190th Meeting of the Vaccines and Related Biological Products Advisory Committee

Advancing CBER's Allergen Extract Standardization Program

Part I: Enzyme-linked monoclonal antibody and aptamer-based assays

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Office of Vaccines Research and Review (OVRR)
Center for Biologics Evaluation and Research (CBER)

October 9, 2025

Desired outcome and problem statement

Desired outcomes:

Updated **methodology** for potency measures of allergen extracts that can be represented by one or two major allergens.

Apply these methods toward increasing the number of standardized allergen extracts.

Problem statements:

1) Current method for measuring major allergen content is outdated.
2) Non-standardized extracts are a regulatory gap that compromises clinical reliability.

Voting questions

Question 1: Scientific Soundness of Mass Concentration Measurements

Does measurement of mass concentrations by ELISA of their major allergens provide a scientifically sound approach for expressing and reporting potencies of cat hair and pelt allergen extracts, and of short ragweed pollen allergen extracts?

Question 2: Appropriateness of Revised Assays for CBER's Allergenic Standardization Program

Are the revised assays for cat hair/pelt and ragweed pollen allergen extracts scientifically appropriate templates for expanding CBER's allergenic standardization program to include major food allergens and environmental allergens?

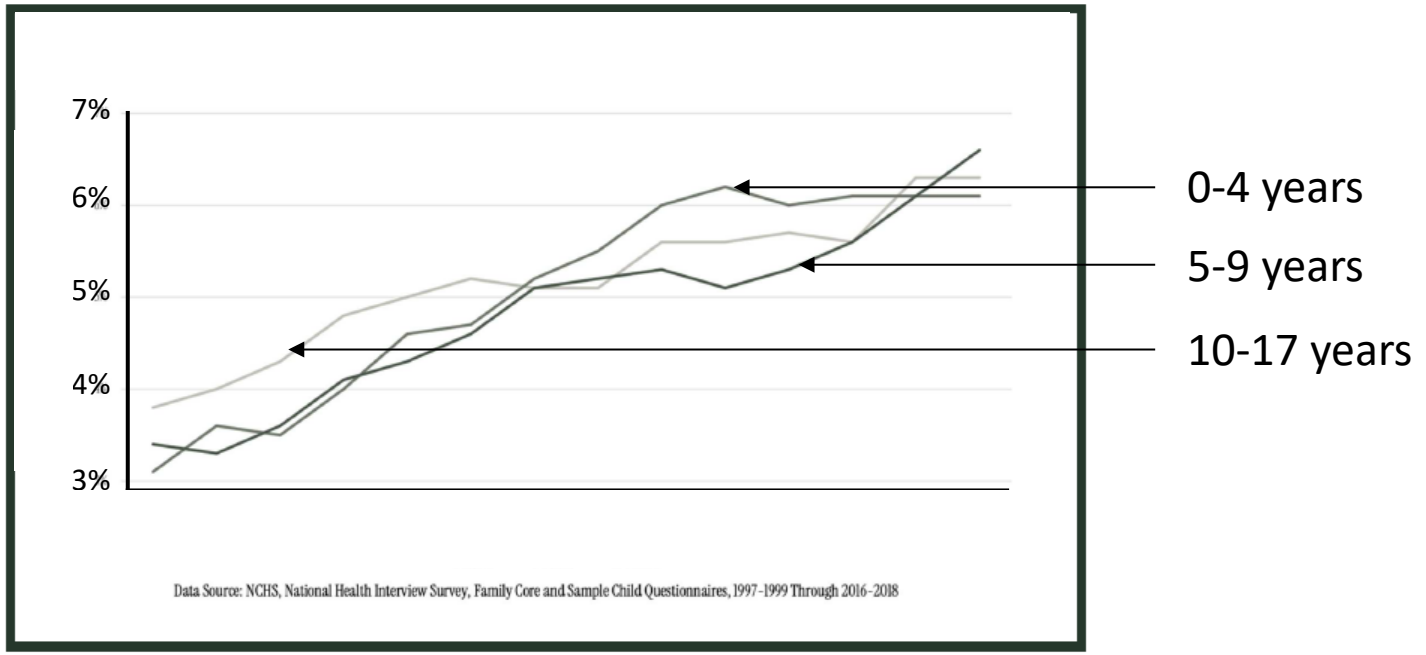
Outline

- Allergen extracts and regulatory framework for their regulation
- Current potency measures of allergen extracts
- Progress in ELISAs and aptamer-based enzymatic assays to replace outdated potency assays in current use
- Need for expanding the list of standardized allergen extracts
- Adoption of ELISA serves as a template for validation of new assays and tech transfer to manufacturers to use for lot release

Asthma and Food Allergy are Common Chronic Diseases

- Over 1 in 4 American children have allergies*
  - Atopic dermatitis
  - Allergic rhinitis/conjunctivitis
  - Asthma
  - Food allergies

*The MAHA Report: Make our Children Healthy Again; February 2025



Center for Biologics Evaluation and Research Regulates Allergenics

- Allergen immunotherapy is the only disease modifying treatment for allergic disease
- 2009 estimate: ~3 million Americans receive allergen immunotherapy
- Immunotherapy for environmental (non-food) allergies is most often administered as subcutaneous injections of allergen extracts
- The basic recipe for allergen extracts has been used for >100 years

Center for Biologics Evaluation and Research Regulates Allergenics

- FDA has not changed its approach towards standardization of allergen extracts in the past 30 years
- Advances in science present an opportunity to bring regulation of allergenics into the 21st century

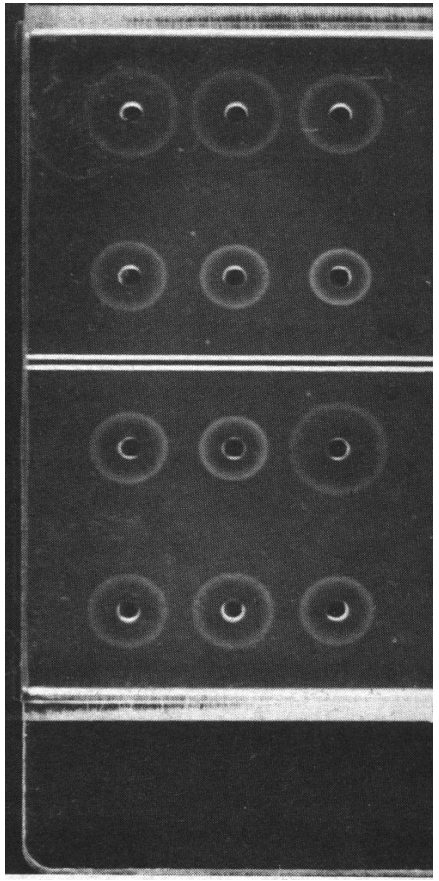
Allergen extracts

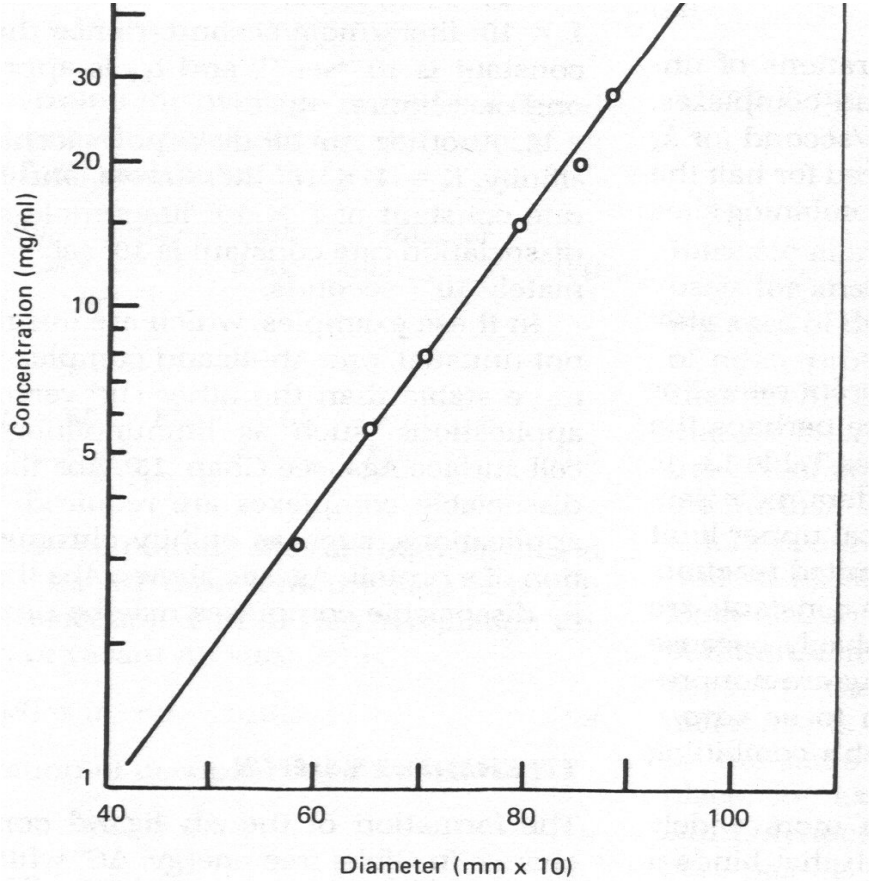
- Sterile aqueous extracts of plant or animal proteins used to diagnose or treat allergic hypersensitivity
- Crude preparations
- Allergenic source materials:
  - Pollen
  - Foods
  - Animal hair/dander

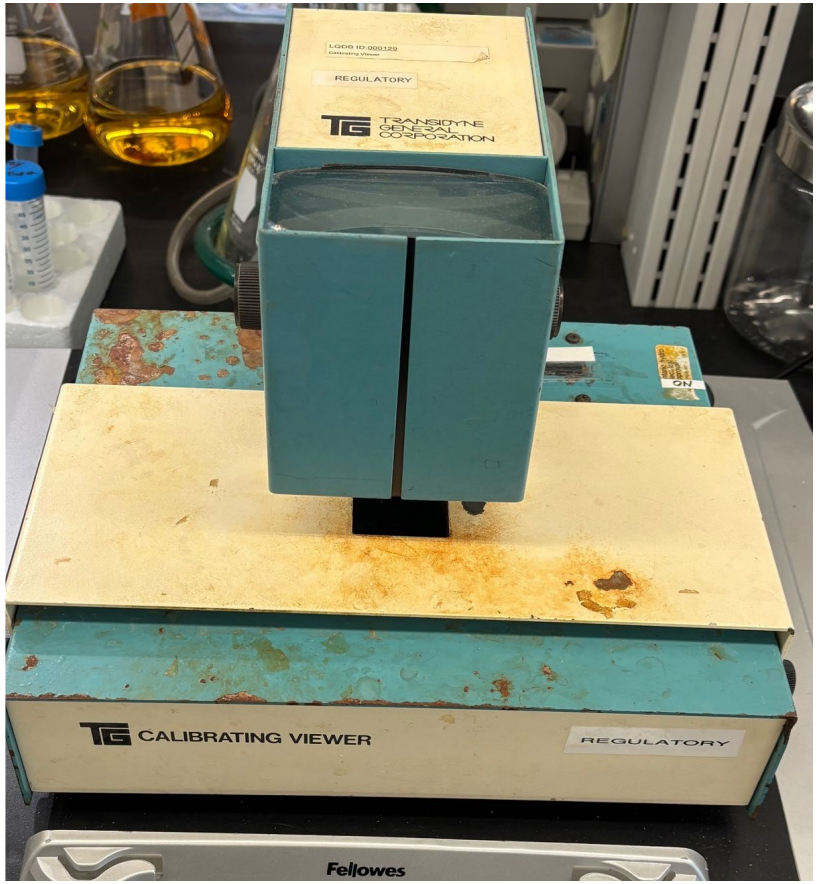
Regulatory Framework for Regulation of Allergen Extracts

- 21 CFR 680.3(e) grants CBER authority to standardize allergen extracts for potency
  - CBER determines standardization methods and potency unitage
  - Once CBER standardizes an extract, it must be distributed as a standardized extract
  - Nineteen allergen extracts were standardized between 1987-1998
    - "Major allergen"
    - "Overall potency"
- Unless standardized by CBER, allergen extracts are non-standardized
  - Protein nitrogen units or weight/volume at time of extraction
  - No indication whether protein is intact

Radial Immunodiffusion Assay Currently Used to Measure Major Allergen Concentrations



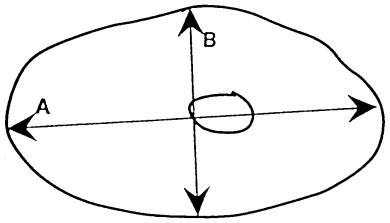


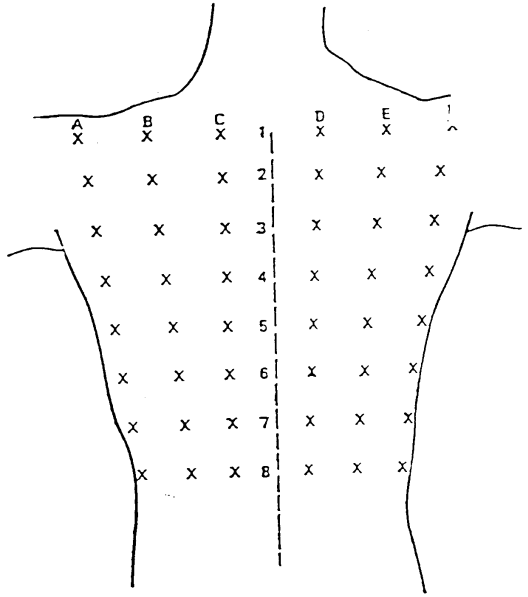


Unpublished LIB photos

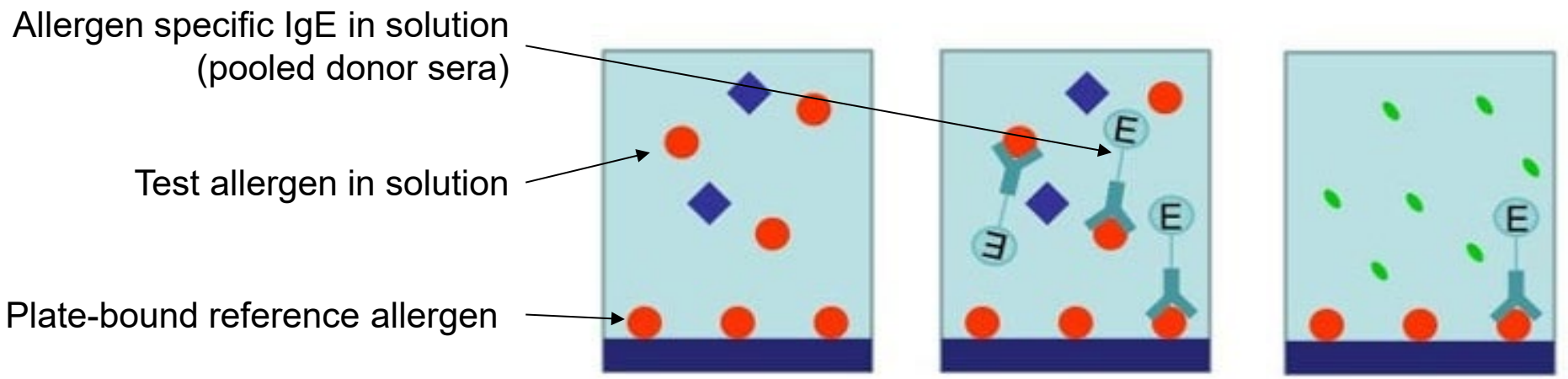
Allergen Standardization by Overall Potency

- ID_{50}EAL testing (Intradermal Dilution for 50 mm Sum of Erythema) Determines <u>Bioequivalent Allergy Units</u>
  - Highly allergic individuals
  - Serial 3-fold dilutions
  - Establish dilution at which SE = 50 mm (D_{50})
  - D_{50} = 100,000 BAU/mL



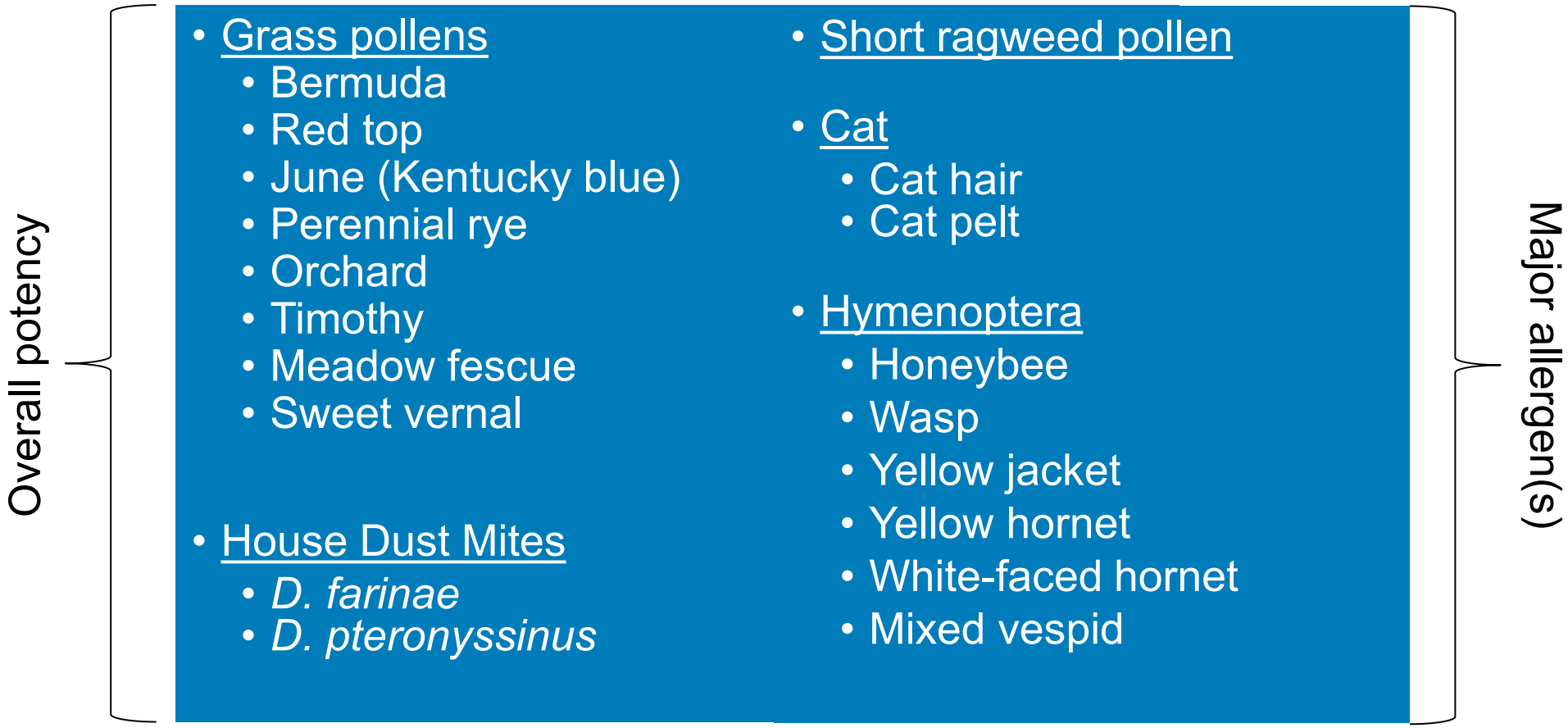


Competitive ELISA is the Surrogate Assay for Overall Potency



- Does not detect compositional differences between extracts
- Serum pools may vary because of differences among donors

Nineteen (19) Standardized Extracts (Hundreds of Non-standardized Extracts)



Summary

- Allergen extracts are crude preparations that are safe and effective and modulate allergic disease
- Nineteen allergen extracts are standardized for potency
  - Major allergen
  - Overall potency
- The remaining hundreds of extracts are non-standardized

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      - Extracts from different manufacturers are qualitatively similar
      - Allergic patients react similarly to the same sets of allergens
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  - No indication whether protein is intact (no efficacy and misdiagnosis)

Question 1: Scientific Soundness of Mass Concentration Measurements
Does measurement of mass concentrations by ELISA of their major allergens provide a scientifically sound approach for expressing and reporting potencies of cat hair and pelt allergen extracts, and of short ragweed pollen allergen extracts?

ELISA Development to Quantify Major Allergens

- Laboratory of Immunobiochemistry (LIB) Reference Reagent Laboratory
  - Devises, qualifies, and validates potency tests for allergen extracts
  - Distributes reference reagents to manufacturers for potency testing for lot release of standardized allergen extracts
  - ISO 17025 certified for competitive ELISA

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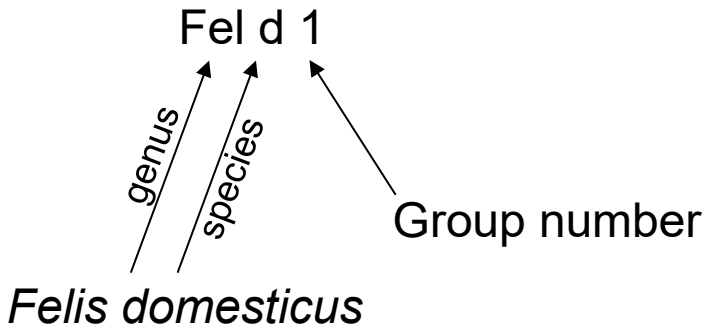

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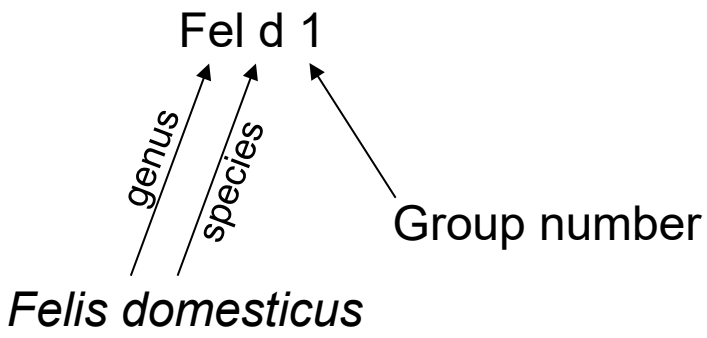
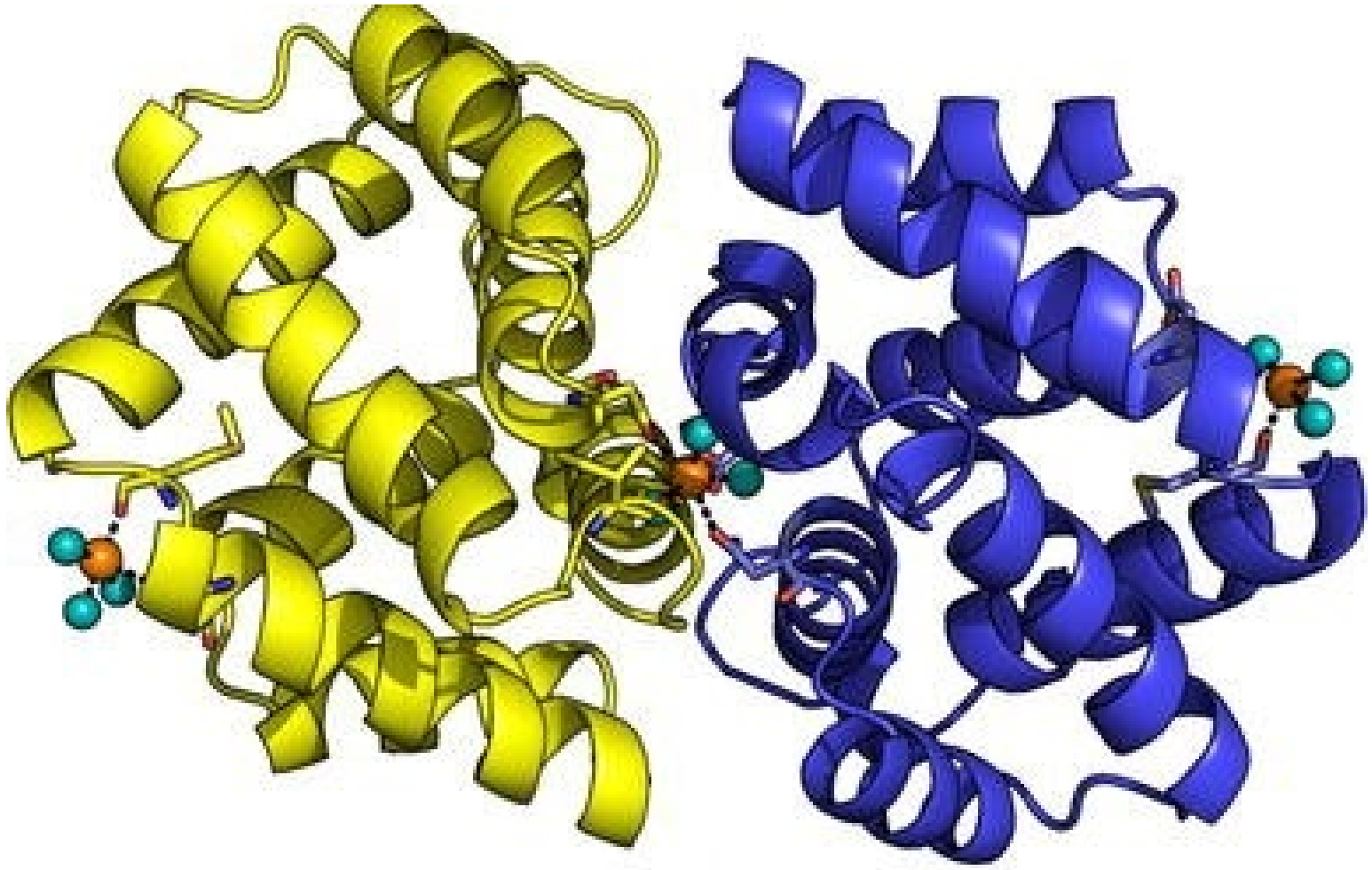
- Major allergens for two allergen extracts are measured by RID
  - Cat hair/pelt: Fel d 1
  - Short ragweed: Amb a 1



Fel d 1 is a "Universal" Allergenic Protein

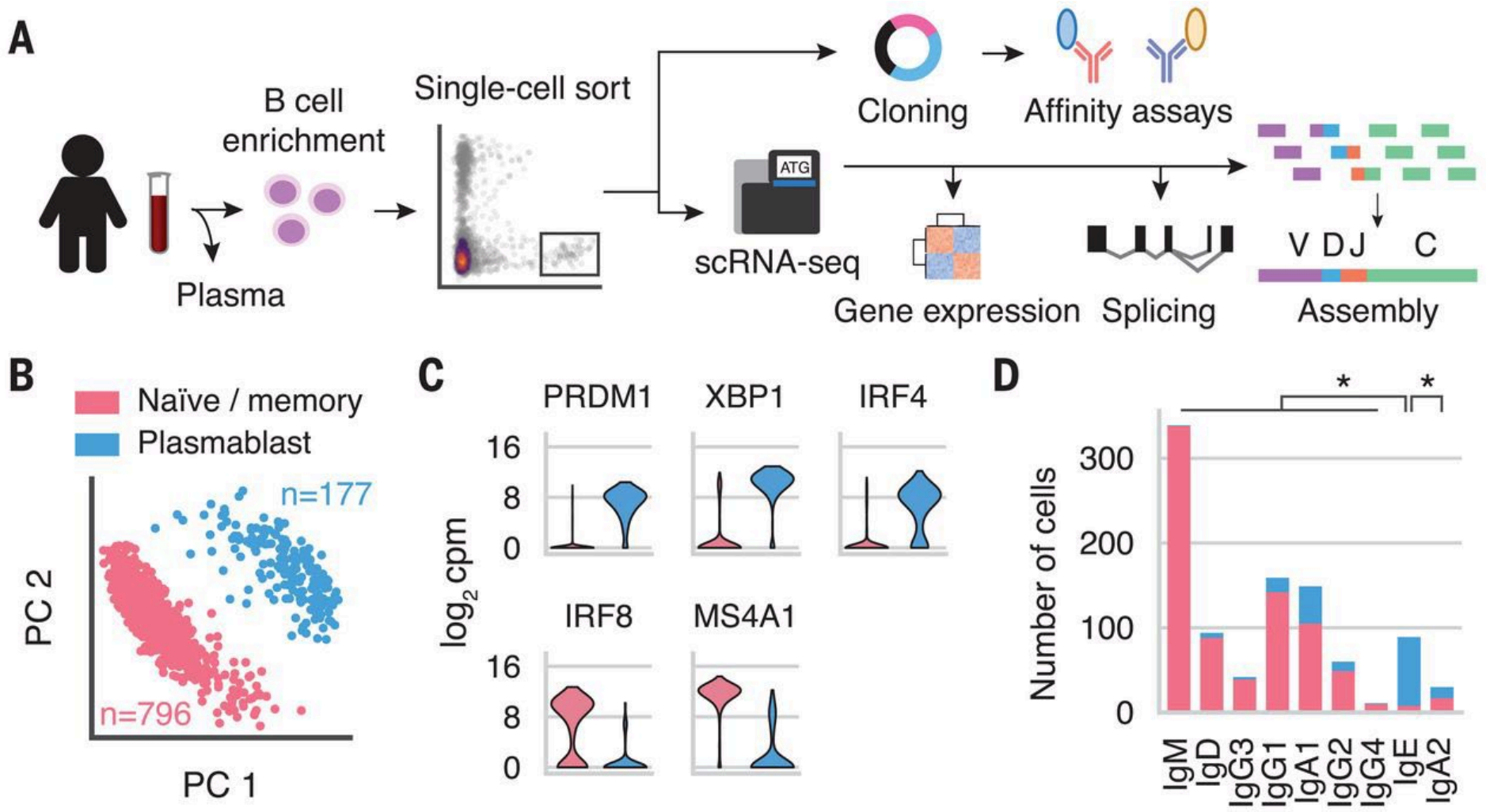
- Secretoglobin protein complex
- Produced by salivary and sebaceous glands
- Tetrameric glycoprotein—two disulfide linked heterodimers (chains 1 and 2); chain 2 is N-linked glycoprotein
- Very stable and "sticky"
- Assigned Fel d 1 "units:" 1 unit = ~4 µg of Fel d 1
- Cat hair/pelt extracts are required to have 10-20 Fel d 1 units/mL
  - 5-9.9 Fel d 1 units/mL = 5,000 BAU/mL
  - 10-19.9 Fel d 1 units/mL = 10,000 BAU/mL

Fel d 1 is a Tetramer Comprised of Two Disulfide Linked Heterodimers



Bonnet, B. et al. *Allergy Asthma Clin Immunol*; 2018 14:14

Cloning Cat Allergen-specific IgE from Allergic Donors



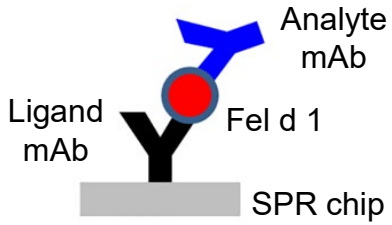
D Croote et al. Science 2018; 362:1306

Choosing Antibody Pairs to Quantify Fel d 1 in Cat Hair and Pelt Extracts by ELISA

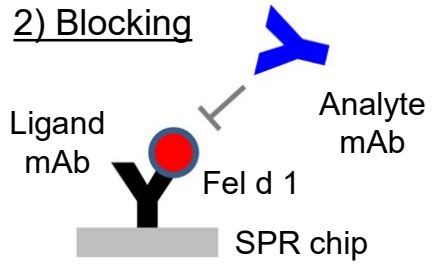
Biologically relevant and high affinity

Two Possible Outcomes of Pairwise Competition

1) Sandwiching



2) Blocking



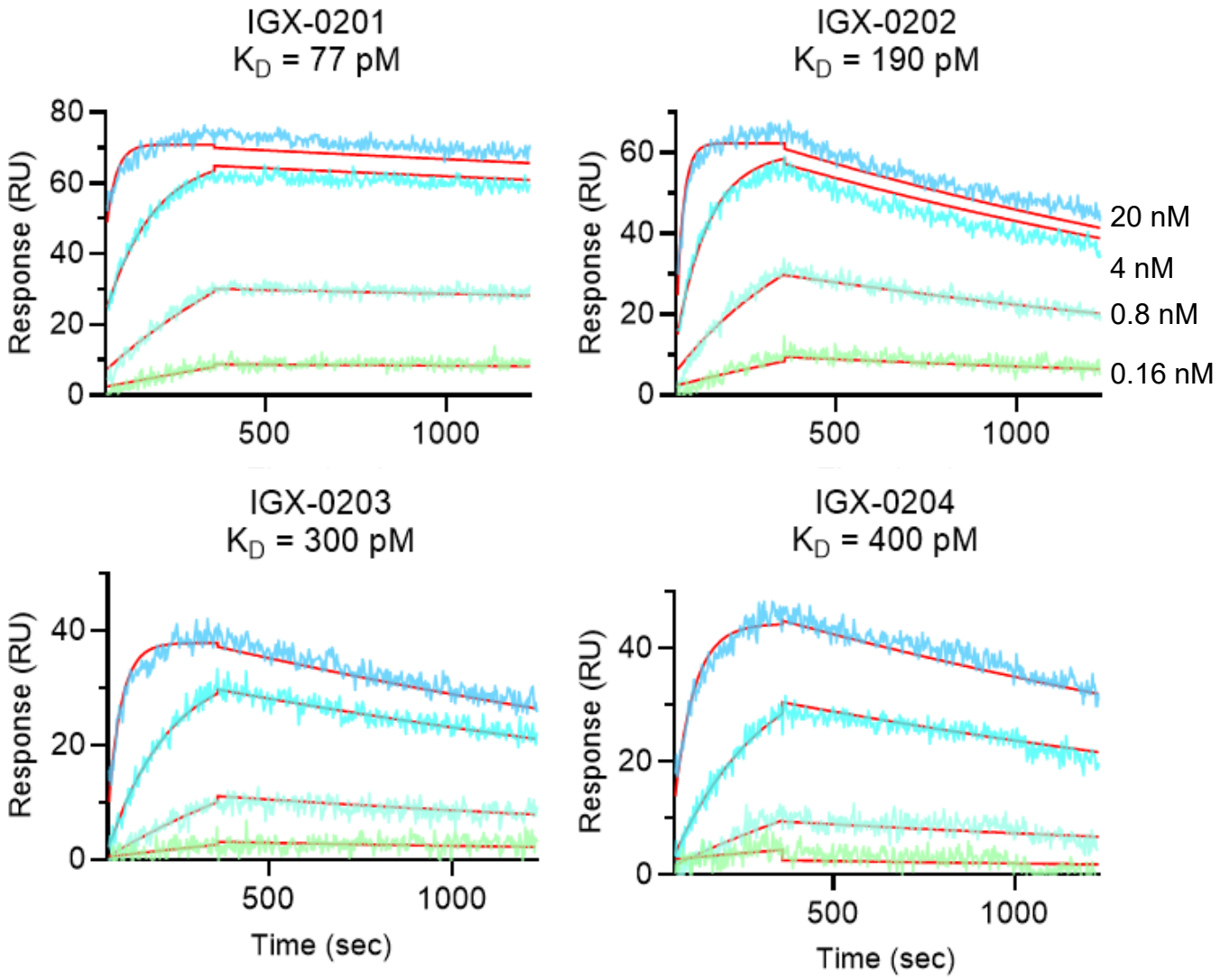
Analyte mAb

Ligand mAb	IGX-0202	IGX-0201	IGX-0204	IGX-0203
IGX-0202		-0.09	3.76	3.88
IGX-0201	0.00		4.31	5.36
IGX-0204	4.00	4.65		0.02
IGX-0203	4.05	4.23	-0.05	

Legend:
Sandwiching
Blocking
Self-blocking

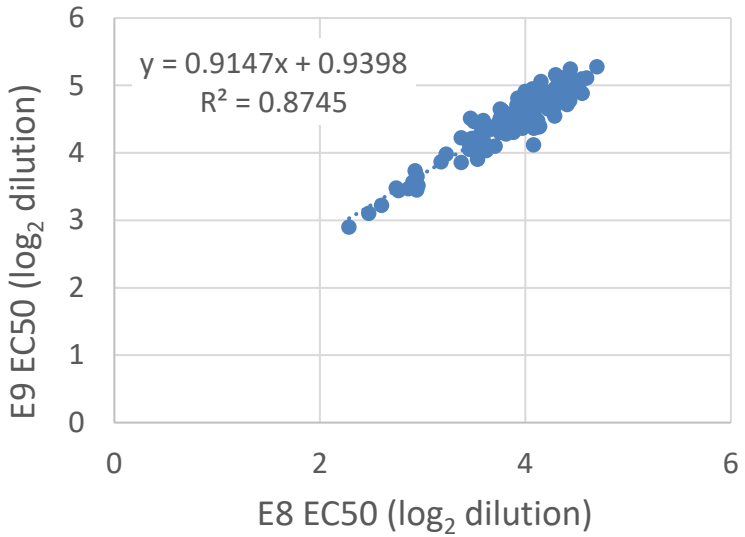
- IGX-0201 & IGX-0202 sandwich Fel d 1 with IGX-0203 & IGX-0204
- Sandwiching mAbs form the basis of a sandwich ELISA

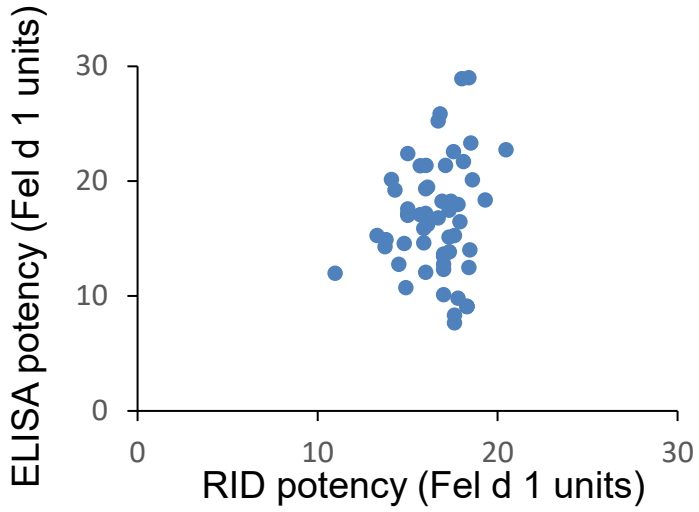
Class-switched IgE mAb have High Affinity for Fel d 1

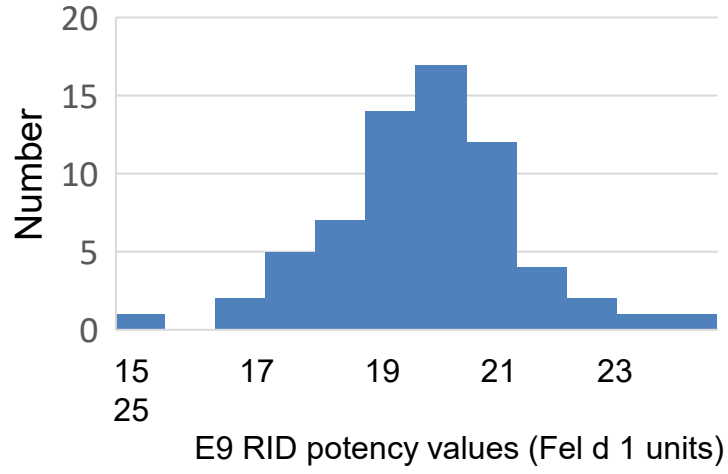


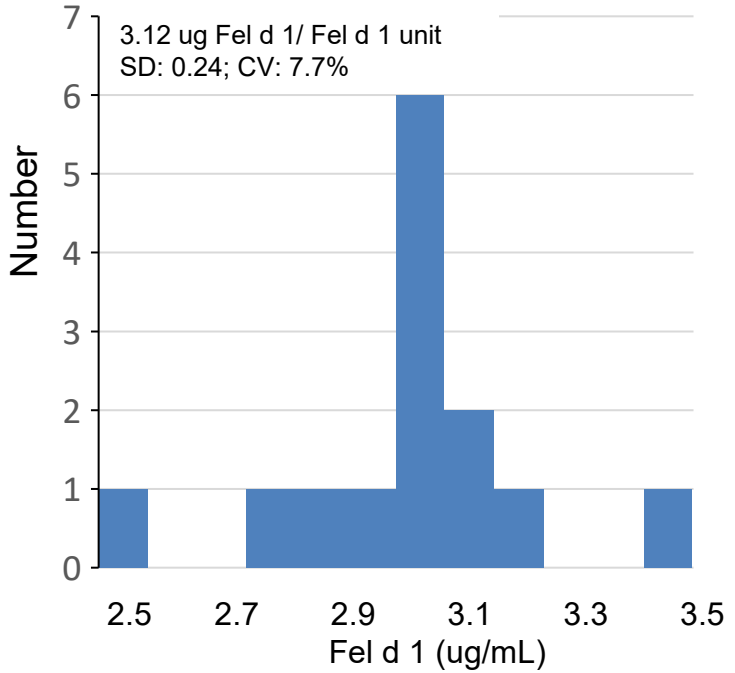
Rabin RL et al., Frontiers Allergy 2024 15:5:1417879

Fel d 1 ELISA is Consistent over Multiple Replicates









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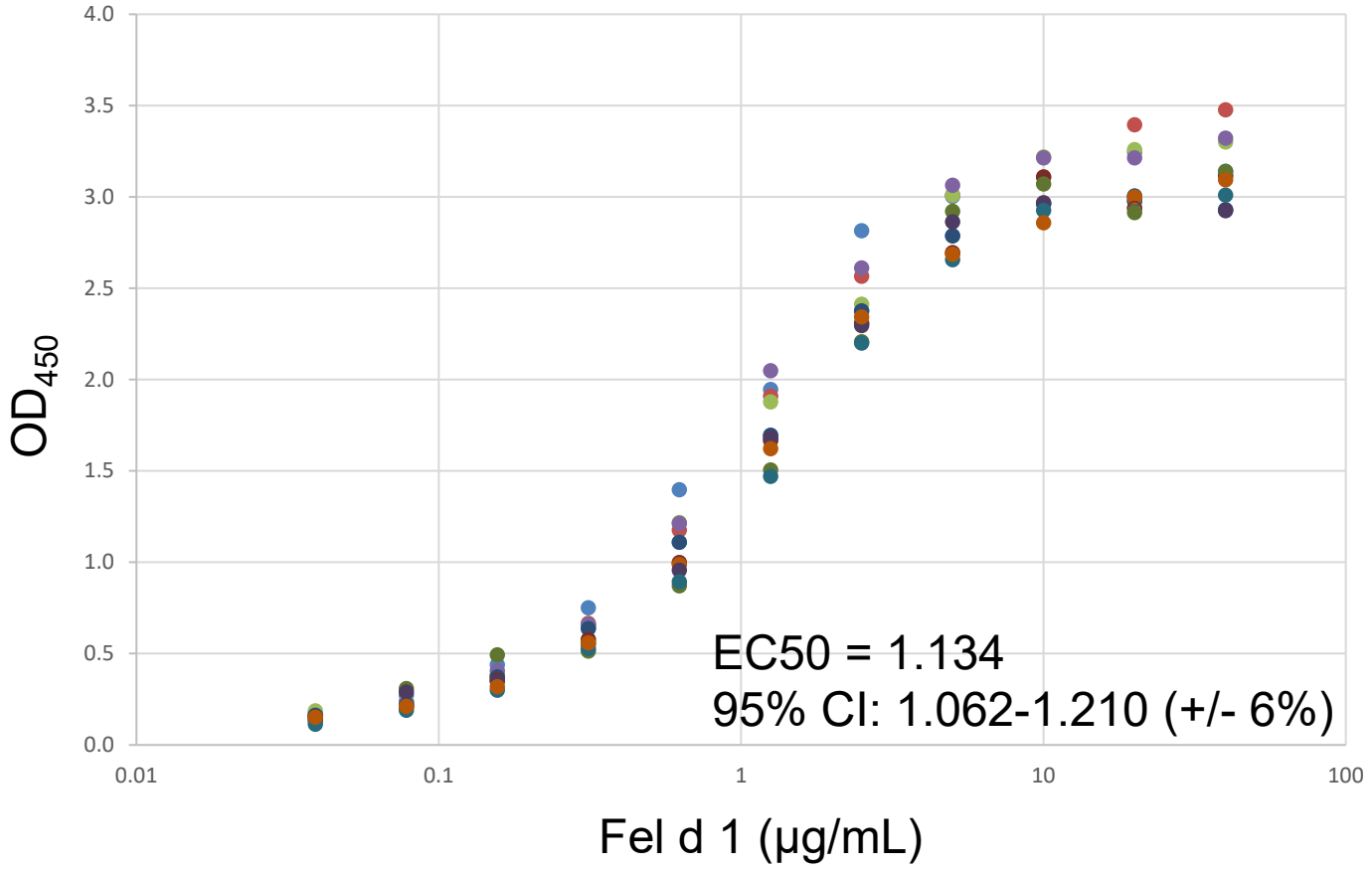
Fel d 1 ELISA is Precise



Laboratory of Immunobiochemistry Reference Reagent Lab; unpublished observations

High-affinity mAb aren’t available for all allergens

→ DNA aptamers

DNA Aptamers are an Alternative to High Affinity mAb

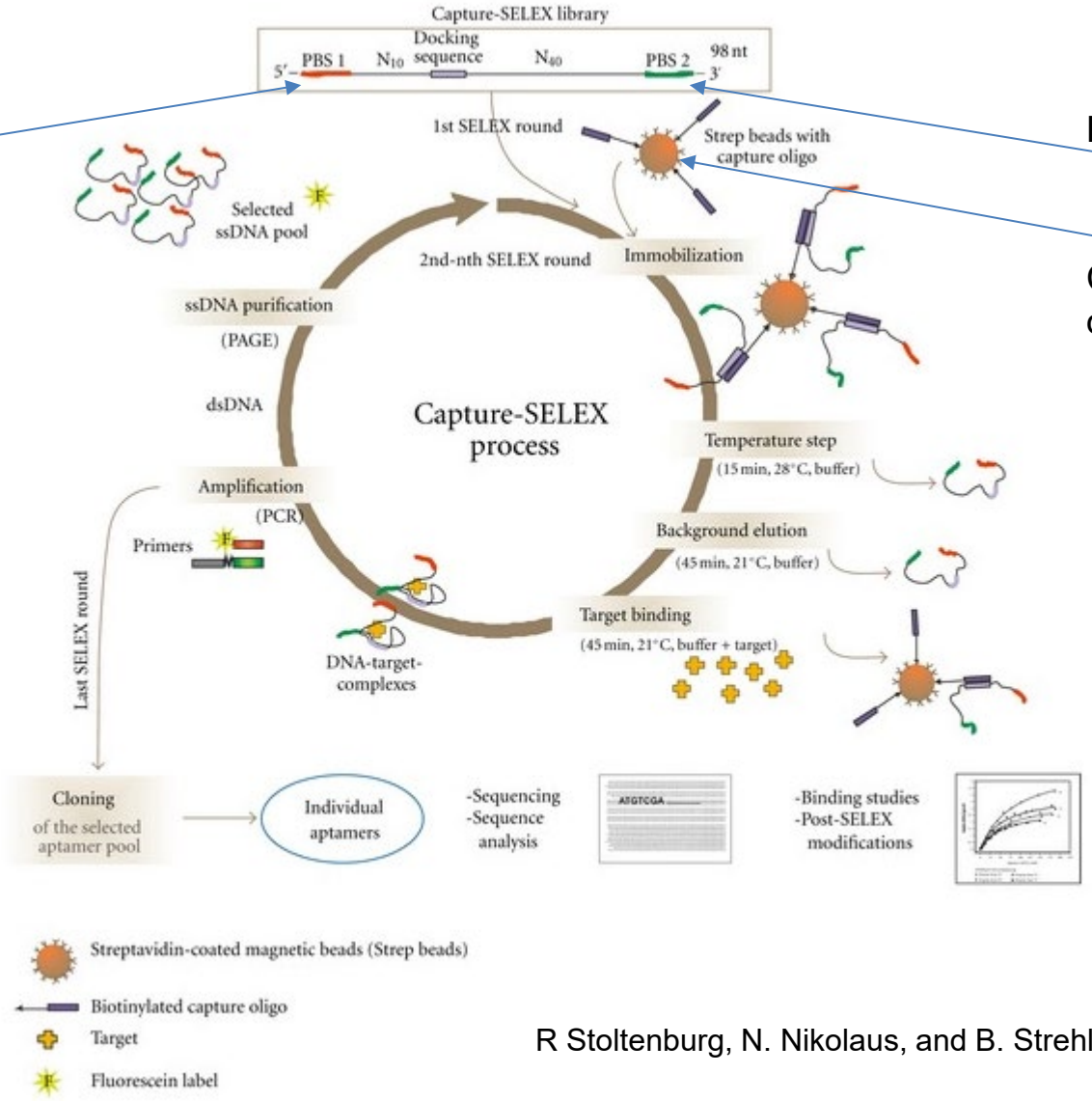
- Synthetically produced oligomers that bind to target molecules with high affinity
- Often referred to as “chemical antibodies”
- Selected from a starting pool of ~10^{14} randomly sequenced ssDNA oligomers
- Affinities and competition determined with (e.g.) surface plasmon resonance
- No animals or human blood are necessary
- Once the optimal pair has been chosen, synthesis is relatively cheap

Aptamer Selection: SELEX–Systematic Evolution of Ligands by EXponential Enrichment

Primer binding site

Primer binding site

Capture sequence complementary to docking sequence



R Stoltenburg, N. Nikolaus, and B. Strehlitz. 2012; J Anal Methods Chem

Advancing CBER's Allergen Extract Standardization Program

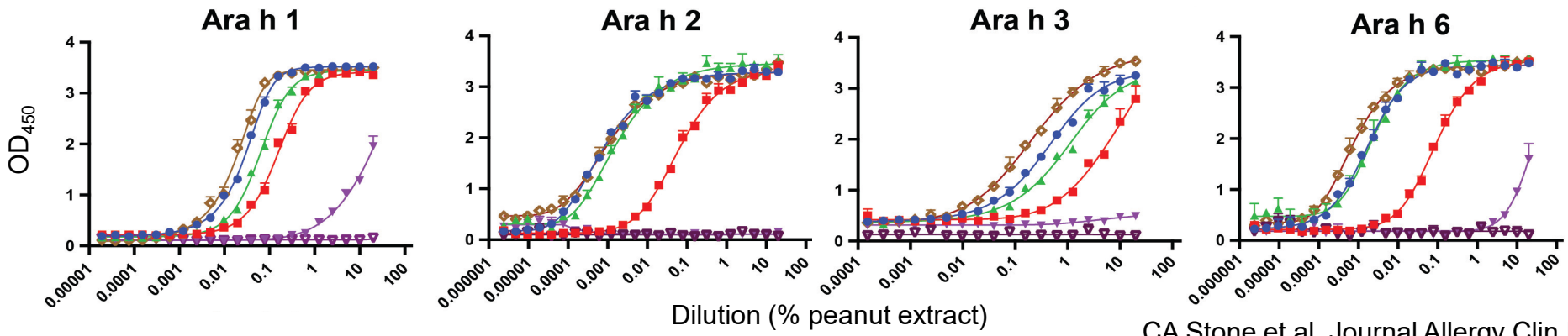
- Use newly developed assays to measure potencies of cat and short ragweed allergen extracts
- Change unitage from Fel d 1 or Amb a 1 units to µg/mL

Question 2: Appropriateness of Revised Assays for CBER's Allergenic Standardization Program

Are the revised assays for cat hair/pelt and ragweed pollen allergen extracts scientifically appropriate templates for expanding CBER's allergenic standardization program to include major food allergens and environmental allergens?

Non-standardized Extracts can Lack Efficacy (i.e., No Allergen) and can be Unsafe

- 2022: Four lots of ALK peanut extract withdrawn because of false negative skin tests
- Some children were misinformed that they were not peanut allergic and subsequently reacted to peanut-containing foods
- 2023: Pecan extract lots recalled because of false negative skin tests



CA Stone et al. Journal Allergy Clin Immunol 2023:3547

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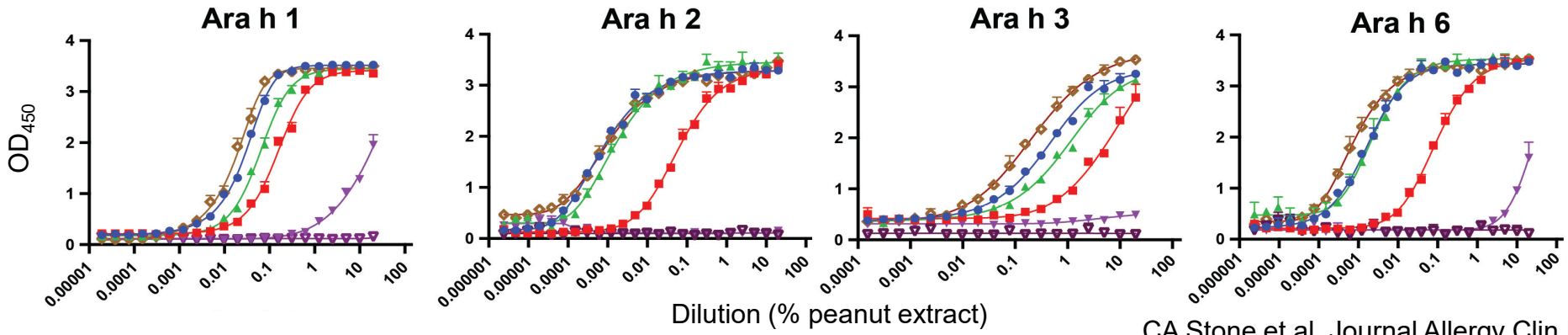


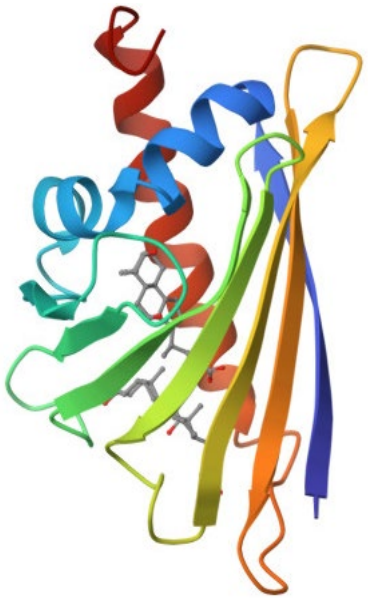
CA Stone et al. Journal Allergy Clin Immunol 2023:3547

- Advocates for expanding CBER's standardization program:
  - Manufacturers
  - Stakeholders (Food Allergy Research and Education), particularly to food allergens
  - →Reference Reagent Lab: ELISA development to replace obsolete assays and to expand standardization program

Tree Pollen Allergen Extracts are Currently Non-standardized

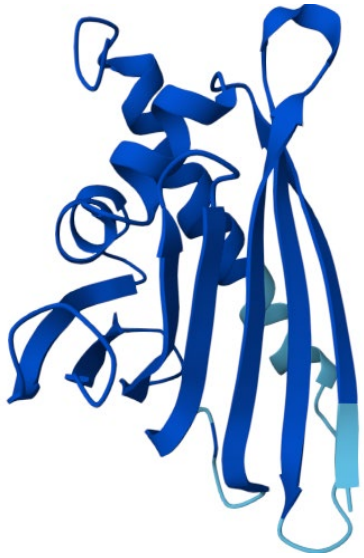
- Tree pollen counts peak in early-late spring
- Oak, birch, cedar pollen allergies are prevalent

Structural Similarity Among Pathogenesis-related Protein-10 (PR10) Family of Allergens



Bet v 1

(Birch)

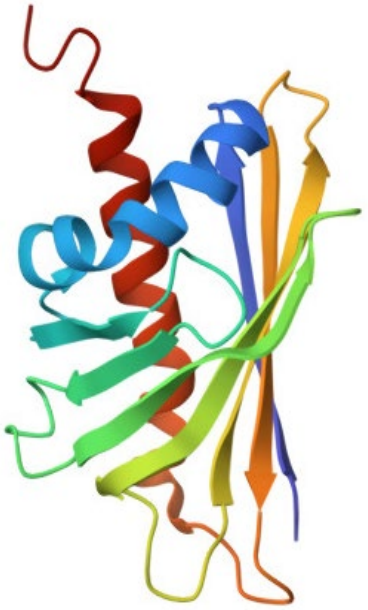


Car b 1

(Hornbeam)

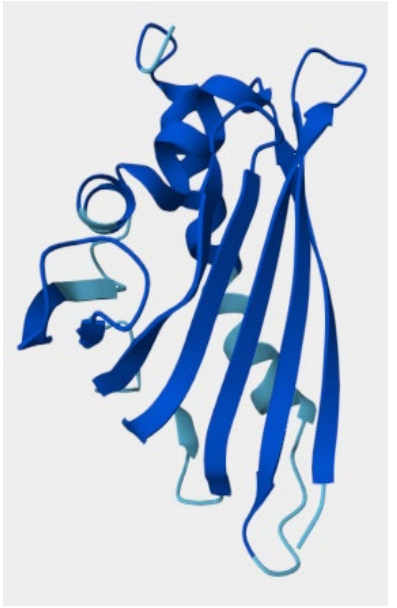
Cas s 1

(Chestnut)



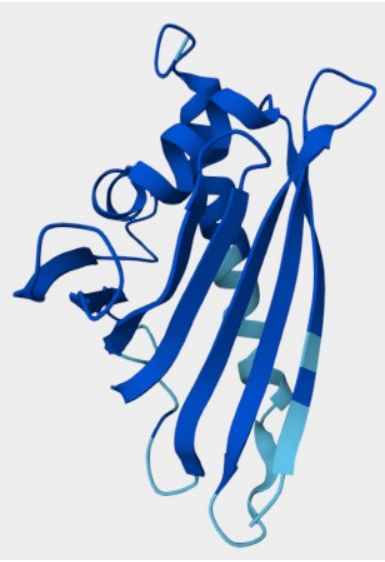
Fag s 1

(European Beech)



Que a 1

(White Oak)

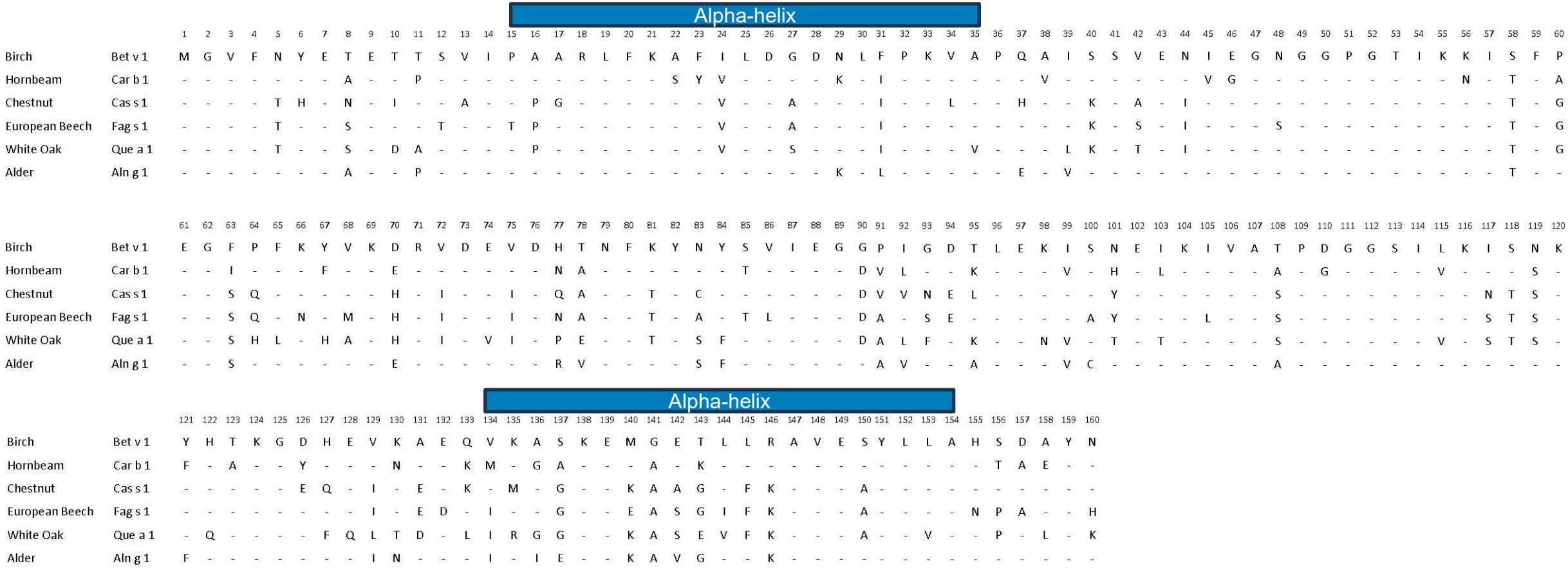


Aln g 1

(Alder)

Ribbon structures downloaded from Uniprot, except Car b 1, which was determined in silico with Alphafold

Similarity Among PR10 Family Allergens Suggests that Common Reagents (mAb or aptamers) can be Used to Standardize Multiple Extracts



Sequences downloaded from Uniprot

Current Status and Next Steps

- Replacement of Fel d 1 RID with the ELISA is progressing towards qualification and validation
- Aptamers are being selected for Amb a 1 enzyme-linked assay
- The Fel D 1 ELISA will serve as a template for qualification, validation and tech transfer to the manufacturers

Assay Validation is Required

Governing Regulations

- 21 CFR Part 211 (Current Good Manufacturing Practice for Finished Pharmaceuticals): Requirements for laboratory controls, including testing and stability
- 21 CFR Part 610 (General Biological Products Standards): Specific requirements for the testing of biological products

Key FDA Guidance Documents

ICH & FDA Guidance For Industry

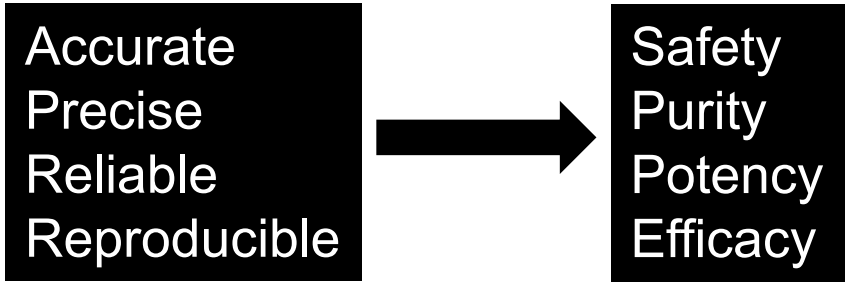
- Analytical Procedures and Methods Validation for Drugs and Biologics - Jul 2015
- Q2(R2) Validation of Analytical Procedures – Mar 2024
- Q14(R2) Analytical Procedure Development – Mar 2024
- ICH Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products – Aug 1999
- Process Validation: General Principles and Practices – Jan 2011

USP Chapters

- 111: Design and Analysis of Biological Assays
- 1010: Analytical Data – Interpretation and Treatment
- 1030: Biological Assay Chapters
- 1032: Design and development of biological assays
- 1033: Biological assay validation
- 1034: Analysis of biological assays
- 1210: Statistical Tools for Procedure Validation

Assay Validation: the Foundation of Quality Control

- Objective is to prove that the assay:
  - Fit for intended purpose
  - Appropriate for stage of development
  - Can be trusted for critical regulatory decisions



- Assays are the foundation of quality control
- Unvalidated or poorly validated assays can give misleading results

Key Performance Characteristics to Test and Document

Validation Characteristics

Accuracy
Closeness to true value

Specificity
Distinguish analyte

Limit of Quantification (LOQ)
Lowest detectable amount with acceptable accuracy & precision

Linearity
Proportional to concentration

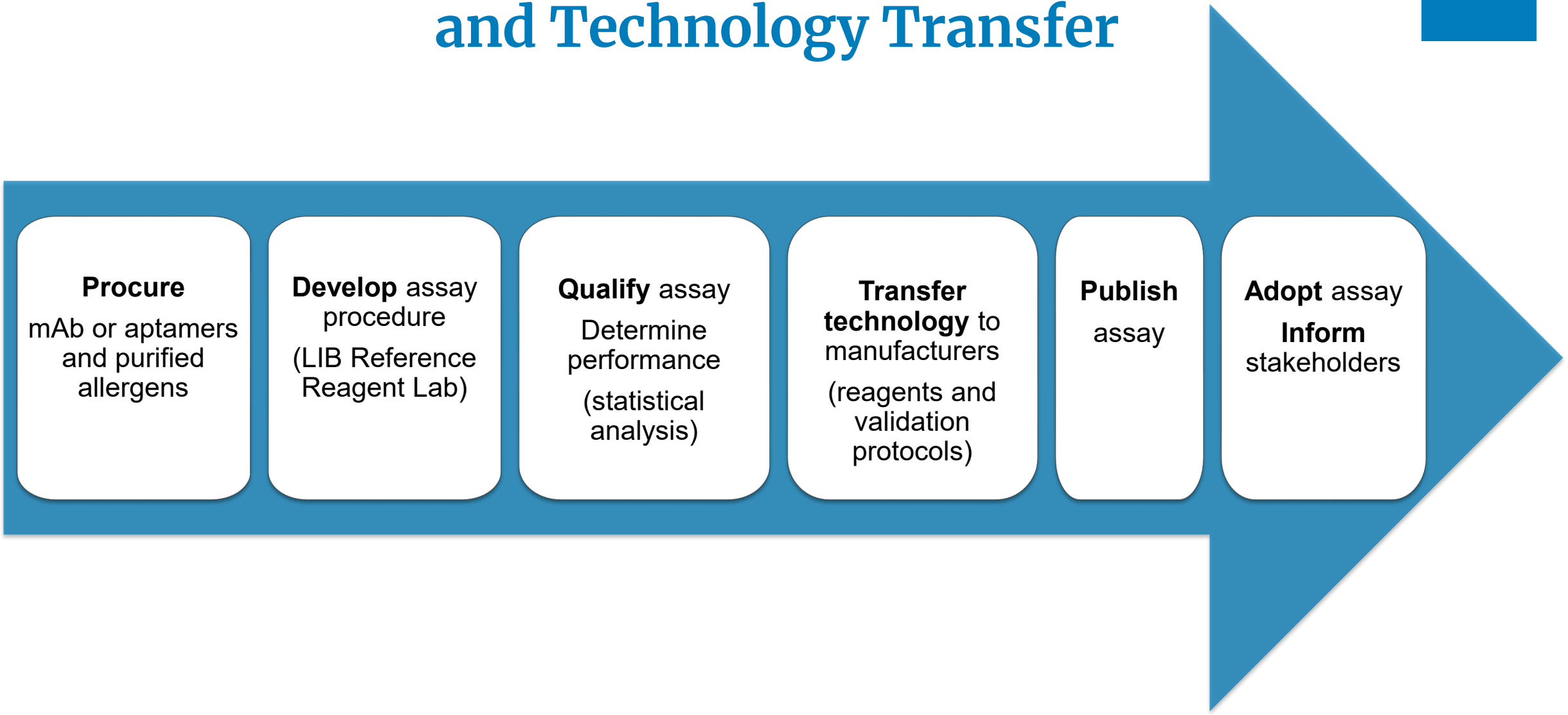
Range
Interval of acceptable linearity, accuracy, precision

Precision
Distinguish analyte

Robustness
Unaffected by variations

System Suitability
System functioning

Template for Qualification, Validation, and Technology Transfer



Acknowledgements

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Sharon Tennant
Jennifer Bridgewater
Leslie Wagner

Johns Hopkins University
Robert Hamilton

IgGenix
Derek Croote
Joyce Jia Wen Wong
Jessica Grossman

Conclusions

- Replacement of Fel d 1 RID is progressing towards qualification and validation
- Aptamers are an alternative to high-affinity antibodies
- The Fel d 1 ELISA provides a template for standardization of currently non-standardized extracts
- Standardizing food allergen extracts is a priority
- Similarity of major allergens may provide a template for standardizing multiple allergen extracts
- Assays for standardization will undergo rigorous validation

Voting Questions

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