and time course of the immunologic response to administration of standardized cat allergen extract. *J Allergy Clin Immunol*. 2004;114(6):1339-1344.
7. Creticos PS, Reed CE, Norman PS, Khoury J, Adkinson NF Jr, Buncher CR et al. Ragweed immunotherapy in adult asthma. *N Engl J Med*. 1996;334(8):501-506.
8. Spangfort MD, Larsen JN. Standardization of allergen-specific immunotherapy vaccines. *Immunol Allergy Clin North Am*. 2006;26(2):191-206, v-vi.
9. Barber D, Polo F, Lombardero M, Villalba M, Rodriguez R. The importance of minor allergens in allergen standardization. *Arb Paul Ehrlich Inst Bundesamt SeraImpfstoffe Frankf A M.* 2006;(95):128-134.
10. Seppälä U, Hagglund P, Wurtzen PA, Ipsen H, Thorsted P, Lenhard T et al. Molecular characterization of major cat allergen Fel d 1: expression of heterodimer by use of a baculovirus expression system. *J Biol Chem*. 2005;280(5):3208-3216.
11. Duffort O, Calabozo B, Gonzalez R, Carpizo JA, Barber D, Polo F. Monoclonal Antibody-Based ELISA to Quantify the Major Allergen of Cynodon dactylon (Bermuda Grass) Pollen, Cyn d 1. *Int Arch Allergy Immunol*. 2004;135(4):277-283.
12. Lombardero M, Garcia-Selles FJ, Polo F, Jimeno L, Chamorro MJ, Garcia-Casado G et al. Prevalence of sensitization to Artemisia allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. *Clin Exp Allergy*. 2004;34(9):1415-1421.
13. Jimeno L, Duffort O, Serrano C, Barber D, Polo F. Monoclonal antibody-based ELISA to quantify the major allergen of Artemisia vulgaris pollen, Art v 1. *Allergy*. 2004;59(9):995-1001.
14. Løwenstein H. Overview: biological standardization in Europe and the USA. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M*. 2003;(94):67-68.
15. Calabozo B, Barber D, Polo F. Studies on the carbohydrate moiety of Pla l 1allergen. identification of a major N-glycan and significance for the immunoglobulinE-binding activity. *Clin Exp Allergy*. 2002;32(11):1628-1634.
16. Lombardero M, Obispo T, Calabozo B, Lezaun A, Polo F, Barber D. Cross reactivity between olive and other species. Role of Ole e 1-related proteins. Allergy. 2002;57 *Suppl* 71:29-34.
17. Calabozo B, Duffort O, Carpizo JA, Barber D, Polo F. Monoclonal antibodies against the major allergen of Plantago lanceolata pollen, Pla l 1: affinity chromatography purification of the allergen and development of an ELISA method for Pla l 1 measurement. *Allergy*. 2001;56(5):429-435.
18. Larsen JN, Houghton CG, Lowenstein H, Lombardero M. Manufacturing and standardizing allergen extracts in Europe. *Clin Allergy Immunol*. 2004;18:433-455.
19. Mogensen JE, Wimmer R, Larsen JN, Spangfort MD, Otzen DE. The major birch allergen, Bet v 1, shows affinity for a broad spectrum of physiological ligands. *J Biol Chem*. 2002;277(26):23684- 23692.
20. G. Plunkett, L. Jimeno, 1 ALK-Abelló, Inc., Round Rock, TX, ALK-Abelló, Madrid, Spain Group 1 Major Allergen, Art v 1, in Artemisia Species Found in the United States. AAAAI March 2006 Miami Abstract 846, JACI (2006).
21. G. Plunkett, N. Noieam, J. Gaswint, R. Lankow; 1 ALK-Abelló, Inc., Round Rock, TX, ALK-Abelló, Inc., Port Washington, NY. Major Allergen Content of Standardized Mite and Grass Allergenic Extracts AAAAI March 2005 San Antonio Abstract 652,JACI 115. No. 2 (2005).
22. Greg Plunkett, J. Gaswint, N. Noieam, ALK-Abelló, Inc, Round Rock, TX. Quality Control of Non-Standardized Allergenic Extracts: Major Allergen and IgE Binding of Birch, English plantain and Olive Pollen. AAAAI, March 2004 Abstract 449.
23. GA Plunkett, WT Caples, Richard Lankow, Peter Hauck, ALK- Abello, Inc, RoundRock, TX and Port Washington, NY. Major Allergen Determination in Mite, Grass, and Cat Hair/Pelt Allergenic Extracts. ACAAI, November 2002 Abstract 11.
24. G. Plunkett. Stability of Allergen Extracts Used in Skin Testing and Immunotherapy. *Curr Opin Otolaryngol Head Neck Surg*. 2008;16(3):285-29.