

Biological Methods for Allergen Standardization

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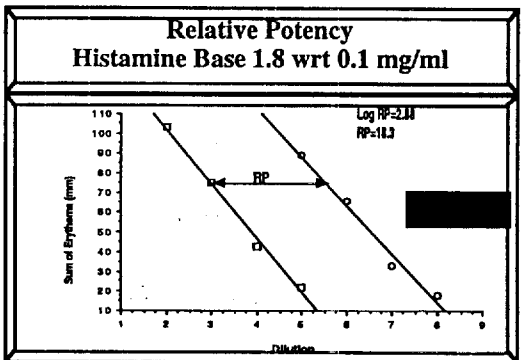
42 USC 262
 Sec. 351. (d) Regulations Governing Licenses
 ...biologicals may be issued only upon a showing that the environment and the products ... meet standards.
 42 USC 263
 Sec. 352. (a)(b) Preparation of Biological Products by the Service
 The Service may prepare and distribute any product described in Sec. 351.
 Panel on Review of Allergenic Products
 Final Report 13 Mar 81
 The Panel, therefore, has recommended that the large proportion of these preparations be retained on the market and made available for continued use pending adequate standardization and further investigation to determine their effectiveness.

US Standard of Potency	In-House/Proprietary Std
1. Common Unit: BAU Interchangeable	1. Proliferation of units: "single of unit" "great confusion" "multiple units per equivalent dose" "standard" to units due to "obvious risks in switching" DeWitt A79 Post Met Int. 1979, VI: 164
2. Cost Effective: Cost/BAU	2. Unlabeled
3. Increased Value of Info: Post Market info applicable to all products: Safety, Efficacy data quickly obtained from all units with common unit.	3. Decreased Value of Info: Applicable only to specific mix. Mixes with small units will not generate data effectively.
4. Promotes Innovation	4. Promotes Marketing
5. Consensus Std: Well Defined Nat'l Criteria.	5. Mix Dependent Std: What are minimal criteria for acceptable Std? Mfr decides.

- ### Properties of Bioassay
- Potency
 - Composition
 - Assignment of Units

Relative Potency

■ Ratio of Doses for Identical Response



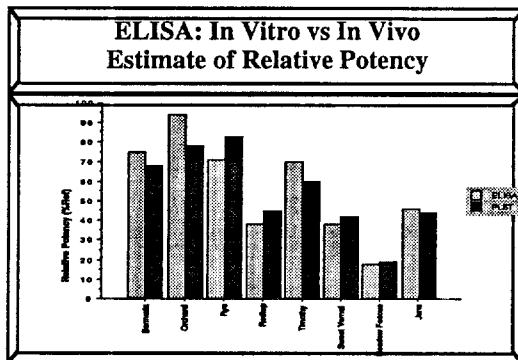
Properties of Acceptable Dose-Response Line

<ul style="list-style-type: none"> ■ <u>Clinical Criteria</u> ■ 4 Serial Dilutions ■ Graded Erythema ■ ΣE Brackets 50mm (includes end-point) 	<ul style="list-style-type: none"> <u>Statistical Criteria</u> $r \geq 0.92$ Slope ≥ 13
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Accuracy and Precision of Parallel-Line Bioassay

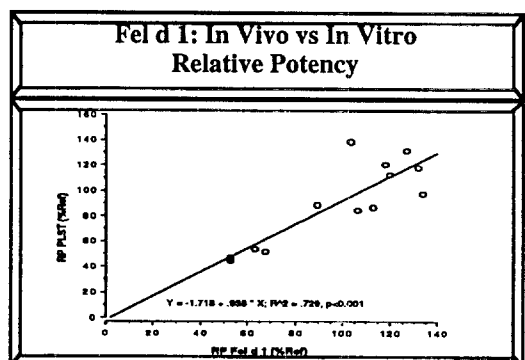
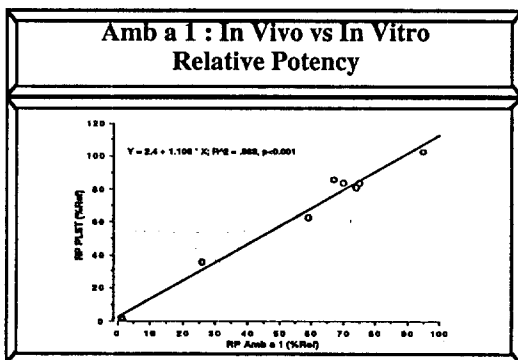
- 95% Limits of RP (%Reference)*
65.7-152.2
- 99% UL of SD of Mean Log RP*
0.76

* N = 4
- Methods of Allergenic Products Testing Laboratory (Nov. 1994)



Major Allergen

- Relative potency based on major allergen content is predictive of relative potency based on parallel-line skin test



"Major Allergen" - Misnomer
Der p 1

1. The determination of the relative potency and relative potency based on Der p 1 was presented to Advisory Committee June 1986.

2. Der p 1 accounts for a negligible percent of the overall allergenic activity of D. pteronyssinus JACI 1968; 81:884.

3. "Major allergen" content is not a measure of overall allergenic activity. Lowenstein in Allergens and Allergen Immunotherapy, 2nd ed, 1999, Marcel Dekker, p.312.

4. In study to determine optimal dose of Der p 1 for immunotherapy, methods section indicates "total allergenic activity was measured by RAST inhibition" not Der p 1. Haugaard, JACI 1993; 91:789.

5. In study to assess stability of D. pteronyssinus slide extracts, Der p 1 content fell off rapidly while the potency assay based on IgE binding using ELISA inhibition showed no significant decrease at room temperature. Annals Allergy Immunol 1996;98:177.

Compositional Differences

- Interpatient variability of RP exceeds upper limit seen with identical extracts
- Relative Potency is patient dependent

**Compositional Differences:
Cat Pelt vs Cat Hair**

Relative Potency (%Reference)

Extract	PLST		
	Fel d 1	Serum-	Serum+
Cat Pelt	[100]	[100]	[100]
Cat Hair	127	132	16

- Cat Pelt 11.8 Fel d 1 U/ml
- Cat Hair 15.0 Fel d 1 U/ml
- Cat Serum- Fel d 1 reactor
- Cat Serum+ non-Fel d 1 reactor

Assignment of Units

- Arbitrary 100,000 IU/ml
- Arbitrary 100,000 AU/ml
- Biologically Based Units

Assignment of Biologically Equivalent Allergy Units (BAU)

■ Potency: Dose for Specified Response - D50

Proficiency Assessment of Mean D₅₀

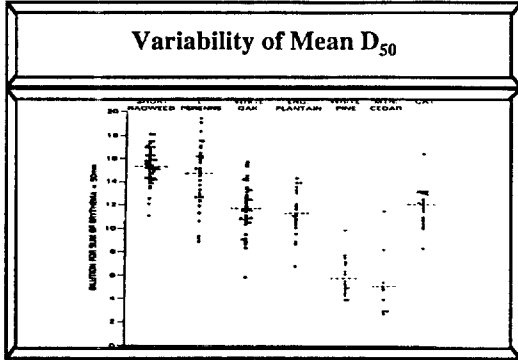
95% Limits of Mean D₅₀*

Histamine base (mg/ml)	
0.1	1.8
3.5-4.5	5.9-6.9

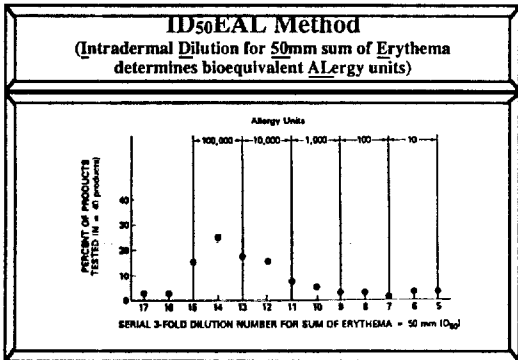
99% UL of SD of Mean D₅₀*

1.01

*N = 4
• Methods of the Allergic Products Testing Laboratory



- ### Subject Selection
- Highly Reactive Subjects:
Puncture $\Sigma E \geq 75\text{mm}$
 - Appropriate Allergic Disease



Percutaneous Reactivity 10,000 BAU/ml

Allergen	Sum of Diameters (mm)	
	Erythema	Wheal
■ Grass	84	15
■ Df	78	15
■ Dp	87	17
■ Cat Hair	71	14
■ Cat Pelt	77	15

- Bifurcated needle
- Df and Dp 10,000 AU/ml = 10,000 BAU/ml

Intradermal Dose for 50mm Sum of Erythema Response (BAU/ml)

■ 0.02*

- *3-14 x 100,000 BAU/ml
- *3-11.9 x 10,000 BAU/ml

- ### Clinical Research Program Proposals
1. Determination of D₁₀ for candidate latex and cockroach ~~reference animals - Ongoing - applicability to future references~~
 2. Determine whether major allergens are predictive of overall allergen activity - Proposed.
 3. Determine whether assays of compositional identity are clinically relevant - Proposed.
 4. Determination of diagnostic and therapeutic safety and efficacy of standardized allergen vaccines - Proposed.
 4. Epidemiologic Studies
 - a. 3rd National Health and Nutrition Examination Survey (NHANES III). Prevalence of allergic disease, percutaneous hypersensitivity to standardized allergens - ongoing.
 - b. Mortality follow up of allergy portion of NHANES II - ongoing.
 - c. IgE Seroprevalence: US population - NHANES III/CDRII - ongoing.
 - d. Impact of allergic disease on fertility - ongoing.