Slides for
APAC
Feb. 10, 2000
from
Dr. Slater

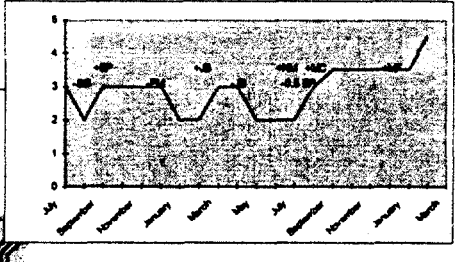
Laboratory of Immunobiochemistry

Operational issues

LIB staffing

- *Full time*
  - *Jay E. Slater, MD - Lab Chief (1.5)*
  - *Lyudmila Soldatova, PhD - Visiting Scientist (2.5)*
  - *Maneesha Solanki - Biologist (4)*
  - *Kristin Morrow - Biologist (<1)*
  - *Melissa Catena - Biologist (<1)*
  - *Mona Febus - Microbiologist (<1)*
- *Part time*
  - *Elizabeth Paupore - Biologist*
  - *Gerald Poley, MD - Guest Worker*
  - *Li-Shan Hsieh, PhD - CDER*

LIB staffing 1998-2000



Operational improvements within LIB

- *Improved competition ELISA*
- *Rapid feedback to specific inquiries*
- *Pro-active reference replacement program*

Division of Bacterial, Parasitic and Allergenic Products (DBPAP)

- *Merger of DAPP and DBP*
- *Drusilla Burns, PhD, Director (Acting)*
- *Carolyn Deal, PhD, Deputy Director*

Merger consequences for LIB

- *Increased access to*
  - *regulatory resources*
  - *administrative resources*
- *Continued*
  - *high level of program support*
  - *research/regulatory balance*

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Regulatory activities

"Routine" regulatory activities

- *Protocol review*
- *Lot release testing*
- *Reference development*
- *Reference distribution*
- *Reference maintenance*
  - *semiannual checks*
  - *replacement*

"Routine" regulatory activities

- *Protocol review (1999)*
  - *477 protocols reviewed*
  - *01 withdrawn on review*
  - *04 lots failed on potency testing*

"Routine" regulatory activities

- *Reference development*
- *Reference distribution*
  - *1999: 1983 vials in 104 shipments sent to manufacturers*
- *Reference maintenance*
  - *semiannual checks*
  - *replacement*

Reference replacement program

- *As of 9/98:*
  - *many out of date (20/24)*
- *Replacement program:*
  - *bring full inventory up to date*
  - *target completion date: August 2001*

Reference replacement procedure

1. *Identify references to be replaced*
2. *Select candidates from recent submissions*
3. *Initial testing in LIB/CBER*
4. *Select provisional reference replacement*
5. *Send provisional replacement to manufacturers*
6. *Review results; confirm selection OR go back to step 2*

Reference replacement activity

- *1998-1999:*

Reference	Date sent for testing	Date released
S4 – D. pteronyssinus/farinae	N/A	11/5/98
C9 – cat	2/1/99	8/2/99
E3 – cat hair	N/A (IEF reference)	3/1/99
E7- D. pteronyssinus	5/11/99	9/27/99

Reference replacement activity

- *References to be replaced in 1999-2000:*
  - *Red top**
  - *Orchard grass**
  - *Meadow fescue**
  - *Sweet vernal*
  - *Short ragweed (S)*

Reference replacement program - switch to lyophilized extracts

- *1999-00 : LIB will lyophilize a portion of all new reference extracts*
- *2000-03 : LIB will assess stability and reliability of lyophilized products*
- *Results and samples will be distributed to APMA membership prior to action*

Laboratory of Immunobiochemistry

Research report

LIB research program summary

- *Allergen structure and function*
  - *Stability of lyophilized references*
  - *Glycosylation*
  - *Enzyme activity*
  - *Identification methods*
- *Immunomodulation*
  - *Epitopes*
  - *LPS*
  - *Cross-sensitization*

SDS-PAGE of glycerinated (gly) and lyophilized extracts with (ly-M) and without (ly) mannitol

Cat pelt lyophilization - Fel d 1

	mean R	SD	units/mL	95% LL	95% UL
E4 cat pelt	8.48	0.29	12.78	11.81	13.82
E4 lyo	8.14	0.24	11.21	10.51	11.95
E4 lyo-man	8.19	0.24	11.43	10.71	12.20

D. pteronyssinus lyophilization

	RP	95% LL	95% UL
E7-Dp	0.7011	0.6005	0.8184
E7- lyo	0.7100	0.4956	1.0172
E7- lyo man	0.9197	0.7618	1.1105

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Allergen structure and function - glycosylation

- *Is the decreased antibody binding of non-glycosylated antigens primarily a function of impaired folding?*
- *What is the biochemical anatomy of the glycosylation requirement?*
- *Can non-glycosylated allergens equal native allergens in immunotherapy?*
- *How can non-glycosylated products be evaluated for diagnosis and therapy?*

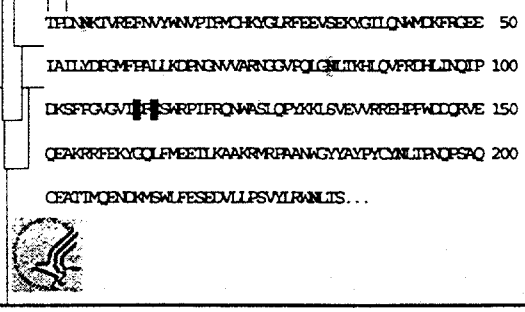
Role of glycosylation in allergen structure and function

- *Model: bee venom hyaluronidase and acid phosphatase*
- *Approach:*
  - *improve/validate current enzyme assays*
  - *express glycosylation mutants in insect cells*
  - *study antibody binding and enzyme activity of native, recombinant and mutant proteins*

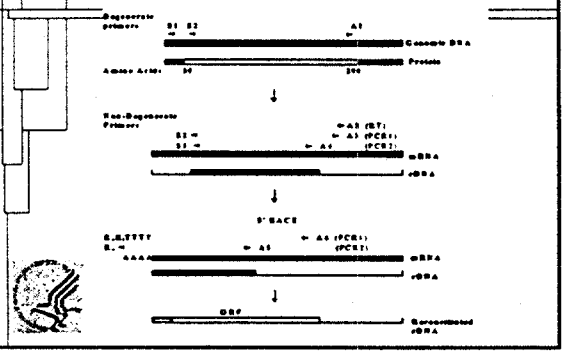
Important Allergens of Honey Bee Venom

Allergen	*Mol. weight, Da*	*% dry weight*	*Toxicity*	*Allergenicity*
Phospholipase A$_2$	19,000	12	membrane-poison	++++
Hyaluronidase	43,000	2	spreading factor	+++
Acid Phosphatase	45,000	1		++
Allergen C	165,000	1		+
Melittin	2,840	50	membrane-poison	+

Hyaluronidase mutation targets



RT-PCR Cloning of Bee Venom Acid Phosphatase



Sequence Homology of Bee Venom Acid Phosphatase

ACID PHOSPHATASE PRECURSOR (Acph-1), lysosomal from fruit fly (Drosophila melanogaster)

Identities = 30/95 (31%), Positives = 46/95 (47%)

HUMAN LYSOSOMAL ACID PHOSPHATASE PRECURSOR (LAP)

Identities = 16/55 (29%), Positives = 27/55 (49%)

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  - Glycosylation
  - Enzyme activity
  - Identification methods
- Immunomodulation
  - Epitopes
  - LPS
  - Cross-sensitization

Allergen structure and function - enzyme activity

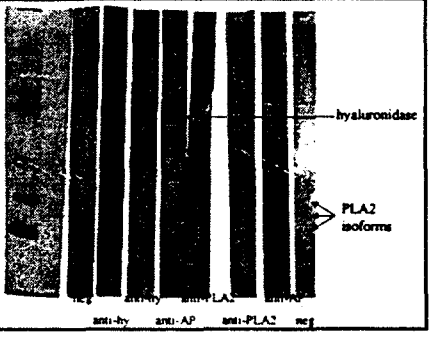
- What is the relationship between enzyme activity and allergenicity?
  - antibody binding
    - in vitro
  - bioavailability
  - antigen processing
- Specific regulatory applications

hymenoptera, mites, latex

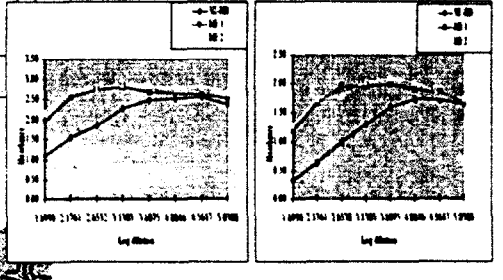
Allergen structure and function - enzyme activity

- Model: bee venom hyaluronidase and PLA2
- Approach:
  - improve/validate current enzyme assays
  - develop immunoassays for hyaluronidase and PLA2
  - compare enzyme activity and antibody binding in fresh, old and inactivated samples

Rabbit anti-hyaluronidase and anti-PLA2



Competition ELISA for PLA2



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  - *Cross-sensitization*

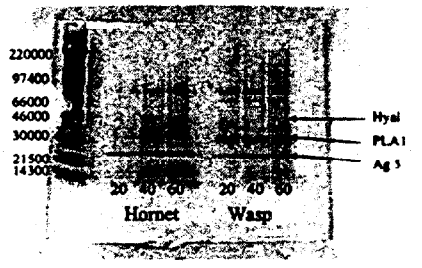
Allergen structure and function - identification methods

- *MALDI-TOF mass spectroscopy*
- *Quantitative SDS-PAGE*
- *Quantitative immunoblot*

Allergen structure and function - identification methods

- *Model: all standardized allergens*
- *Approach:*
  - *evaluate allergens with sequential SDS-PAGE and MALDI-TOF mass spectroscopy*
  - *identify common features among lots and manufacturers (normative phase)*
  - *identify specific allergens with immunoblots*

SDS-PAGE of venoms



Allergen identification techniques - additional questions

- *Can we develop a quantitative profile of natural allergen preparations?*
- *Can we use MALDI-TOF mass spectroscopy to carefully assess the glycosylation of recombinant allergens?*

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Immunomodulation: lipopolysaccharides

- *LPS co-administered with Hev b 5/MBP accentuates*
  - *anti-Hev b 5 IgE and IgG responses*
  - *anti-Hev b 5 and anti-MBP splenocyte proliferation*

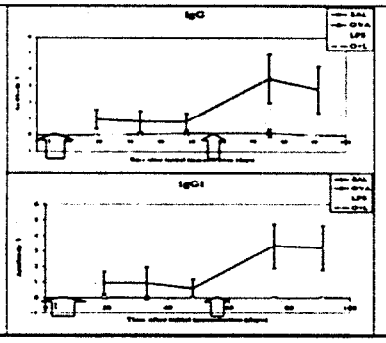
Immunomodulation: lipopolysaccharides

- *Model: airway immunization in mice*
- *Approach:*
  - *verify Hev b 5 results using ovalbumin*
  - *assess functional responses (plethysmography)*
  - *identify anatomic specificity/requirements*

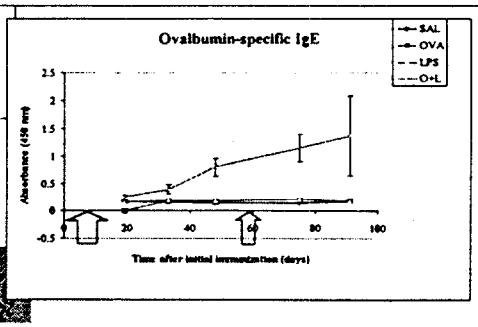
Nasal immunization protocol

- *BALB/c mice are anesthetized with methoxyflurane*
- *receive either **saline, LPS, antigen, or LPS + antigen** (10 μg each)*
- *god doses on **days 1-12** and **days 57-61***

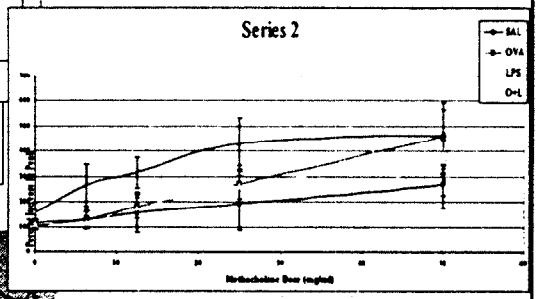
Anti-ovalbumin antibody responses



Anti-ovalbumin IgE antibody responses



Methacholine responses



LPS adjuvancy study - next experiments

- *Separate upper and lower airway delivery of antigen*
- *Plethysmographic responses to methacholine and antigen*
- *Cellular responses (bronchoalveolar lavage)*

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  - LPS
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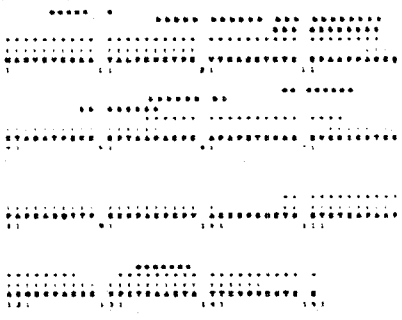
Epitope analysis of Hev b 5



Site-directed mutagenesis of Hev b 5

CCA AAG AAT GAG ACA CCT GAG GTG ACC
... P K_{15} N E T P E V T
CCA
P

AAG GCT GAG GAG ACG AAG ACC GAA GAA...
K_{23} A E E T K_{28} T E E ...
CCA CCA
P P

Publications from LIB staff, 1999-2000 (abstracts)

- Slater JE, Pastor RW. The determination of equivalent doses of standardized allergen vaccines. *J Allergy Clin Immunol* 2000: *in press.*
- Poley GE Jr., Slater JE. Mice immunized with avocado have high titer specific IgG antibody to natural rubber latex proteins. *J Allergy Clin Immunol* 2000; *in press.*
- Solanki MD, Slater JE. The effects of lipopolysaccharide (LPS) on lower airway and immune responses in mice. *J Allergy Clin Immunol* 2000; *in press*
- Soldatova LN, Bakst JB, Hoffman DR, Slater JE. Molecular cloning of a new honey bee venom allergen, acid phosphatase. *J Allergy Clin Immunol* 2000, *in press*
- Paupore EJ, Slater JE. Site-directed mutagenesis of the latex allergen Hev b 5. *J Allergy Clin Immunol* 2000; *in press*

Publications from LIB staff, 1999-2000 (reviews/chapters)

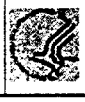
- Slater JE, Gam AA, Solanki MD, Burk SH, May FM, Pastor RW. Statistical considerations in the establishment of release criteria for allergen vaccines. *Arb Paul Ehrlich Inst.Bundesamt Sera Impfstoffe Frankf A M*, 2000, in press.
- Soldatova LN, Biological Activity of Recombinant Bee Venom Allergens Expressed in Baculovirus-infected Cells. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M*, 2000, in press

Publications from LIB staff, 1999-2000 (refereed articles)

- Slater JE, Paupore EJ, O'Hehir RE. Murine B-cell and T-cell epitopes of the allergen Hev b 5 from natural rubber latex. *Mol Immunol* 1999; 36 135-143
- Soldatova LN, Paupore EJ, Burk SH, Pastor RW, Slater JE. The stability of house dust mite allergens in glycerinated extracts. *J Allergy Clin Immunol* 2000, in press
- Slater JE, Pastor RW. The determination of equivalent doses of standardized allergen vaccines. *J Allergy Clin Immunol* 2000, in press
- de Silva HD, Sutherland MF, Suphioglu C, McLellan SC, Slater JE, Rolland JM, O'Hehir RE. Human T cell epitopes of the latex allergen Hev b 5 in health care workers. *J Allergy Clin Immunol* 2000, in press

Allergen standardization

Current standardized allergens



Allergen standardization

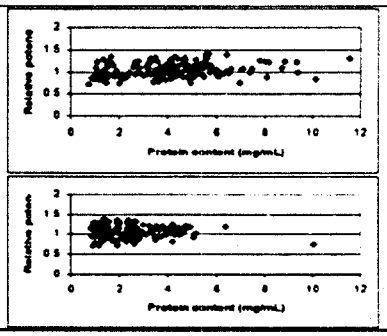
- *Scientific results*
- *QC/QA results*
- *Building on our experience*
- *Planning the next steps*

Allergen standardization - scientific results

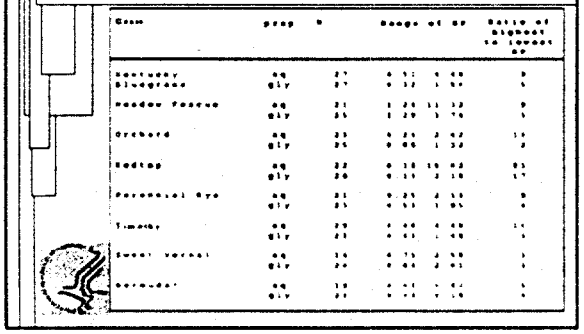
- *Protein ≠ potency*
- *Clinical testing is essential*
- *In vitro tests can serve as surrogates for skin tests*
- *Different in vitro tests may be optimal for different allergens*
- *50% glycerol substantially improved stability*
- *Stability assessment may be method-dependent*

Potency does not correlate with protein content

- *Grass pollens (100,000 BAU/mL)*
- *Mites (10,000 AU/mL)*



Potency of non-standardized allergen extracts



Allergen standardization - scientific results

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- *50% glycerol substantially improved stability*
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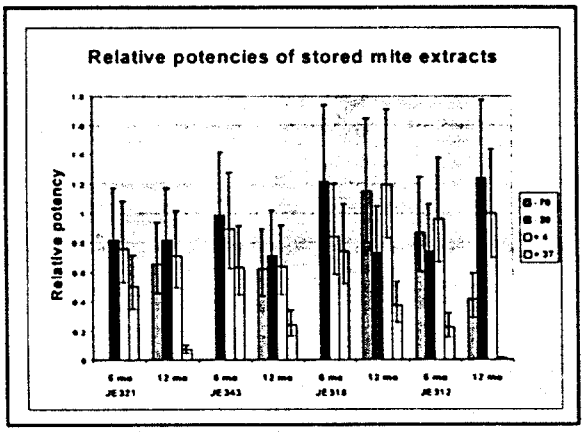
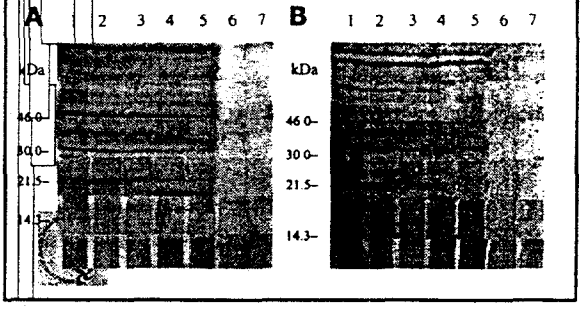
Mite stability study

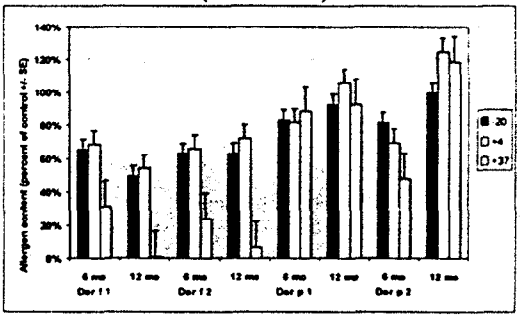
- *Liu T, Lin Y. Ann Allergy Asthma Immunol* 1998;80:177
- Soldatova LN, Paupore EJ, Burk SH, Pastor RW, Slater JE. *J Allergy Clin Immunol* 2000, in press.
  - *Lyophilized mite extracts*
  - *Time 0 - reconstitute in 50% glycerol*
  - *Store at -70°C, -20°C, +4°C and +37°C*
  - *Check RP, immunoblots, and specific allergen content at 6 and 12 months*



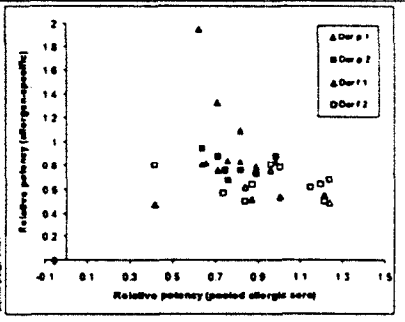
Immunoblots of Der f Mite Extracts Stored for 6 Months and Developed with Allergic Serum Pool



Group 1 and Group 2 allergen content (12 months)



Relative potency vs. allergen-specific RP



Conclusions

- RP stable at -20 °C and 4°C
- Degradation of specific allergens observed at 4°C
- Protease inhibitors did not contribute to stability
- Lyophilized extracts are more stable that glycerinated
- The stability of glycerinated extracts is enhanced at lower temperatures

Allergen standardization - QA/QC results

- US standards of potency
  - common industry-wide unitage
- SOPs for testing methods
  - QA/QC possible
- Stability monitoring program

Advantages of a US standard

- Increases safety and consistency
- Facilitates scientific studies
- Enhances product choices

Allergen standardization - QA/QC results

- *US standards of potency*
  - *common industry-wide unitage*
- *SOPs for testing methods*
  - *QA/QC possible*
- *Stability monitoring program*

Allergen standardization - building on our experience

- *Continue standardization*
- *Continue US standard of potency*
- *Keep clinical testing as the gold standard*

Allergen standardization - planning the next steps

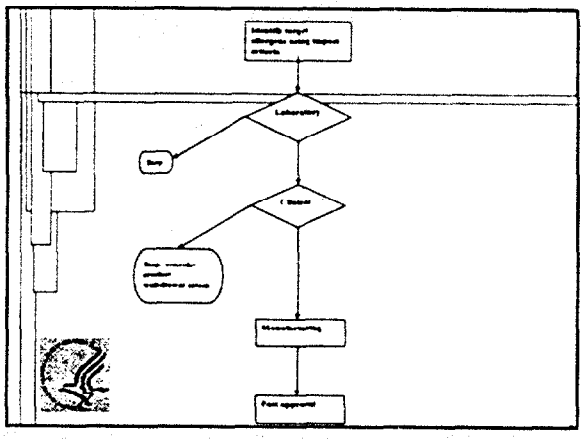
- *Build consensus*
- *Construct a transparent process*
- *Identify specific decision points to abort a specific campaign and/or withdraw product*
- *Consider increased role for industry and other collaborations*
- *Choose the most stable references possible*
- *Consider clinical data in setting release limits*
- *Consider enlisting USP to handle references*

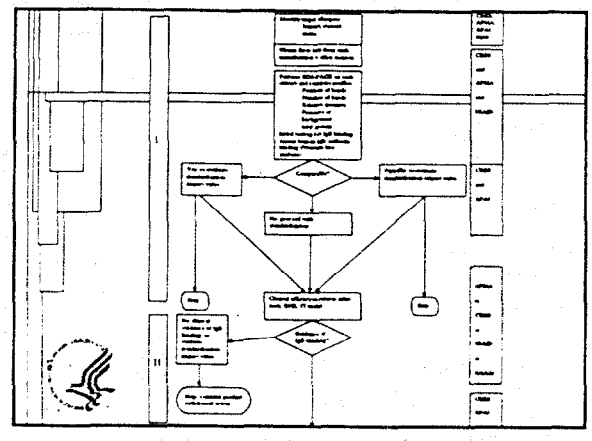
Purpose

- Build upon prior successes
- Establish priorities and procedures
- New:
  - State clearly the criteria for allergen selection
  - Delineate responsibilities
  - Set exit points

Impact criteria

- Availability of stable, preferably lyophilized material for use as long-term reference extracts
- Consistency of currently marketed product
- Widespread use as a diagnostic and/or therapeutic reagent in the U S
- Number of manufacturers producing the product
- Potential use in immunotherapy (higher score) or diagnostics (lower score)
- Public health impact of correct diagnosis and/or adequate treatment





Preliminary

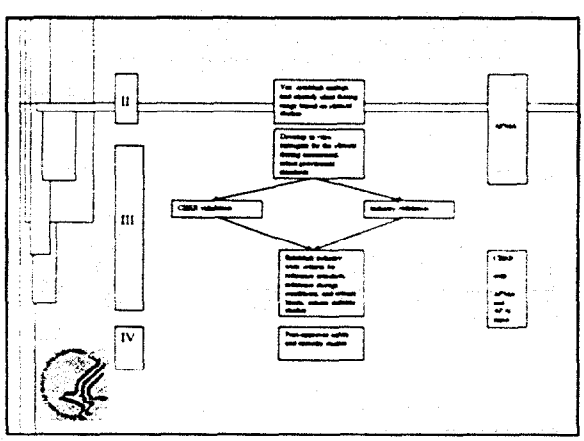
- *Identify target allergens*
- *Obtain multiple lots for study*
  - *three from each manufacturer*
  - *other sources, including*
    - *academics (universities, NIH)*
    - *industry R & D*
    - *LIB*

Phase I - Laboratory

- *Develop/adapt methods for allergen determination*
- *Compare allergen content of different lots*
- *If commercial products are highly consistent, re-consider impact*
- *If commercial products are comparable to "best" material, re-consider impact*

Phase II - Clinical

- *skin testing, histamine release, IT data*
- *if no evidence of IgE-mediated disease, re-consider impact and consider product withdrawal*
- *establish biological unitage and ideal dosing ranges*



Phase III - Manufacturing

- *Develop in vitro surrogate testing*
- *Select provisional reference standard*
- *Validation by CBER and industry*
- *Select reference, establish release limits, initiate stability testing*

Phase IV - Post-approval

- *Stability*
- *Safety*
- *Equivalent dosing - possible revision of release limits*

Prior allergen standardization priority targets

- *latex*
- *cockroach*
- *tree pollens*
- *peanut*
- *molds*
- *dog*

DHHS Asthma Initiative

March 1999

- *Department-wide effort*
- *NIH, FDA, EPA, CDC in lead roles*
- *CBER represented by Paul Turkeltaub*

Role of allergen standardization

- *Improved diagnosis*
- *Allergen avoidance*
- *Better and safer immunotherapy*
- *Better science*

Allergens and asthma

- *Indoor allergens*
  - *dust mites**
  - *cat**
  - *cockroach*
  - *molds*
  - *dog*
- *Outdoor allergens*
  - *molds*

* = already standardized

Allergens to standardize in response to the asthma initiative

- Cockroach
- *Aspergillus fumigatus*
- *Alternaria alternata*