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

Advancing CBER's Allergen Extract Standardization Program

Part II: Standardizing and advancing complex allergen extracts with tandem mass spectrometry

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October 9, 2025

Desired outcome and problem statement



Desired outcomes:

Updated **methodology** to better characterize complex allergen extracts from **relevant source materials** to improve product quality.

Problem statements:

- 1) Current methods are outdated and do not adequately assess complex allergen extracts.
- 2) Source materials may not represent clinically relevant allergen exposure.

Voting questions

Question 3: LC/MS/MS Analytics to Improve Product Quality

Does LC/MS/MS technology, compared with the currently used analytic technology, provide sufficient fit-for-purpose analytical capability for better characterization of complex allergen extracts to improve product quality?

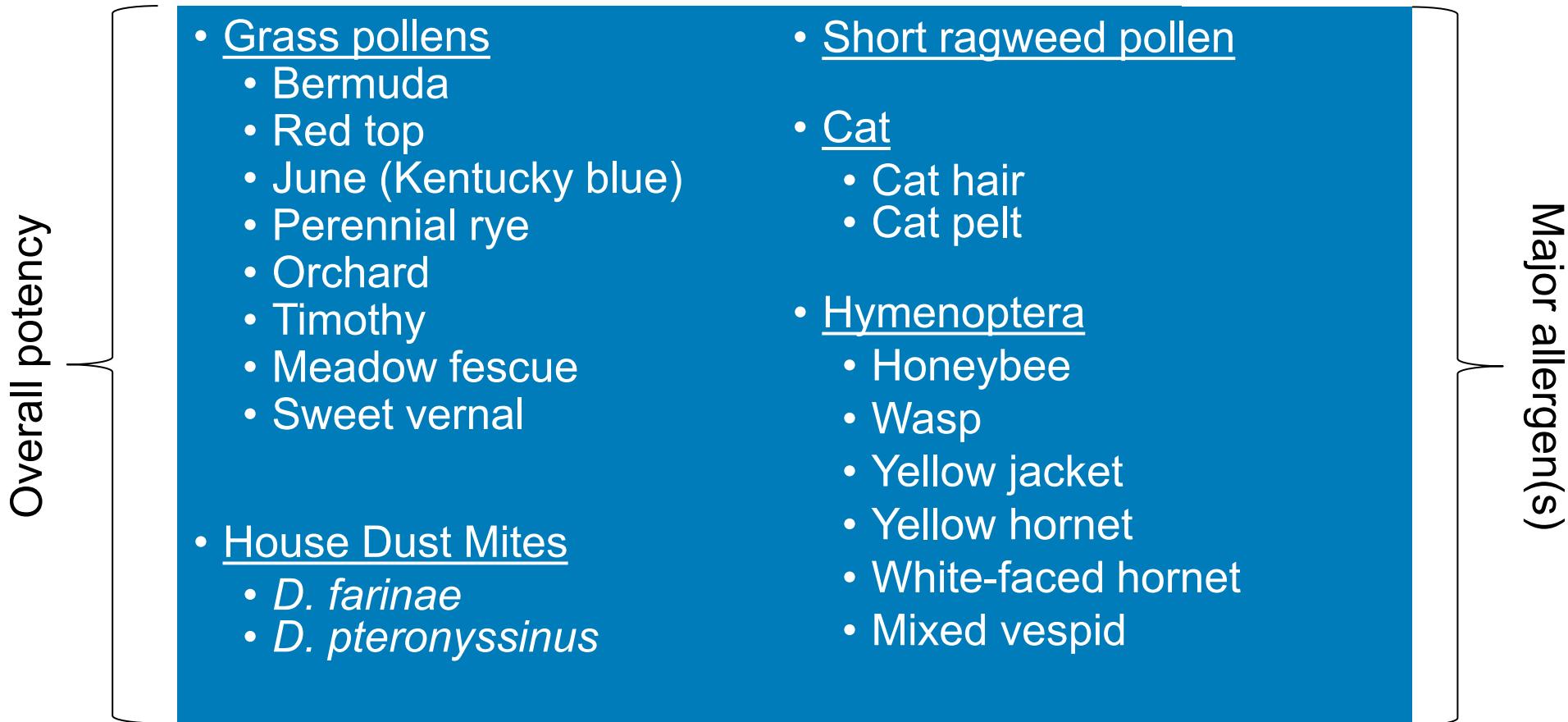
Question 4: House Dust Mite (HDM) Source Material Optimization

Do the available data support inclusion of both house dust mite (HDM) bodies and fecal pellets as source materials for HDM allergen extracts to more adequately mimic clinically relevant allergen exposure?

Outline

- Existing standardization method for complex allergen extracts has limitations and may be considered obsolete
- Surrogate assays of complex allergen extracts do not detect compositional differences among extracts from different manufacturers or lots from the same manufacturer
- LC/MS/MS is a transformative platform that enables comprehensive proteomic characterization
- LC/MS/MS reveals potential for improvements in manufacturing methods (i.e., source materials) for complex allergen extracts

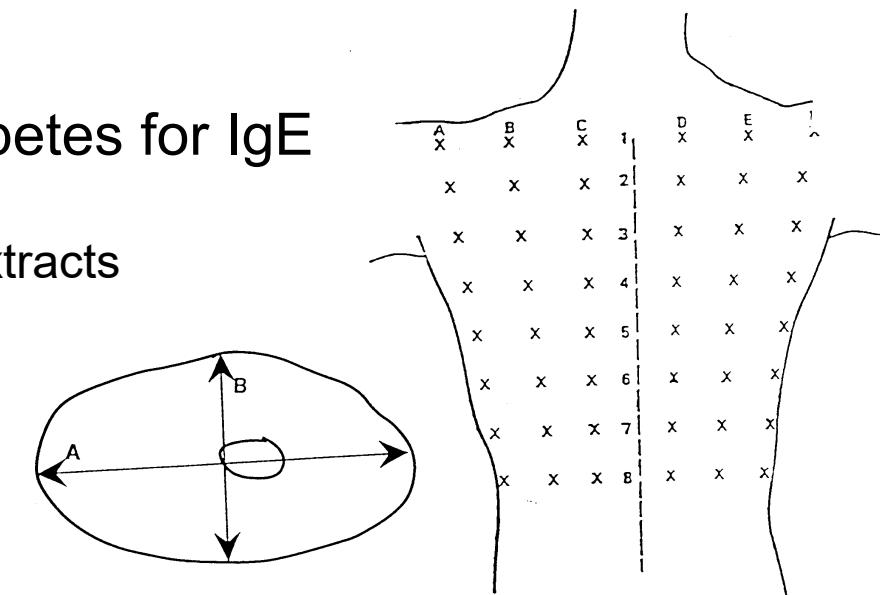
Nineteen (19) Standardized Extracts (Hundreds of Non-standardized Extracts)



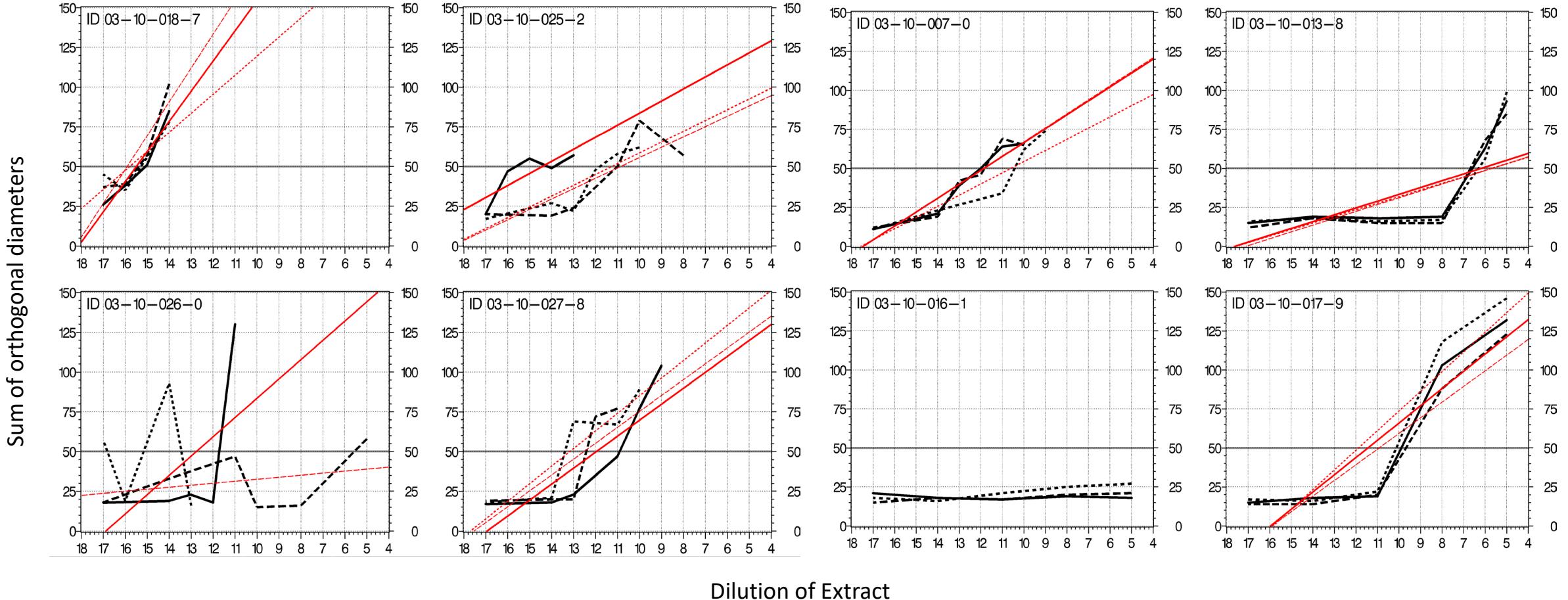
Allergen standardization by overall potency



- ID₅₀EAL testing (Intradermal Dilution for 50 mm Sum of Erythema) Determines Bioequivalent Allergy Units
 - Highly allergic individuals
 - Serial 3-fold dilutions
 - Establish dilution at which SE = 50 mm (D₅₀)
 - D₅₀ = 100,000 BAU/mL
- Surrogate assay is a competitive ELISA (allergen competes for IgE in human serum pools)
 - cELISA does not detect compositional differences between extracts
 - Serum pools may vary because of differences among donors
- ID₅₀EAL testing and cELISA rely on two assumptions
 - Extracts from different manufacturers are qualitatively similar
 - Allergic patients react similarly to the same sets of allergens



Failed Attempt to Standardize Cockroach Allergen Extracts for Overall Potency



With permission; NIAID Inner City Asthma Consortium

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Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)



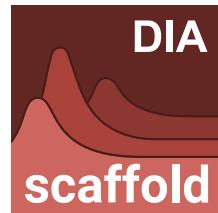
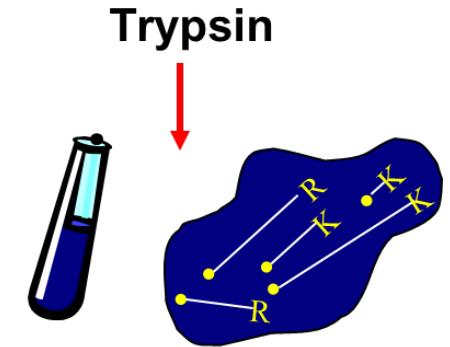
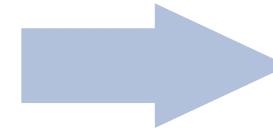
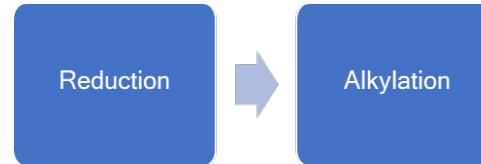
- Generates peptide fragmentation spectra for qualitative and quantitative information
 - Proteomic analyses of complex samples
 - Relative quantification of proteome
 - Allergen profile characterization
 - Compare source materials (i.e., hyphae versus spores)
 - Compare extracts between manufacturers and lots from the same manufacturer
- Necessary first step for absolute quantification by Parallel Reaction Monitoring
 - Use heavy isotope “surrogate peptides” absolute measurement of allergens
 - Measure potency of complex allergen extracts

→Promote manufacture of extracts that are specific, potent, and consistent

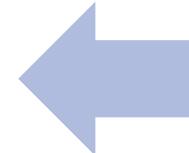
Tandem Mass Spectrometry (LC/MS/MS) Workflow



Biological samples
▪ Source materials
▪ Allergen extracts

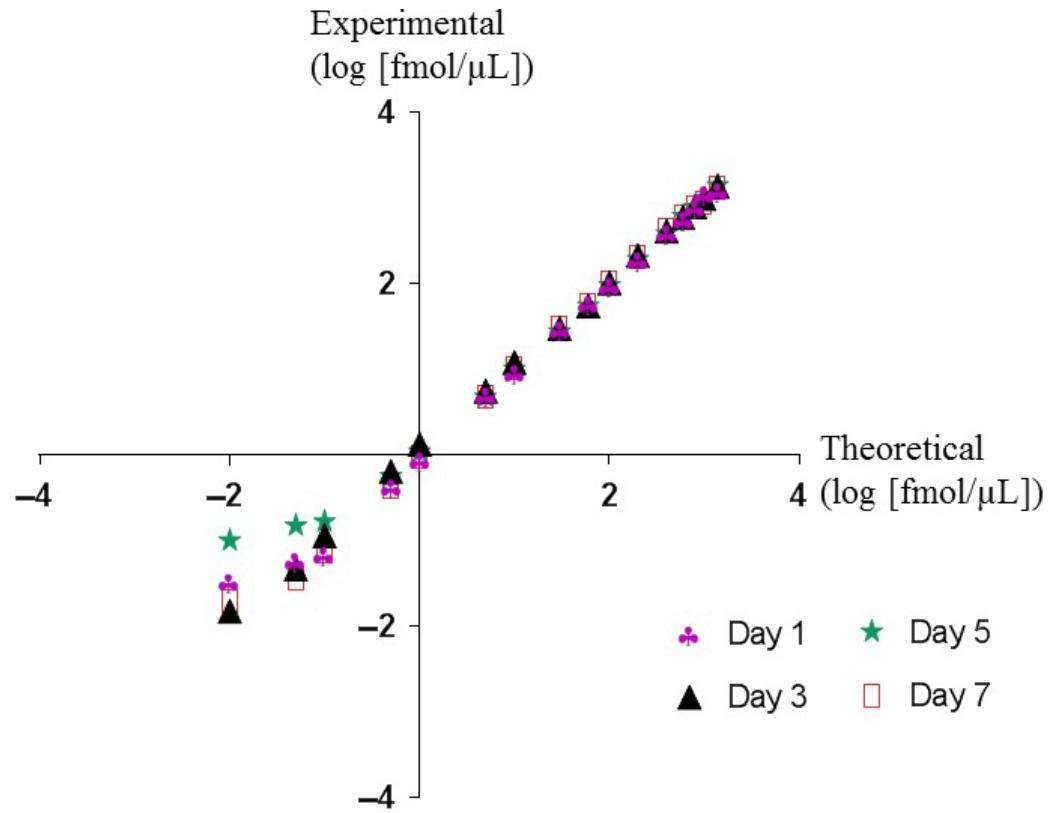


Data are searched against
translated genomic
databases for IDs and
relative abundance



Analyze via LC/MS/MS

Tandem Mass Spectrometry is Precise, Accurate and Reproducible



Repetitive analysis of 5 German cockroach allergens with tandem mass spectrometry using heavy peptides for absolute quantification

Mindaye et al. Clin Experimental Allergy, 2017; 47:1661

Alternaria alternata is a Common Mold that Causes Allergic Asthma

- Mainly outdoors, but can be (poorly ventilated) indoors
- Disseminates in dry air, temperate climates
- Spore counts increase in summer
- Allergen extracts are “variable and insufficiently standardized as they are often a random mixture of allergenic ingredients and coincidental impurities”



*Kustrzeba-Wójcicka et al. Clin Rev Allergy Immunol 47:354

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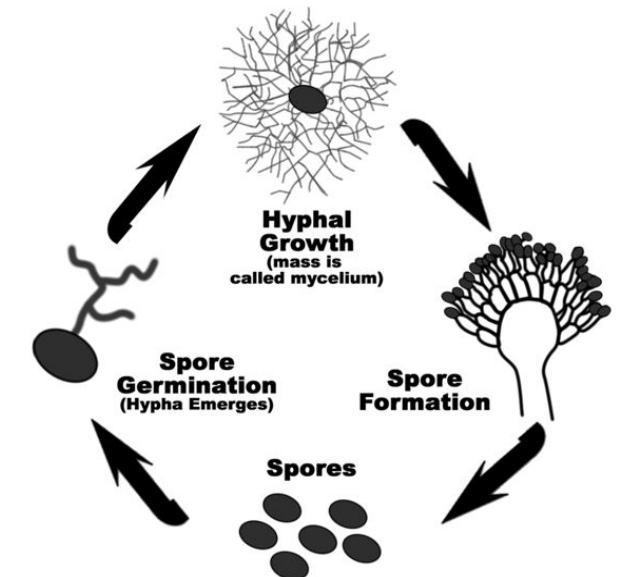


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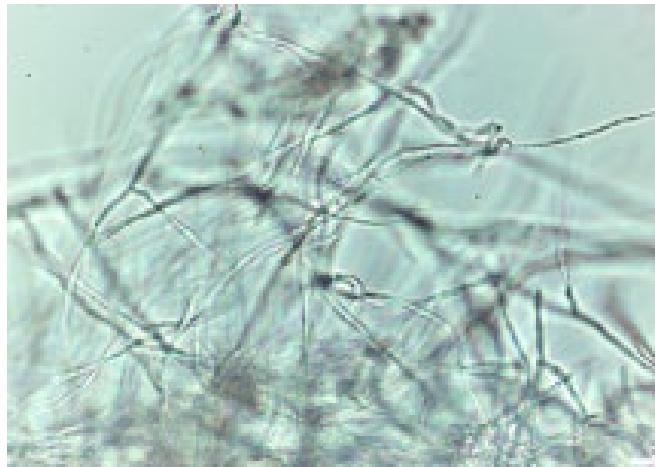
- Allergen expression differs with:
 - developmental stage
 - structural origin
 - growth media

Life Cycle of Mold



*Kustrzeba-Wójcicka et al. Clin Rev Allergy Immunol 47:354

Life Cycle Stages of *Alternaria alternata*



Hyphae



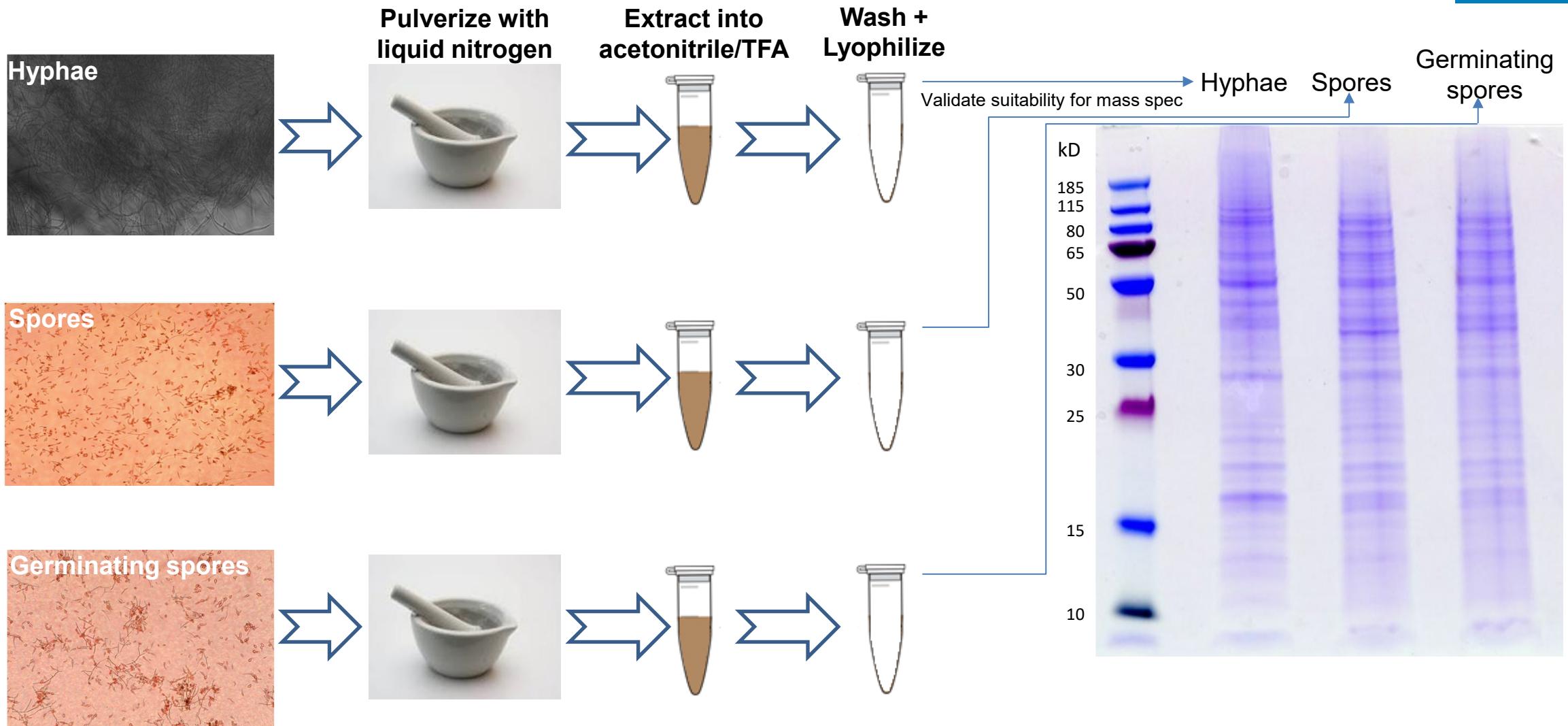
Non-germinating spores



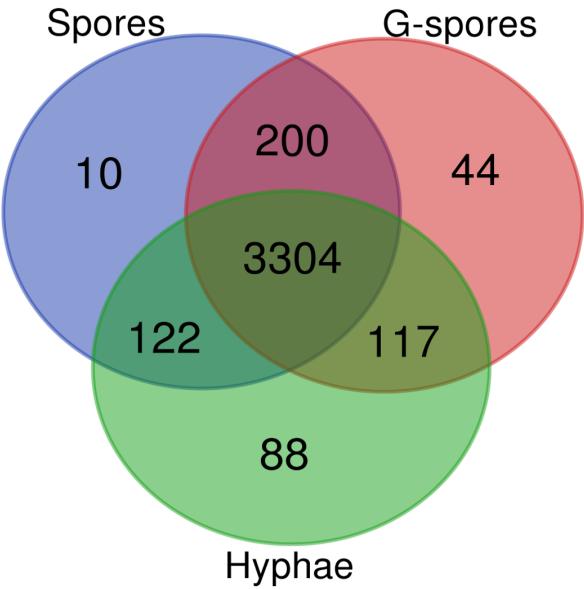
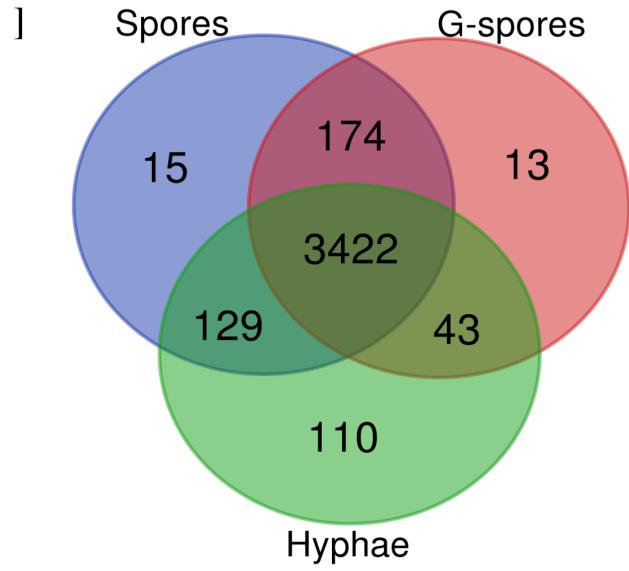
Germinating spores

Strader MB et. al., J Allergy Clin Immunol. 2024; 154:424

Alternaria alternata Spores and Hyphae Extraction



Biological Replicates were Consistent



Sample	# Proteins
Non-germinating spores	3740
Germinating spores	3652
Hyphae	3704
Total proteins identified in all samples	3906

Study 1

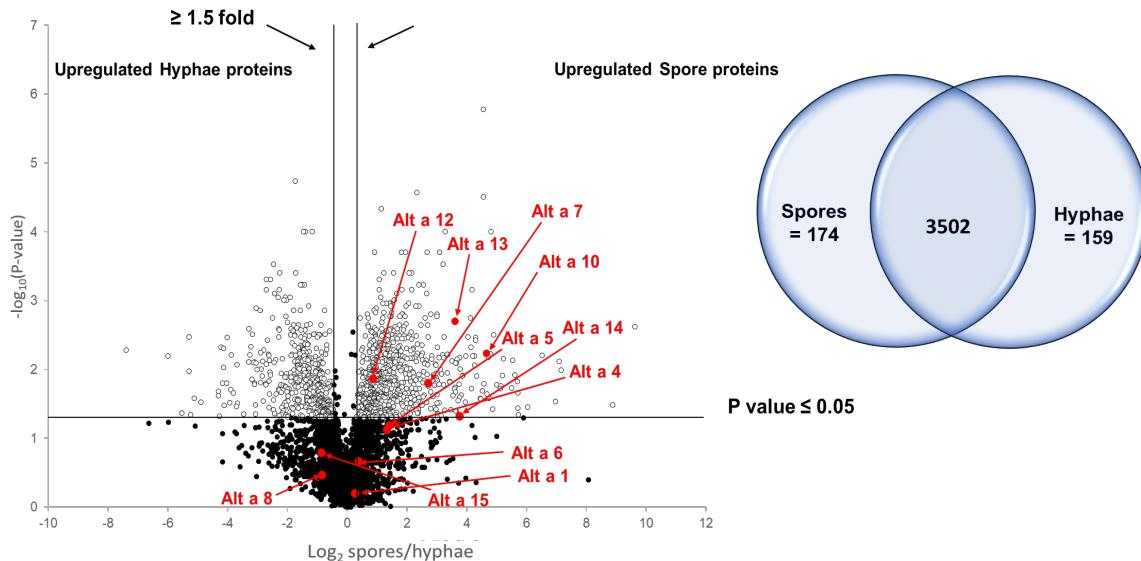
Sample	# Proteins
Non-germinating spores	3636
Germinating spores	3665
Hyphae	3661
Total proteins identified in all samples	3885

Study 2

Most Allergens are Most Abundant in Nongerminating Spores

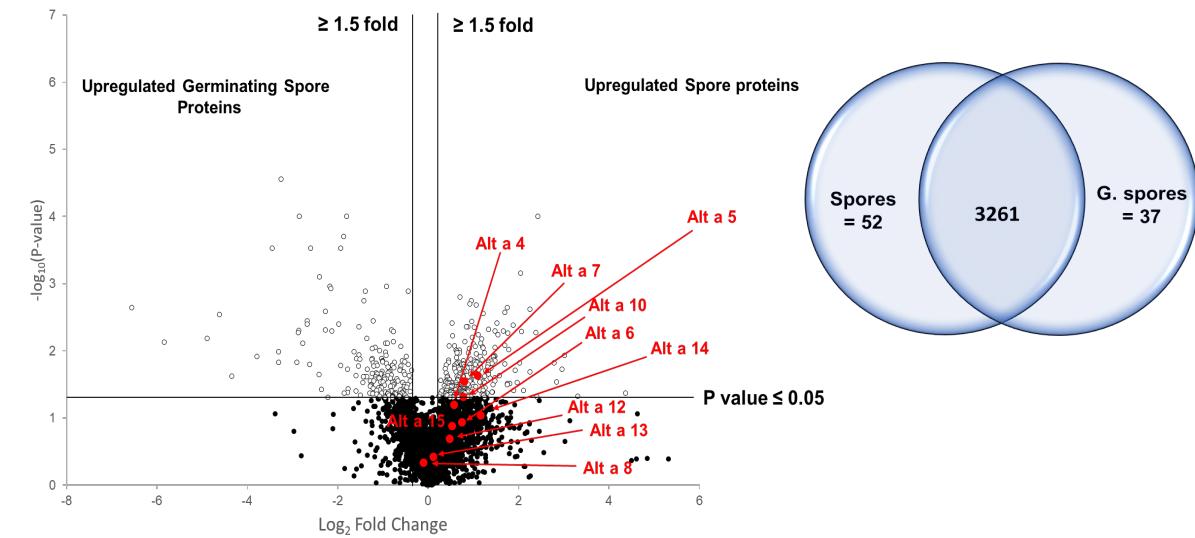


Hyphae versus spores



3502 *Alternaria alternata* proteins
Alt a 3 identified only in spore samples

Germinating spores versus spores



3261 *Alternaria alternata* proteins
Alt a 3 and Alt a 1 identified only in spore samples

- Most known allergens are more prevalent in non-germinating spores.
- A major allergen Alt a 1 is in the growth media (!)
- This analysis is used to select surrogate heavy peptides for absolute quantification (potency measurement).

Commercial *A. alternata* Extracts are Compositionally Heterogeneous



Variability of proteins identified among three lots from each manufacturer

Manufacturer	CV
ALK	33.70%
Greer	49.40%
JHS	35.70%

Allergens relative quantities (geometric means) in three lots from each manufacturer

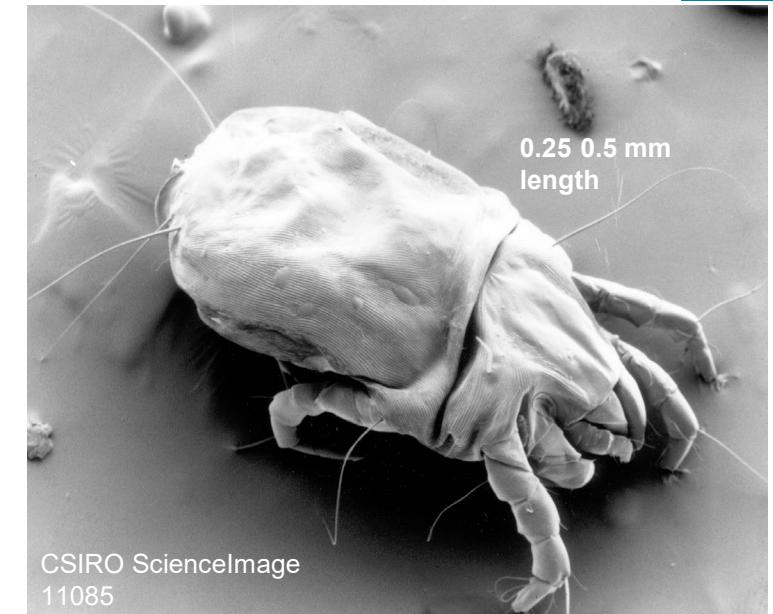
Allergen	ALK	Greer	JHS
Alt a 1	2.6 ± 0.7	1.0 ± 0.4	2.0 ± 0.2
Alt a 3	1.0 ± 1.7	17.7 ± 17.1	2.0 ± 1.3
Alt a 6	—	6.8 ± 6.8	—
Alt a 8	—	21.8 ± 19.5	—
Alt a 10	—	2.2 ± 1.1	—
Alt a 14	—	16.7 ± 8.3	—

Question 4: House Dust Mite (HDM) Source Material Optimization

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House Dust Mites (HDM)

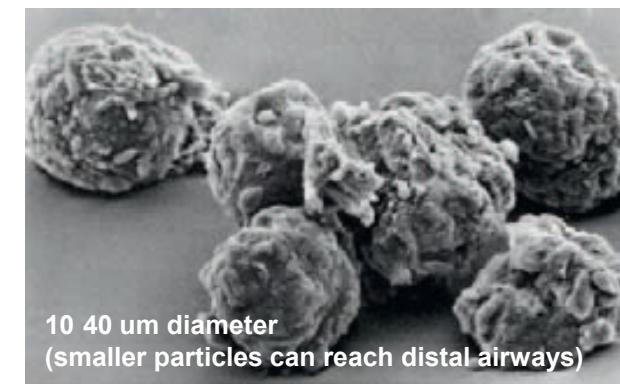
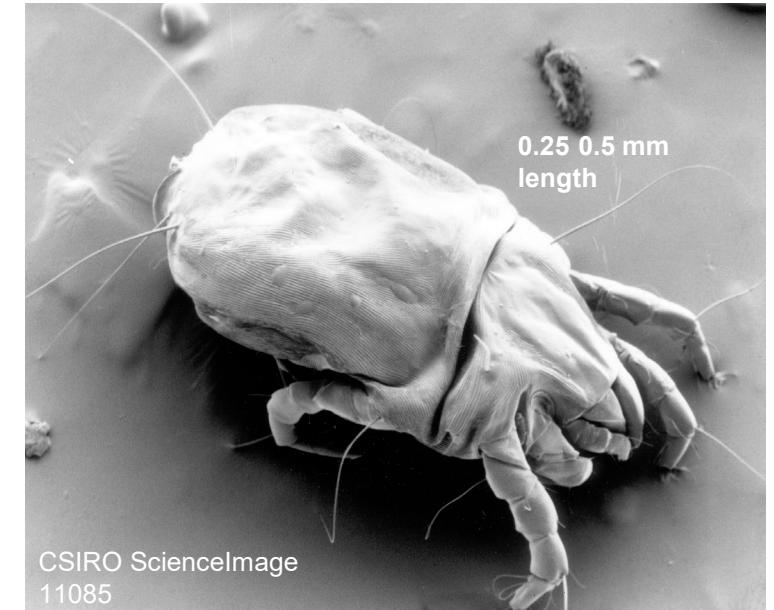
- Ubiquitous indoor allergens (except in cold or dry regions)
- Two predominant species in northern hemisphere
 - *Dermatophagoides pteronyssinus*
 - *Dermatophagoides farinae*
- Eat shed mammalian epithelium
- Live in bedding, upholstered furniture and carpets
 - ~10% weight of a 2-year-old pillow is mite bodies and feces
 - Often the first respiratory allergen, initiates the “allergic march”



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 - Often the first respiratory allergen, initiates the “allergic march”
- Coprophagic: fecal pellets are structurally formed and contain digestive enzymes that are also allergenic proteins
- Allergens are present in mite bodies and fecal pellets
- Fecal pellets are small; inhaled into lower airways
- Like *Alternaria alternata*, HDM are cultured, conditions of which can affect allergen composition
- In the US, HDM extracts are derived from mite bodies

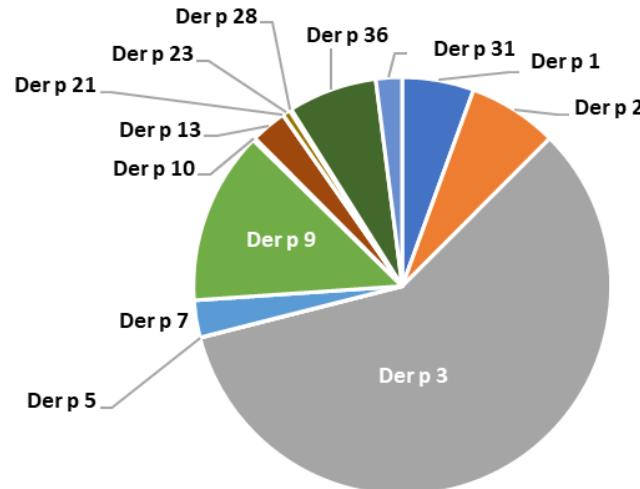


HDM Bodies and Fecal Pellets Differ in Allergen Content

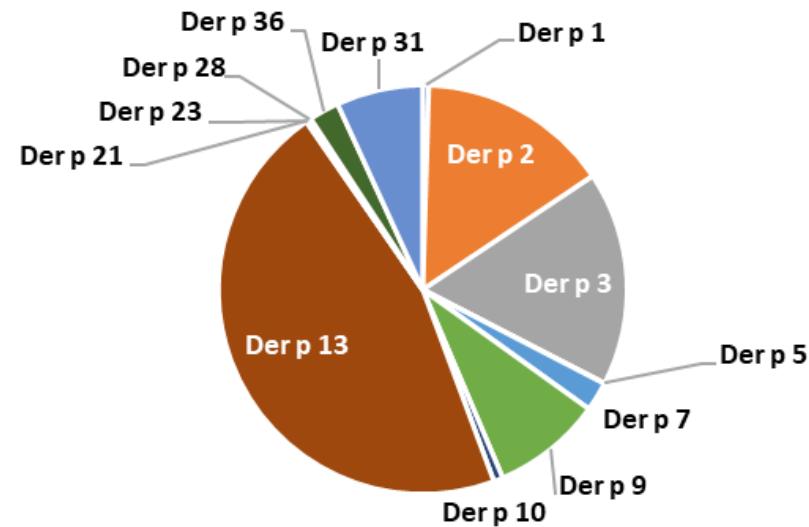


D. pteronyssinus

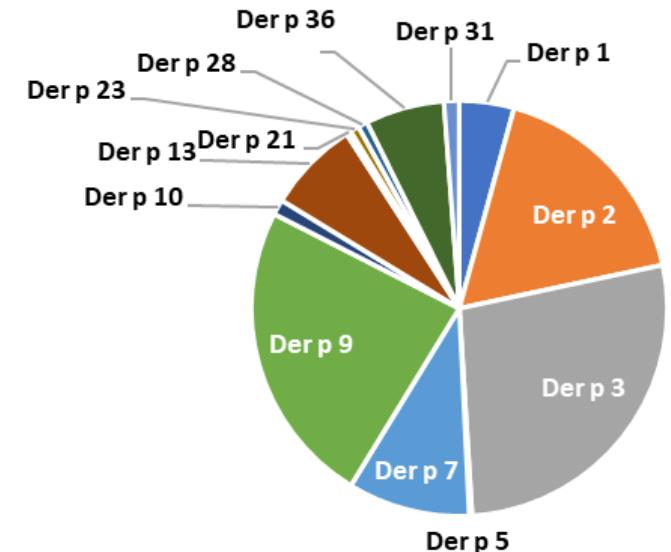
Feces



Bodies



Greer HDM extract



Allergens represent 7% of total protein*

Allergens represent 3% of total protein*

Allergens represent 2% of total protein*

*Based on summed allergen peptide ion current measurements
MB Strader, unpublished observations

Fecal Pellets are Enriched with Major Allergens



Protein	Function	Average Feces	Average Bodies	Average Greer HDM
Der p 1	cysteine protease	18.2 ± 0.2	1.0 ± 0.1	5.9 ± 0.6
Der p 2	MD-2 lipid recognition domain protein	1.0 ± 0.2	1.3 ± 0.3	1.1 ± 0.3
Der p 3	trypsin-like protease	5.4 ± 0.2	1.0 ± 0.4	1.1 ± 0.4
Der p 5	group 5/21 protein	1.7 ± 0.4	1.0 ± 0.1	8.8 ± 0.14
Der p 7	bacterial permeability protein	5.0 ± 0.9	1.0 ± 0.13	3.8 ± 0.2
Der p 9	Trypsin-alpha 3 protease	4.1 ± 1.1	1.0 ± 0.2	3.8 ± 0.2
Der p 10	tropomyosin	1.0 ± 0.1	1.2 ± 0.14	2.1 ± 0.1
Der p 13	fatty acid binding protein	1.0 ± 0.8	9.1 ± 0.5	3.2 ± 0.3
Der p 21	group 5/12 protein	1.0 ± 0.02	1.1 ± 0.12	11.5 ± 0.1
Der p 23	peritrophin-like protein domain	4.1 ± 1.2	1.0 ± 0.11	1.7 ± 0.2
Der p 28	heat shock protein Hsp70	1.4 ± 0.3	1.0 ± 0.13	5.1 ± 0.11
Der p 31	cofilin	3.3 ± 0.9	6.3 ± 0.3	1.0 ± 0.14
Der p 36	C2 domain containing protein	7.6 ± 1.0	1.0 ± 0.12	1.1 ± 0.13

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Summary

- Mass spectrometry is precise, accurate, and reproducible, and identifies proteotypic surrogate peptides
- Parallel reaction monitoring with isotopically labeled surrogate peptides allows for absolute quantification of allergens
- When used to improve product quality, CBER's Reference Reagent Lab will transfer technology and reagents to the manufacturers for lot release
- Mass spectrometry can guide manufacturing of complex extracts
 - Include spores in *Alternaria alternata* extracts
 - Include fecal pellets in HDM extracts
- Mass spec and parallel reaction monitoring are mature technologies that will promote manufacture of extracts that are specific, potent, and consistent

Voting questions

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