

Immunotherapy Allergen Extracts

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Disclosures:

Lectureship, Research Grants and Reagents:

Themofisher Scientific/Phadia
Siemens Healthcare

Scientific Advisory Board:

Hycor Biomedical
U.S. Diagnostic Standards
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Learning Objectives

- Discuss preparation and status of USA allergen extracts used in immunotherapy
- Overview USA extract standardization strategy
- Illustrate the process involved in preparing an allergen extract vial

Prerequisite for Effective Immunotherapy

Specific Allergen Diagnosis to Define the Patient's Allergen Sensitization Pattern

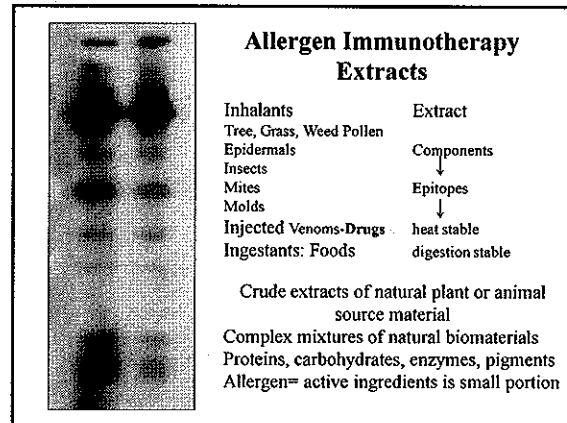
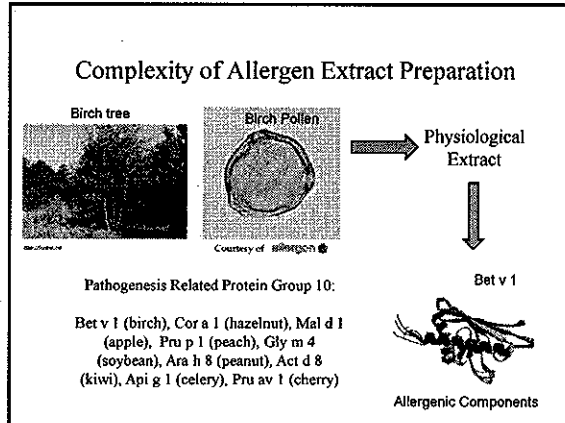
- *in vivo* challenge [skin test, ocular or respiratory challenge]
- *in vitro* [serological measurement of IgE antibody, basophil histamine release or lymphocyte stimulation]

Allergen

Molecules that elicit allergic reactions in susceptible and genetically predisposed individuals

Naturally occurring proteins, glycoproteins derived from plants or animals, synthetic compounds, metals, small molecules

Preparation and Status of Allergen Extracts Used in Immunotherapy in the USA



- ### Allergen Extracts in USA
- All current US allergenic products were considered safe but licensed before FDA's efficacy requirement established in 1972.
 - 0.4% 19/480 IT extracts are considered standardized
 - Labeled by extraction ratio (w/v) or protein nitrogen unit designation (Kieldahl method; PNU/ml).
 - Little correlation between w/v or PNU/ml units and biological measures of allergen potency.

FDA Allergenic Extract Efficacy Review

Panel 1, 21 CFR 601.25 (1974-1979): 773 extracts
 Panel 2, 21 CFR 601.26 (1982-1983): 752 extracts
 Internal FDA Review 2004-2006: >1500 allergen extracts

– Duplicate removal: 1269 non-standardized extracts
 Animal: 28, Molds 180, Dusts: 6, Plants 16, Foods 277, Pollens: 708
 Insects: 34

Table 1: Use in Diagnosis and Treatment Addressed in Literature (480)
 Table 2: Food: Use in Diagnosis Addressed in Literature (134)
 Table 3: Non-food: Use in Diagnosis Addressed in Literature (73)
 Table 4: Minimal or no literature related to Diagnosis or Treatment (566)
 Table 5: Potential Safety Issues (16): e.g. house dust, monkey pelt
 Slater JE et al JACI 129:1014-9, 2012.

Status of U.S. Allergen Extracts Used For Diagnosis and Immunotherapy

19 standardized allergen extracts controlled for potency and stability
D. farinosa, *D. pteronyssinus*, cat hair, cat pelt, short ragweed, Hymenoptera (HBV, PWV, YJV, YHV, WEHV, mixed vespidae venom), Grass pollens (Bermuda, red top, June, perennial rye, orchard timothy, meadow fescue, sweet vernal)

1269 non-standardized allergen extracts: no defined potency or stability (expiration date)
 Hundreds of extracts lack evidence of efficacy

Slater JE, et al. The US FDA review of the safety and effectiveness of non-standardized allergen extracts. JACI 129:1014-9, 2012.

U.S. Allergen Extract Standardization Strategy

**Purpose of Extract Standardization:
To Reduce Lot to Lot Variation and
Provide a Measure of Potency**

- Extract inter-lot variation may be considerable
- Some allergen specificities have greater consistency because of their inherent nature: pollen & pure mite cultures > mold, insect extracts
- Manufacturers: control collection, storage and processing of raw materials, setting expiration dates

**Comparison of Allergen Immunotherapy
Extracts in USA and Europe**

Parameter	USA-FDA	Europe-EMA
Standardization Method	ID ₅₀ EAL or major allergen content	Nordic
Test Technique	Intradermal	Percutaneous
Endpoint	Extract dilution \Rightarrow sum erythema of 50 mm ² of allergen content	Extract dilution \Rightarrow wheal = histamine control
Potency determination	Comparison with CBER reference control	Compared with manufacturer's in house reference
Future focus	Overall allergenicity \Rightarrow multiplex microbead array	Major allergen content
Potency Units	BAU, w/Vol, PNU; FDA units of major allergen for ragweed, cat	Varies with each company; IR, SQ-T, ug major allergen
Formulation	Multiple allergens	Generally 1 allergen

**Maximizing Extract Consistency in USA
"19 Standardized Extracts"**

- Must measure potency of each lot of extract
- Market only lots that fall within acceptable potency ranges
- FDA-21 CFR 680.3(e): when appropriate reference reagent and potency test exists, manufacturers must test each lot of allergen extract for potency prior to release

Allergen Standardization Program in USA

- Select a particular allergenic extract to serve as a US reference preparation from which manufactured products (of that specificity) are cross-standardized
- Assign specific "bioequivalent" unitage to the reference by in-vivo (ID₅₀EAL) skin testing
- Select an *in vitro* assay procedure to compare a manufactured product to the reference extract
- Assign units based on relative potency with respect to the reference by in vitro assay analysis

US Standardized Grass Extracts

Allergen Extract	Current In-Vitro Assay	Published Unitage	Average Standard (Comparative to Reference)	Method to establish bioequivalence (relative to reference)
Bermuda grass (<i>Cynodon dactylon</i>)	Competition ELISA	BAU/ml	E6-Ber (2004)	Competition ELISA
Red top grass (<i>Ligustrum album</i>)	IEP		E3-Rt (1999)	
Timothy grass (<i>Phleum pratense</i>)	Protein		E3-Rb (1994)	
Parennial ryegrass (<i>Lolium perenne</i>)			E12-Rys (1995)	
Orchard grass (<i>Dactylis glomerata</i>)			E3-Or (1999)	
Timothy grass (<i>Phleum pratense</i>)			E8-Tt (2004)	
Meadow fescue grass (<i>Festuca elatior</i>)			E3-Mf (1999)	
Sweet vernal grass (<i>Anthriscanthus odoratum</i>)			E6-Sv (2000)	

Standardized US Dust Mite and Cat Extracts

Allergen Extract	Current In-Vitro Assay	Published Unitage	Current Standard (Comparative to Reference)	Method to establish bioequivalence (relative to reference)
Dust mite (<i>Dermatophagoides farinae</i>)	Competition ELISA	ALU/ml (equivalent to BAU/ml)	E4-Df (2000)	Competition ELISA
Dust mite (<i>Dermatophagoides pteronyssinus</i>)	Protein		E7-Dp (1999)	Competition ELISA
Cat pet (F1) (<i>Felis domesticus</i>)	Fel d 1 (RID)	BAU/ml	E1-Cat pet (1996)	RID, IEP
Cat hair (<i>Felis domesticus</i>)	IEP	5-9.9 Fel d 1 U/ml = 5000 BAU/ml 10-19.9 Fel d 1 U/ml = 10,000 BAU/ml	E4-cat hair (2001) C10-cat (calibration set, 2001)	RID, IEP

Complexity of Cat Allergen Extracts Despite Standardization against Fel d 1



- Cat: (*Felis domesticus*)
- Fel d 1 (uteroglobulin)
 - Fel d 2 (albumin)
 - Fel d 3 cystatin
 - Fel d 4 lipocalin
 - Fel d 5 IgA
 - Fel d 6 IgM
 - Fel d 7 Lipocalin
 - Fel d 8 Laterin (sweat)



US Standardized Ragweed and Venom Extracts

Allergen extract	Control by reference test	Initial units	Current standard date placed in service	Method to establish equivalence to previous standard
Short ragweed (<i>Ambrosia artemisiifolia</i>)	Amb # 1 (RID)	Amb # 1 units	E15-Ras C14-Ras (calibration rev. 2004)	RID
Yellow hornet (<i>Vespa sp.</i>)	Hyaluronidase and phospholipase activity	µg protein	V2-HB	
Wasp (<i>Vespa sp.</i>)				
Honey Bee (<i>Apis mellifera</i>)				
White footed hornet (<i>Vespa sp.</i>)				
Yellow jacket (<i>Vespa sp.</i>)				
Black fly (<i>Simulium sp.</i>)				

Overall Allergenicity Assessment by CBER (ID50EAL)

- IntraDermal Dilution for 50 mm sum of Erythema from serial extract dilutions determines the bioequivalent Allergy units
 - ID skin test with 3 fold dilutions: log dose producing 50 mm sum erythema (D50); 1:4.8 million dilution (3^{-14})=100,000BAU/ml
 - Potency = $3^{-14 \text{ (mean D50)}} * 100,000 \text{ BAU/ml}$
- Subsequent comparisons of extracts from same source material: parallel line bioassay

CBER Methods of Allergenic Products Laboratory Fed Register 1994; 23:59(225)

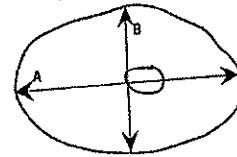


Figure 3: Diameters are measured from the inner margins of the pennaed outline. Longest (A) plus midpoint orthogonal diameters (B) and summed (A + B).

Rabin, Renn, Slater: Manual of Molecular and Clinical Laboratory Immunology, ed 8, 2014

D50 Calculation

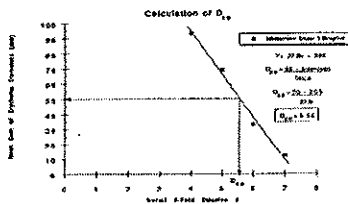


Figure 1. Sample calculation of D_{50} . Serial three-fold dilutions of test material — in this example, histamine base — were injected and the EE responses were plotted against the negative log dilutions. The D_{50} is determined from the best-fit line using the formula: $D_{50} = (50 - \text{Intercept})/\text{slope}$. The calculated D_{50} of 5.56 corresponds to a value of $3^{-14.25} = 100,000 \text{ BAU/ml} = 9.4 \text{ BAU/ml}$.

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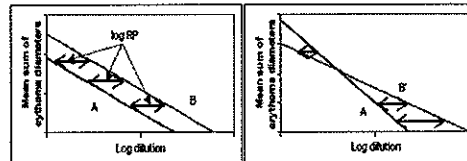
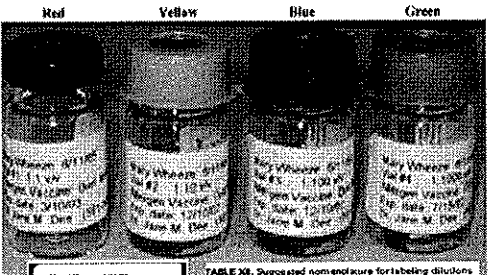


Figure 2: Hypothetical parallel line bioassay curves. In panel A, the bioassay curves are parallel, and the difference of log dilutions resulting in the same diameters is constant at all diameters. The log relative potency (log RP) of test sample B compared to reference A is represented by the difference. In panel B, the curves are not parallel, and the differences vary with the strength of the reaction. Thus, the log RP of B' compared to A cannot be calculated.

Rabin, Renn, Slater: Manual of Molecular and Clinical Laboratory Immunology, ed 8, 2014

Immunotherapy Extract Vial Preparation



Red Yellow Blue Green

May Whorse 01145
Vial #1 1.1ml
Allergen Vaccine, Dog Mites
Exp. Date 12/15/02
Dr. Jose M. Del Oro (913-345-0987)

May Whorse 01145
Vial #2 1.1ml
Allergen Vaccine, Dog Mites
Exp. Date 12/15/02
Dr. Jose M. Del Oro (913-345-0987)

May Whorse 01145
Vial #3 1.1ml
Allergen Vaccine, Dog Mites
Exp. Date 12/15/02
Dr. Jose M. Del Oro (913-345-0987)

May Whorse 01145
Vial #4 1.1ml
Allergen Vaccine, Dog Mites
Exp. Date 12/15/02
Dr. Jose M. Del Oro (913-345-0987)

TABLE XI. Suggested home use for labeling dilutions from the maintenance concentrate

Dilution from maintenance concentrate	Vial label	No.	Color
AU/mg or AU/ml	1:1	1	Red
10:1	1:10	2	Yellow
100:1	1:100	3	Blue
1000:1	1:1000	4	Green
10000:1	1:10000	5	Silver

Principal Immunotherapy AeroAllergen Extracts

- Grasses:** Bermuda, Red-top, Kentucky Blue-June, Meadow Fescue, Orchard, Timothy, Rye, Sweet Vernal: [BAU/ml]
- Dust mites:** *D. pteronyssinus*, *D. farinae* [AU/ml=BAU/ml]
- Weeds:** Ragweed-[Ambal units], Plantain, Pigweed, Lamb's Quarters, Sagebrush, Sheep Sorrel
- Trees:** Oak, Beech, Maple, Elm, Birch, Ash, Sycamore, Poplar, Hickory, Walnut, Mulberry, Juniper
- Fungi:** *Aspergillus*, *Penicillium*, *Mucor*, *Fusarium*, *Alternaria*, *Helminthosporium*, *Cladosporium*, *Botrytis*
- Epithelia-Insects:** Dog, Cat-[10-19.5 Feld1 U/ml=10,000 BAU/ml], Cockroach

Effective U.S. Extract Doses Immunotherapy Practice Parameter 3rd Update

L Cox et al., J Allergy Clin Immunol 2011;127, S1-S55

Allergen Extract	Labelled Potency	Probable Effective Dose	Dose Based on Major Allergen
<i>D. pteronyssinus</i> <i>D. farinae</i>	10,000 & 30,000 AU/ml	500-2000 AU	1,000-1,500 AU
Cat	5,000 & 10,000 BAU/ml	1,000-4,000 BAU	3,750 BAU
Norther Pasture Grasses	100,000 BAU/ml	1,000-4,000 BAU	3,000 BAU
Bermuda Grass	10,000 BAU/ml	300-1,500 BAU	1,000 BAU
Short Ragweed	1:10 w/v (aqueous) 1:20 w/v (glycerin)	6-12 µg Amb a 1 Amb a 1	12 µg Amb a 1
Non-standardized Dog	1:10-1:100 w/vol	15 µg of Can f 1	15 µg of Can f 1
Non-standardized extracts	1:10-1:40 w/vol 10,000-40,000 PNU/ml	Highest tolerated dose	

Allergen Immunotherapy Prescription Order

Prescription Set I	0.9ml inject	conc per ml	allergen stock	ml vol add
DF Mite	900	1800	10,000 AU/ml	1.8ml
DP Mite	900	1800	10,000 AU/ml	1.8ml
Cat	1800	3600	10,000 BAU/ml	3.6 ml
AP Dog	900	1800	38,000 PNU/ml	0.47 ml
Computation (1,800 AU/ml / 10,000 AU/ml) * 10 ml = 1.8 ml				
Extract total				7.67 ml
Diluent				2.33 ml
Vial: 9ml - 6.67ml = 2.33 ml diluent in vial				
Total				10ml

Allergen Group Reactivity

Allergen groups (species within the genus) listed below show strong cross-reactivity within the associated group. Using one member of the group for the allergy immunotherapy extract may be adequate to protect the patient against the entire group.

Group	Members	Notes
Grasses	Red-top, Kentucky Blue, June, Meadow Fescue, Orchard, Timothy, Rye, Sweet Vernal	Strong cross-reactivity within group
Dust Mites	<i>D. pteronyssinus</i> , <i>D. farinae</i>	Strong cross-reactivity within group
Weeds	Ragweed, Plantain, Pigweed, Lamb's Quarters, Sagebrush, Sheep Sorrel	Strong cross-reactivity within group
Trees	Oak, Beech, Maple, Elm, Birch, Ash, Sycamore, Poplar, Hickory, Walnut, Mulberry, Juniper	Strong cross-reactivity within group
Fungi	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Mucor</i> , <i>Fusarium</i> , <i>Alternaria</i> , <i>Helminthosporium</i> , <i>Cladosporium</i> , <i>Botrytis</i>	Strong cross-reactivity within group
Epithelia-Insects	Dog, Cat, Cockroach	Strong cross-reactivity within group

L Cox et al., J Allergy Clin Immunol 2011;127, S1-S55

Protease-containing Extracts				
Allergenic Extract	Insects	Fungi	Mites	Comments
Insects	⊖	⊕	⊕	Whole-body insect extracts contain very high protease levels; susceptible to endogenous proteases unless stored in 50% glycerin
Fungi	⊕	⊕	⊕	Fungal extracts do not appear to be adversely affected by proteases.
Mites	⊖	⊖	⊕	Mite allergens resistant to insect and fungal proteases if stored in ≥ 10% glycerin.
Pollens	⊖	⊖	⊕	Pollen extracts susceptible to insect and fungal proteases; compatible with mite extracts when stored in ≥ 10% glycerin.
Cat hair/epithelia	⊕	⊕	⊕	Fel d 1 in cat extract is highly resistant to fungal and insect proteases
Dog hair/epithelia	⊕	⊖	⊕	Dog allergens susceptible to most fungal extracts, but more stable when mixed with insect extracts.

Allergen immunotherapy: What can and cannot be mixed?
Robert E. Esch, PhD Lenoir, NC J Allergy Clin Immunol 2008;122:659-60.

Allergen Immunotherapy Extract Preparation Manual

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• Michael R Nelson MD Ph.D., Linda Cox, MD

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