Indoor Air Quality ASSOCIATION INC.

12th Annual Meeting & Indoor Air Expo
February 24-26, 2009 • Fort Worth, TX
Omni Fort Worth & Fort Worth Convention Center

Indoor Allergens

Health Effects, Exposure Assessment and Intervention

Martin D. Chapman, PhD
Eva M. King, PhD
INDOOR Biotechnologies Inc., Charlottesville VA

The IAQ Industry's Largest Annual Convention

Indoor Allergens:

Risk Factors and Health Effects

Martin D. Chapman, PhD
President/CEO
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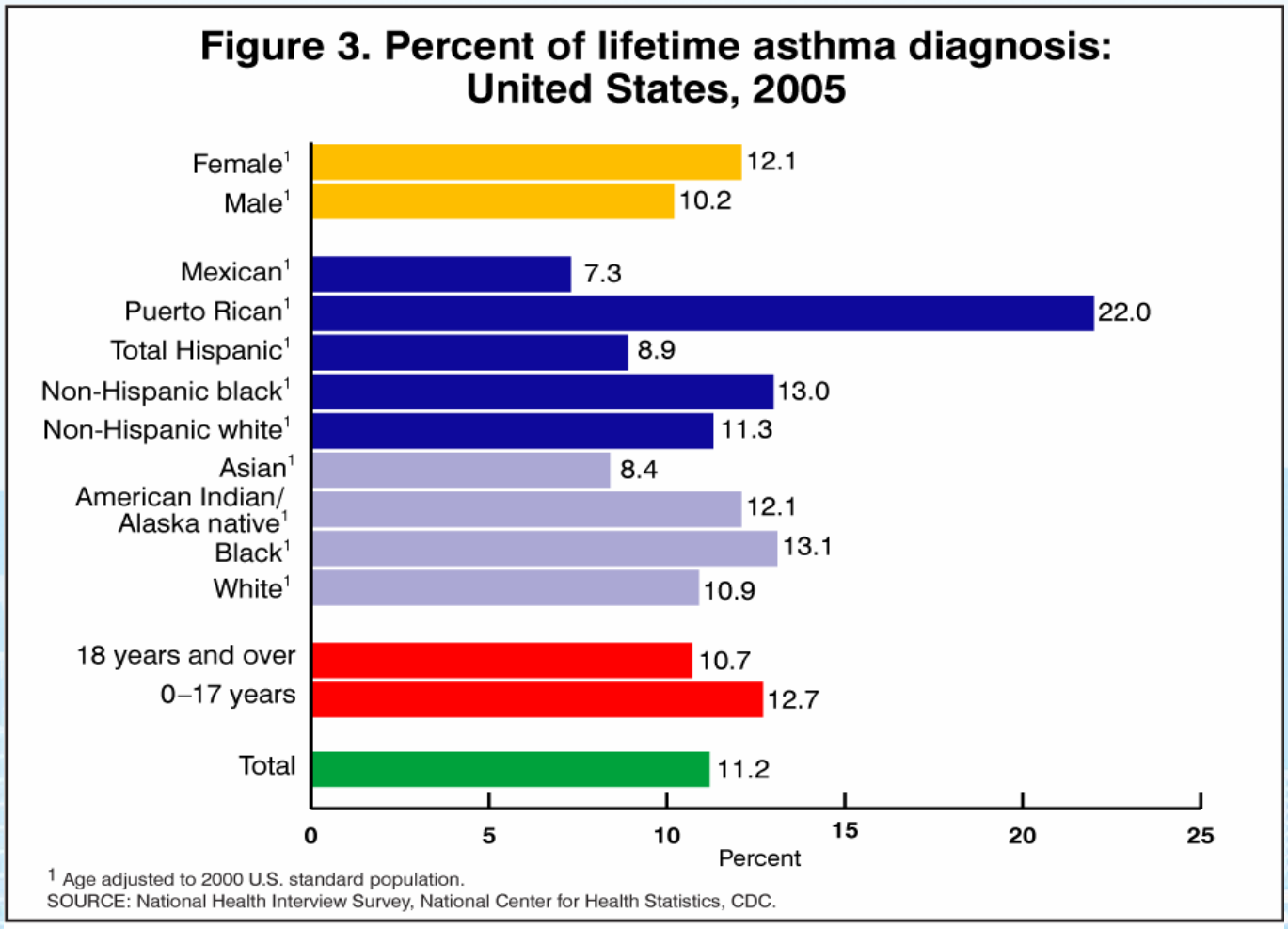
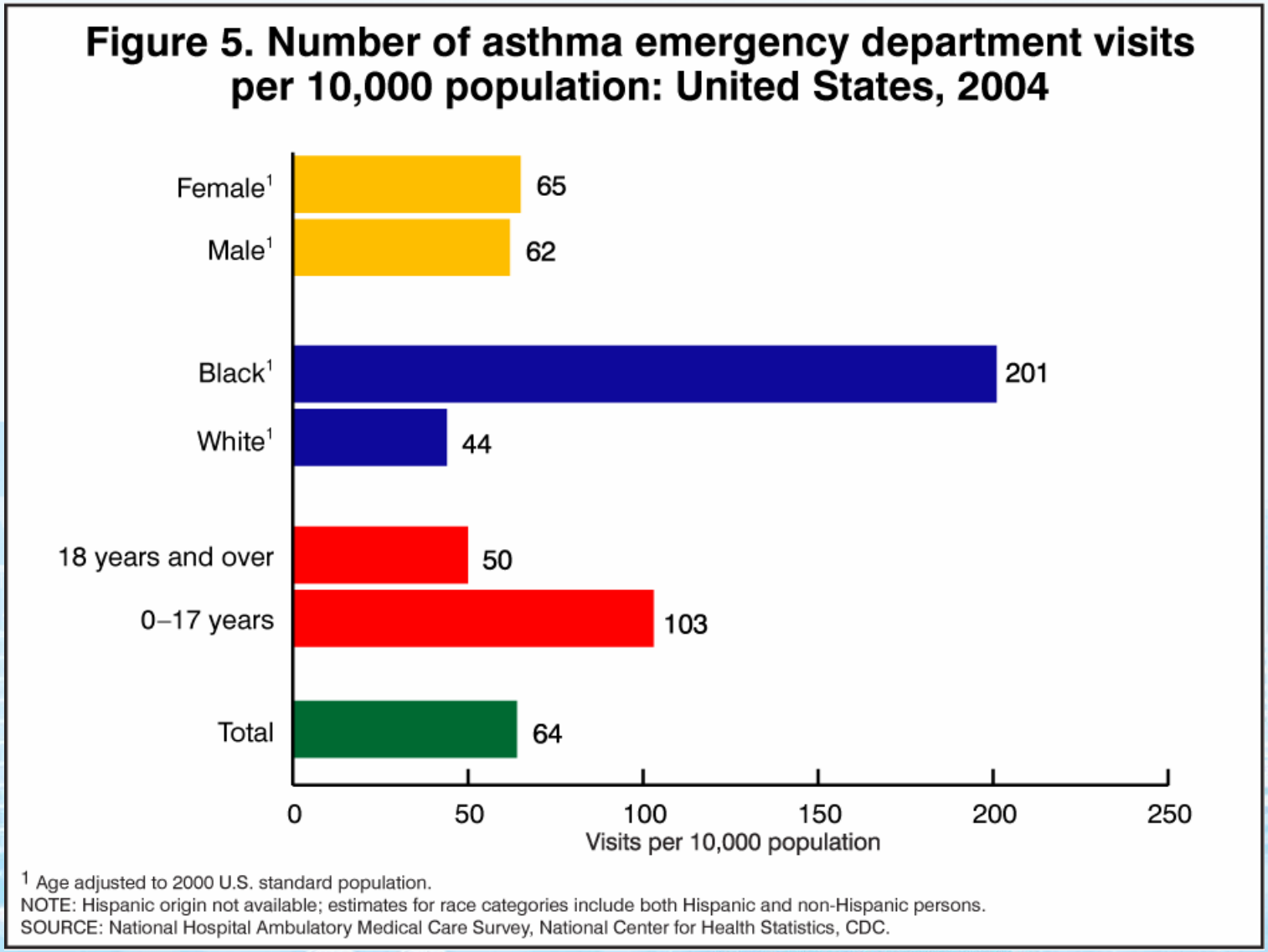
Asthma: A Public Health Problem

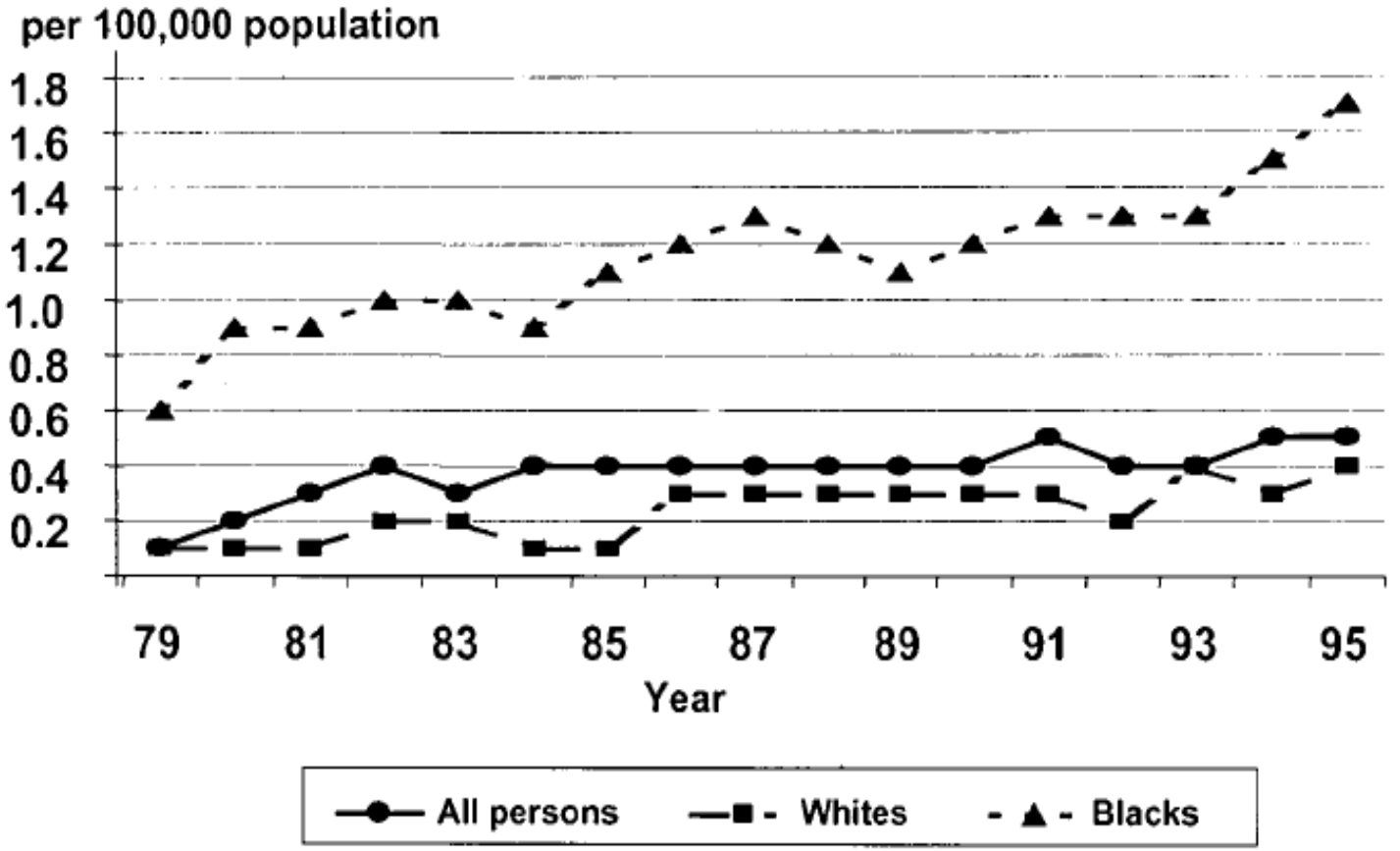


Courtesy of Herman Mitchell, Rho, Inc.

Asthma: A Public Health Problem



Asthma Mortality Rates in the US



Grant et al, JACI 1999; 104: S1-9

Risk Factors for Asthma

- Family history of asthma or allergy
- Allergen exposures
- Viral infections
- Obesity
- Tobacco smoke
- Air pollution

Association between Indoor Allergens and Asthma

- IgE-mediated sensitization to indoor allergens is a major risk factor for asthma:
  - Case control studies (e.g. in Emergency Rooms and schools)
  - Prospective studies in high risk cohorts
  - Longitudinal population based cohort*

*Sears et al, NEJM 2003;349:1414-22

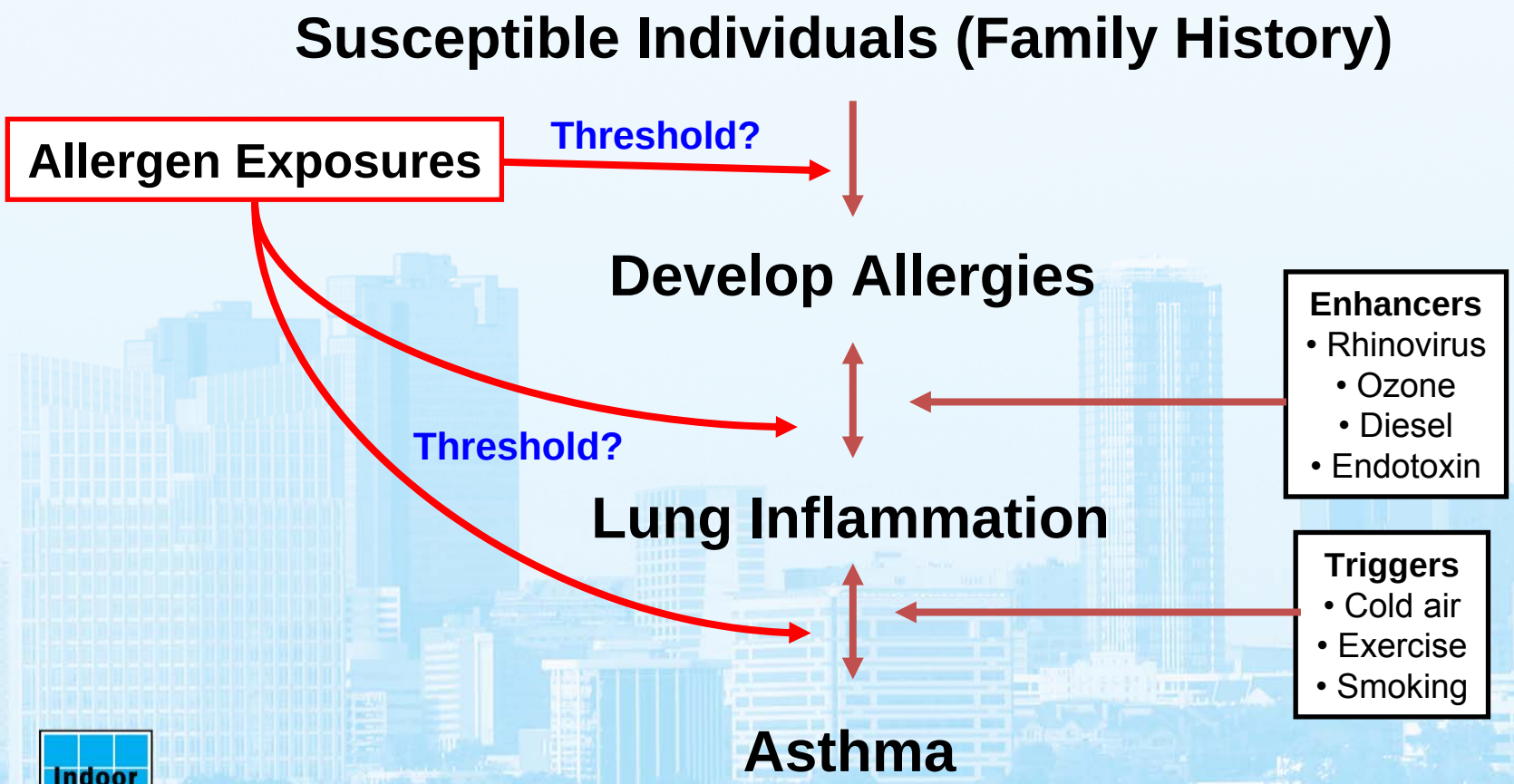
A longitudinal, population-based, cohort study of childhood asthma (onset-26 yr)*

Multivariate Model	Persistence		Relapse	
(Significant Risk Factors)	O.R.	P Value	O.R.	P Value
PC20 ≤ 8mg/ml or BDR > 10% at any assessment (9-12 yr)	3.00	<0.001	3.03	<0.001
Positive mite Skin Test at 13yr	2.41	0.001	2.18	0.01
Female sex	1.71	0.03	----	----
Smoking at 21yr	1.84	0.01	----	----
Age of onset of wheezing	----	----	0.89	<0.001

*Odds Ratios for factors predicting persistence or relapses of wheezing

Sears et al., NEJM, 2003: 329:1414-22

Development of Allergic Asthma



What are Allergens?

- Small proteins or glycoproteins
- 10 to 50,000 kDA molecular size
- Readily soluble ⟶ penetrate membranes
- Have different biologic functions
- Cause allergic immune responses (IgE antibody)

Skin Prick Test

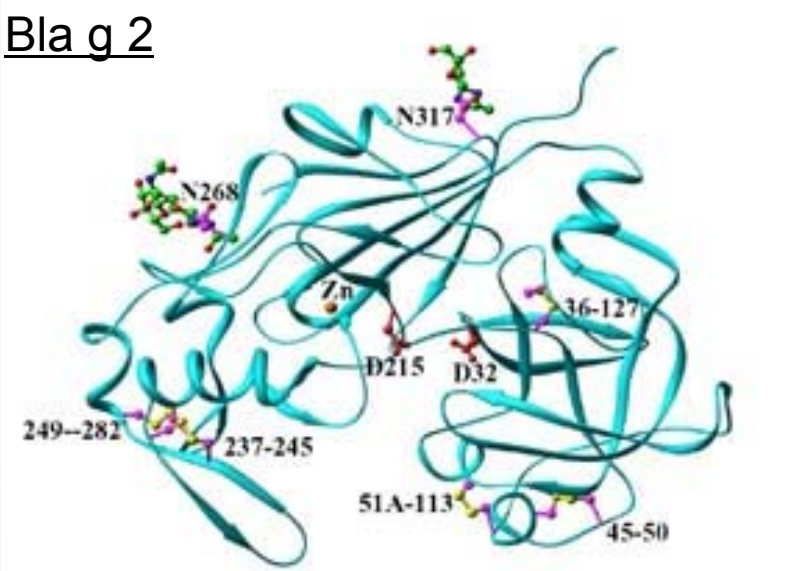
Naming of Allergens

Examples:

- 1st allergen of cat (***Fel****is* ***d****omesticus*): **Fel d 1**
- 2nd allergen of dust mite ***Der****matophagoides* ***p****teronyssinus)*: **Der p 2**

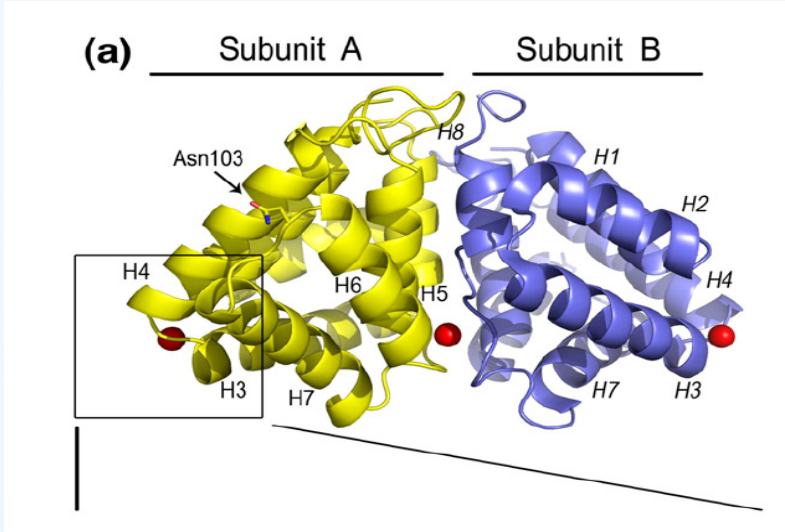
International Union of Immunological Societies' (IUIS) Allergen Nomenclature Subcommittee (www.allergen.org)

Bla g 2



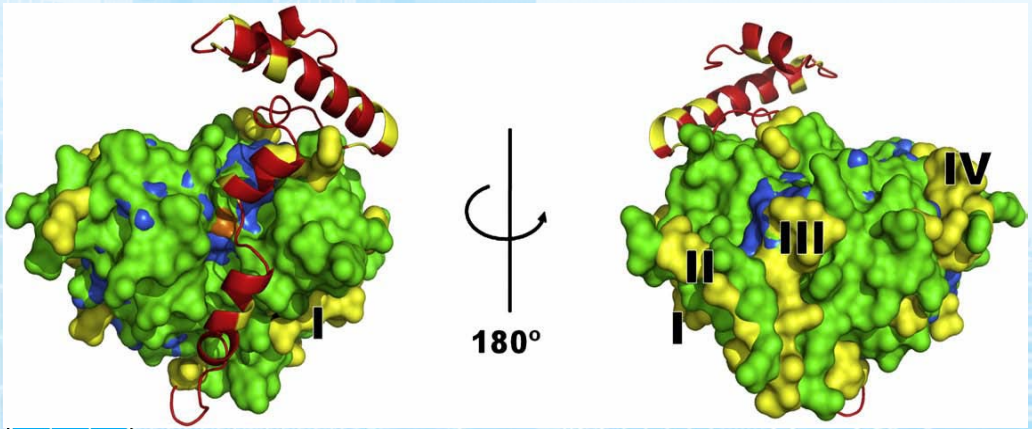
Gustchina et al J Mol Biol, 2005

Fel d 1



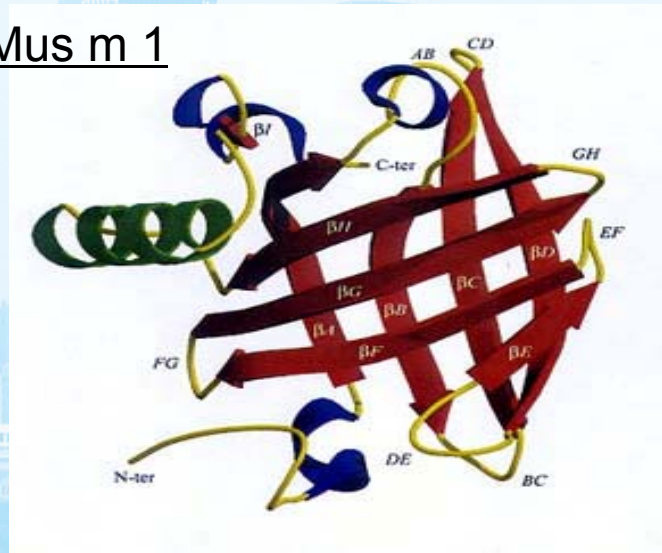
Kaiser et al, J Biol Chem 2003

Der p 1, Der f 1



Chrusczc et al J Mol Biol 2009

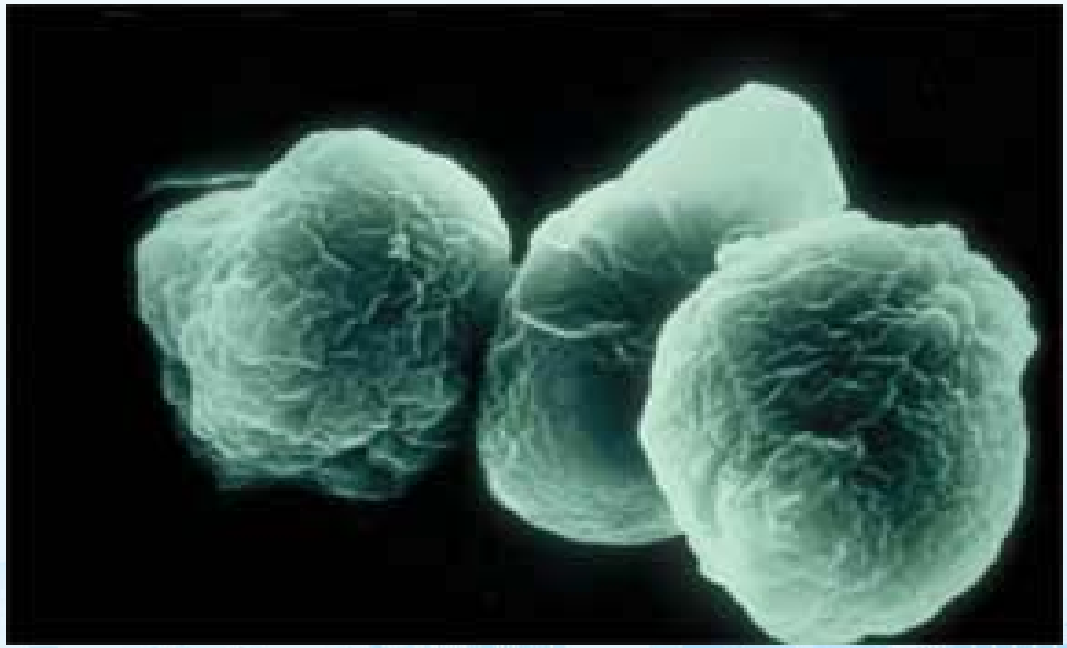
Mus m 1



Kuser et al Acta Cryst 2001

Mite Feces is Loaded with Allergens and Adjuvants

- Allergens
- Proteases
- Endotoxin
- Bacterial DNA
- Mite DNA
- Chitin
- Evolutionary distance



Platts-Mills JACI 119:297-302, 2007, Reese et al, Nature 447: 92-6, 2007

Dust Mites Associated with Asthma

Domestic	Distribution
✓ *Dermatophagoides* spp.	"Worldwide" (low altitude)
✓ *Euroglyphus maynei*	"Worldwide"
✓ *Blomia tropicalis*	Tropics

Occupational:	
✓ *Lepidoglyphus destructor*	Farms, farming communities,
✓ *Tyrophagus putrescentiae*	"Barn Allergy"
✓ *Acarus siro*	Europe & USA
✓ *Tetranychus urticae*	Orchards, greenhouses:
✓ *Panonychrus ulmi*	Spain, Germany, Korea*

*Burches et al, Clin Exp Allergy 26:1262 1996; Kim et al, JACI 107:244, 2001

Risk Factors for Asthma in US Inner Cities

- Race (African American, Hispanic)
- Lower socio-economic status
- Sensitization and exposure to allergens, especially cockroach

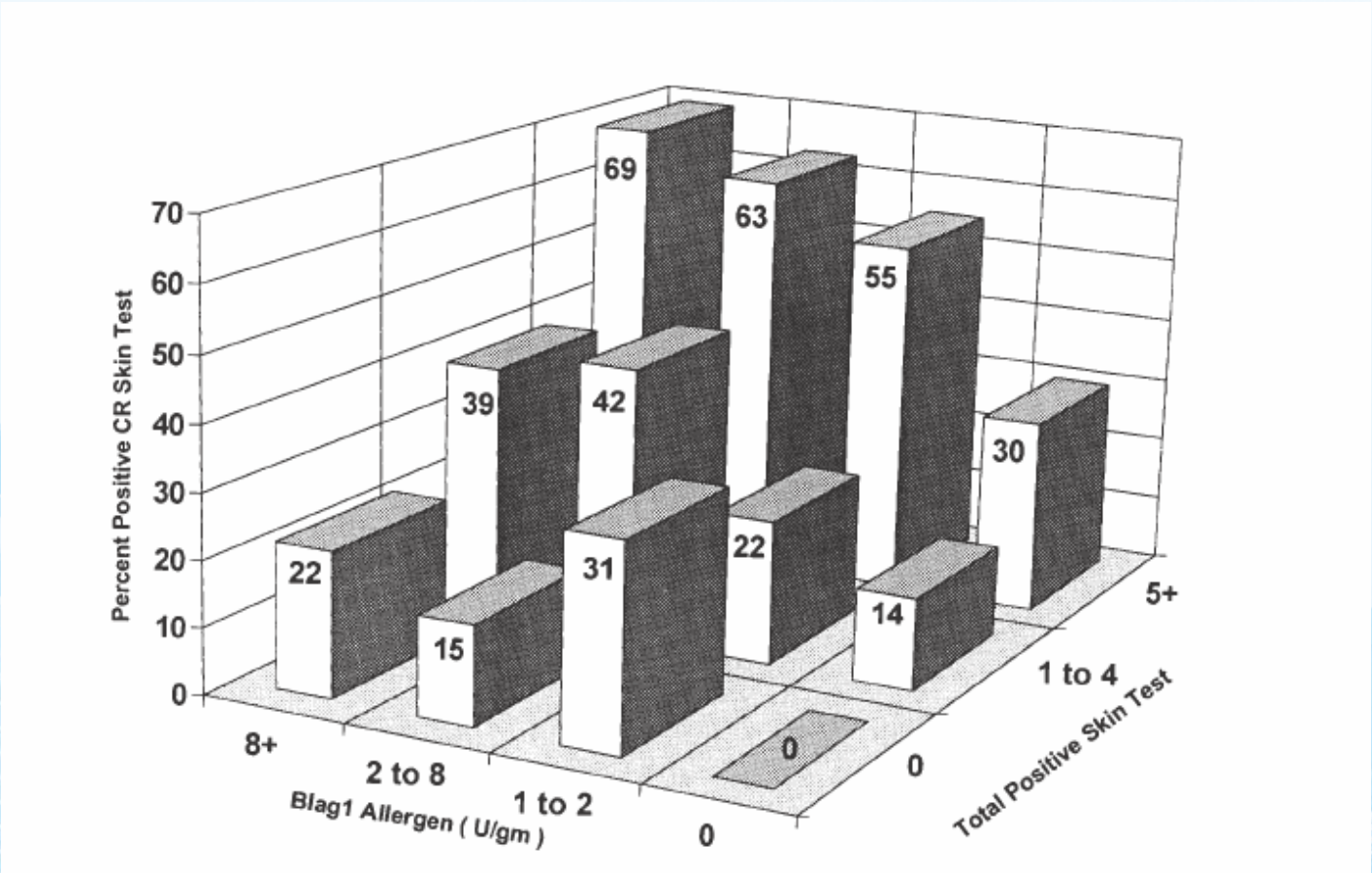
NIH - Inner-City Asthma Study

Inner City Asthma Study

- 937 children (aged 5-11)
  from Seattle, Dallas, Tucson, Boston, St. Louis, New York, Bronx
    - Skin Tests
    - Allergen Exposures
- Sensitization
    - Cockroach: Bronx, New York, Dallas (~80%)
    - Mite: Dallas, Seattle (~80%)
- Exposure
    - Cockroach: >2U/g Bla g 1, New York, Bronx, Chicago
    - Mite: >2µg/g, Dallas, Seattle

Gruchalla et al, JACI 115:478, 2005

Dose Response Relationship between Sensitization and Exposure to Cockroach



Eggleston et al, JACI 1998; 102: 563-70

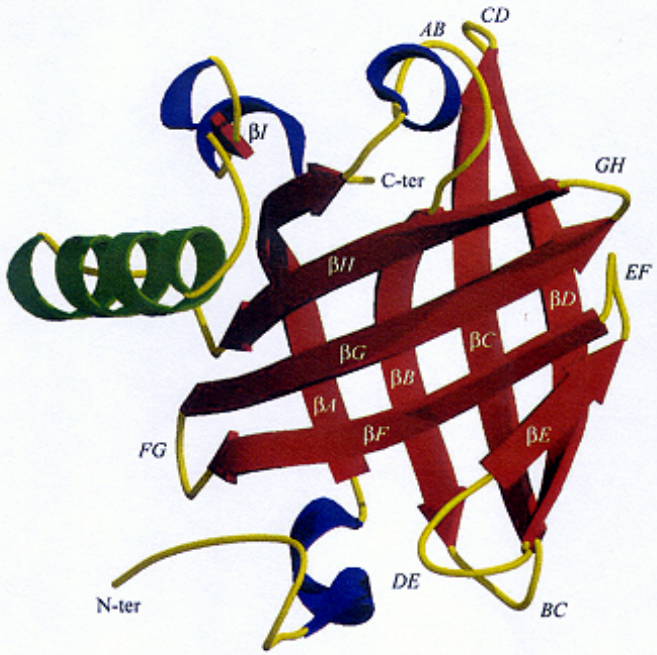
Animal allergen exposure (cat & dog)

- Is ubiquitous
  - occurs in homes without pets (1-10μg/g)
  - occurs in schools, daycare centers, offices and other public places
  - is passively transferred on clothing
- In schools:
  - airborne cat allergen increased 5-fold in classes with cat owners
  - indirect cat exposure increases the risk of asthma exacerbation (9-fold)

Almqvist et al., AJRCCM 2001; 163:694-8

Mouse allergen: Associated with inner-city asthma

- Prevalence of sensitization:
  11-27% in inner-city children with asthma (in 8 US cities)
- Mouse allergen (Mus m 1) found in >75% of low income homes - median 1.6μg/g.
- Risks of sensitization associated with Mus m 1 exposure



Phipatanakul et al., JACI 2000:106:1070-80

National Allergen Survey of Allergen Exposure in US Homes

- 831 housing units surveyed in 75 locations across the US
- Estimated that 23% (~ 22 million) of U.S. housing units contain high levels (>10μg/g) of Group 1 mite allergen
- Cat and dog allergens present in all homes
- Levels of mouse allergen associated with sensitization (>1.6μg/g) found in 22% of homes

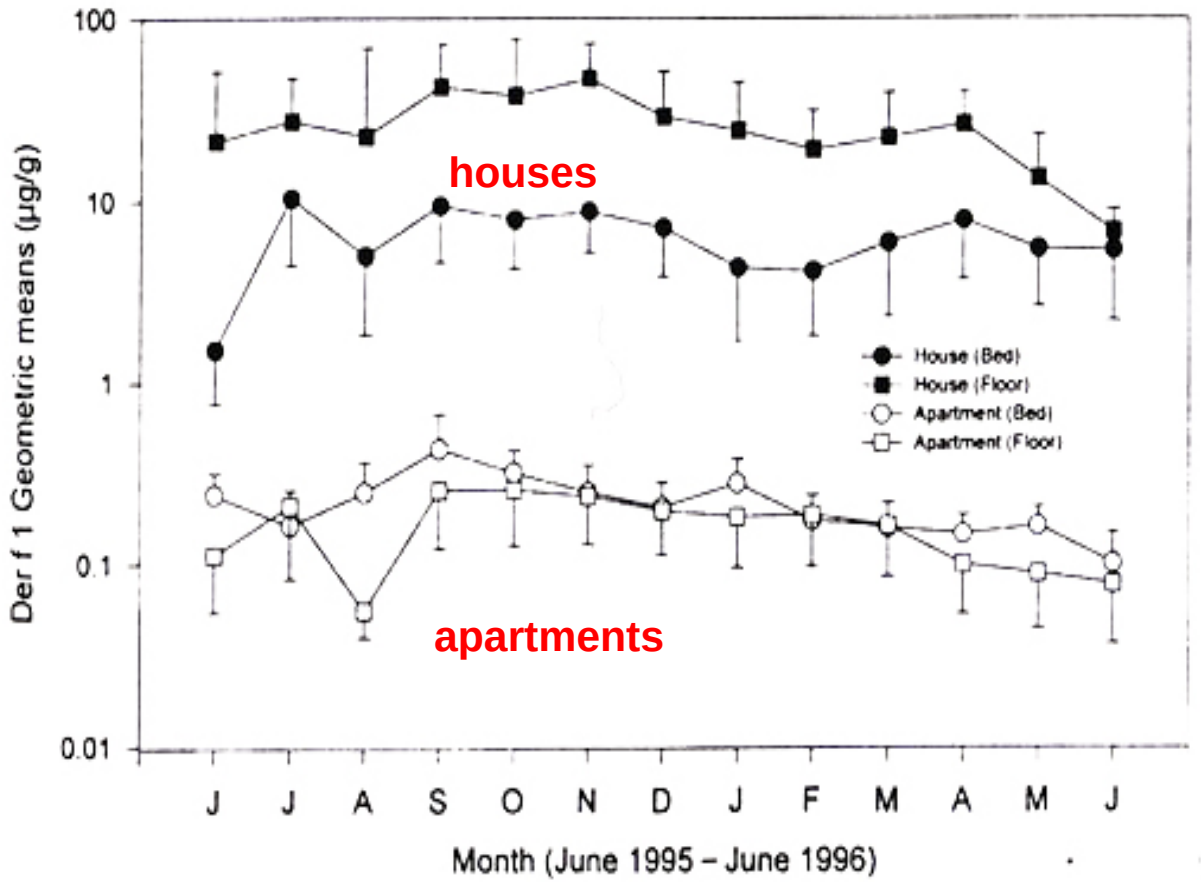
Studies conducted by National Institute of Environmental Health Sciences and Dept. Housing and Urban Development

Arbes et al, J Allergy Clin Immunol 114;111-7, 2004

Allergen Exposure Guidelines for Developing Allergic Sensitization

		Allergen level in dust
Mite:	Der p 1, Der f 1	2-10 µg/g
Cat:	Fel d 1	1-10 µg/g
Dog:	Can f 1	1-10 µg/g
Cockroach:	Bla g 1	1-8 U/g
	Bla g 2	>1µg/g
Mouse:	Mus m 1	1-2 µg/g

Monthly Der f 1 levels in houses and apartments in Boston, MA



Chew et al, Allergy 54:1058-66, 1999

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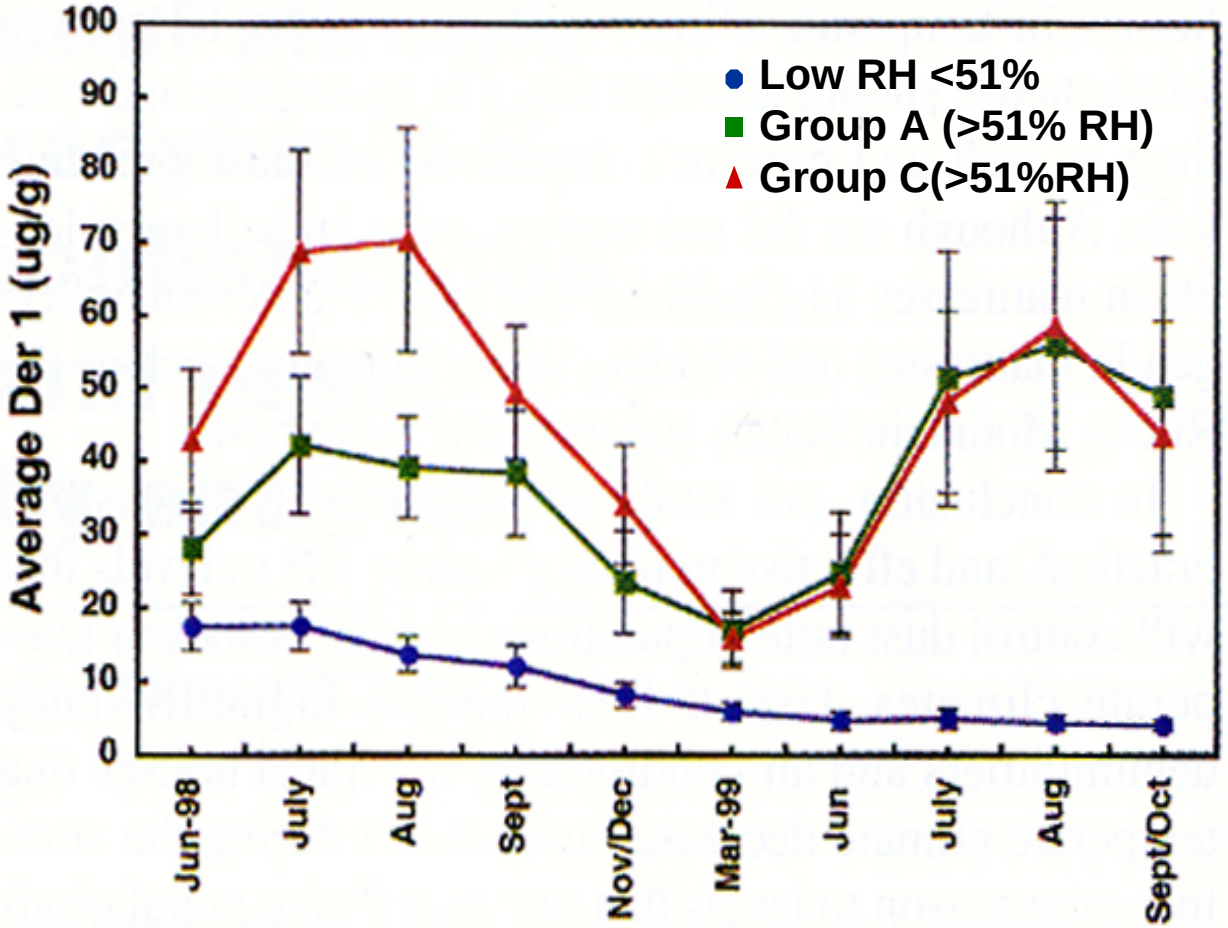
Effect of Relative Humidity on Mite Allergen Levels in Homes



Arlian et al., J Allergy Clin Immunol 2001; 107:99-104

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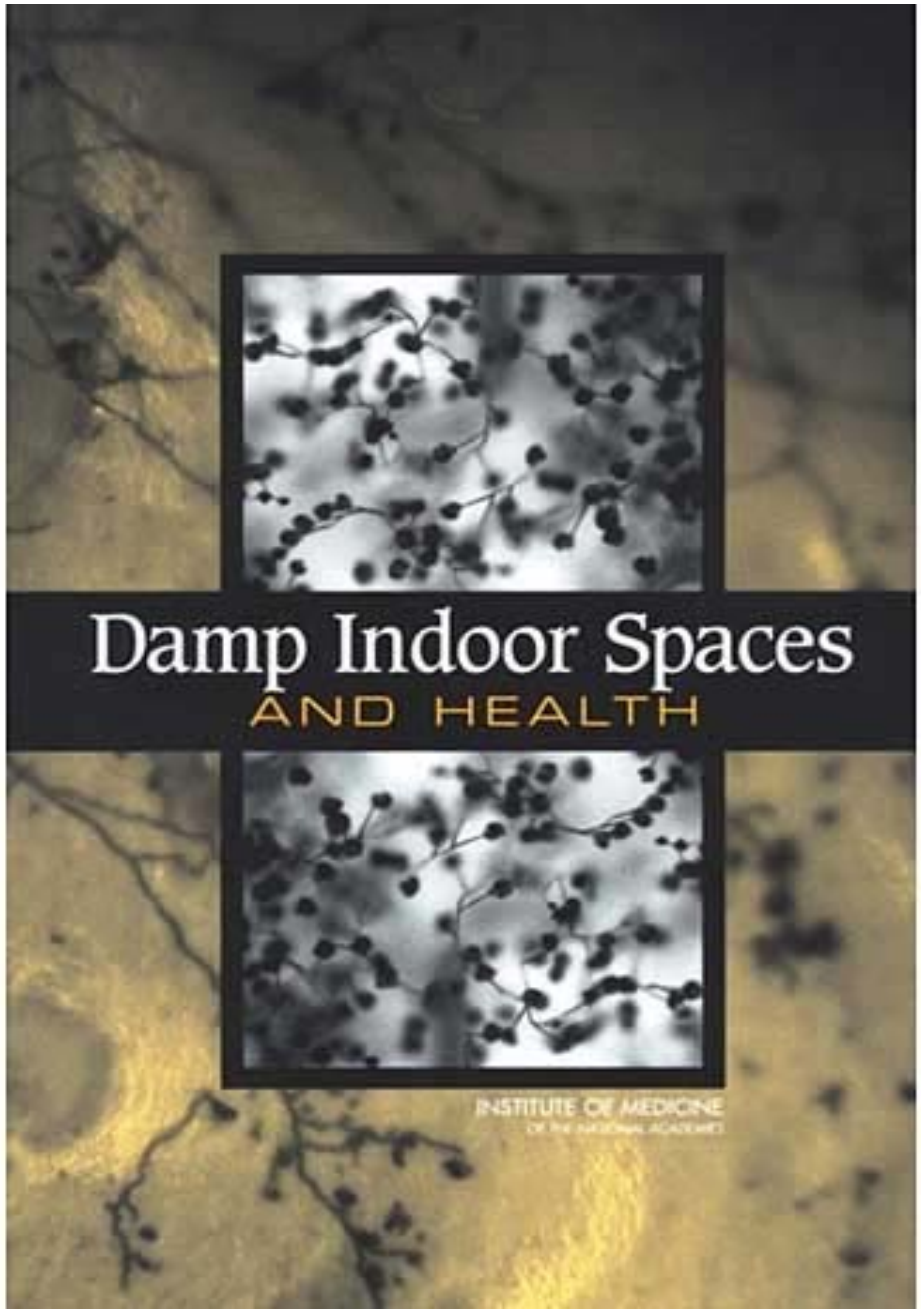
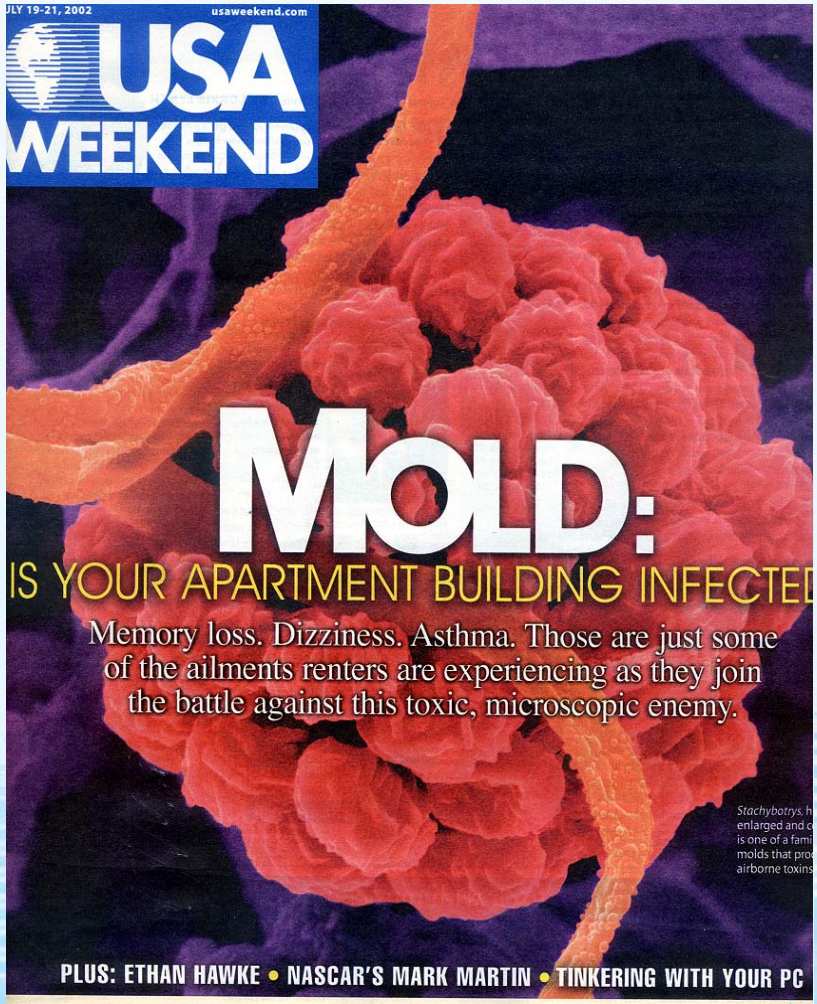

Damp Indoor Spaces
AND HEALTH
INSTITUTE OF MEDICINE

Challenges Associated with Mold:

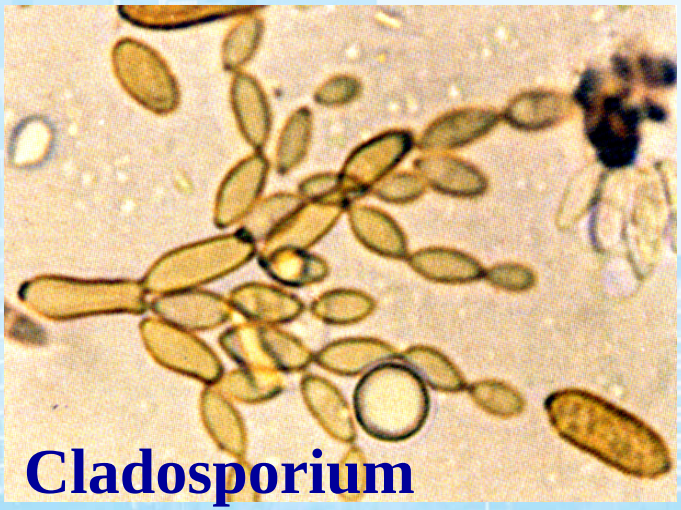
- assessments of sensitization and exposure
- diverse health effects
- establishing a relationship between mold exposure and disease



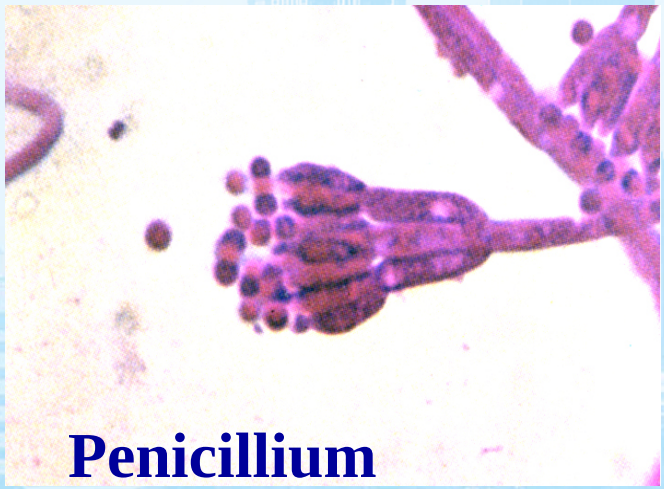
Molds Associated with Asthma

Alternaria

Aspergillus



Cladosporium



Penicillium

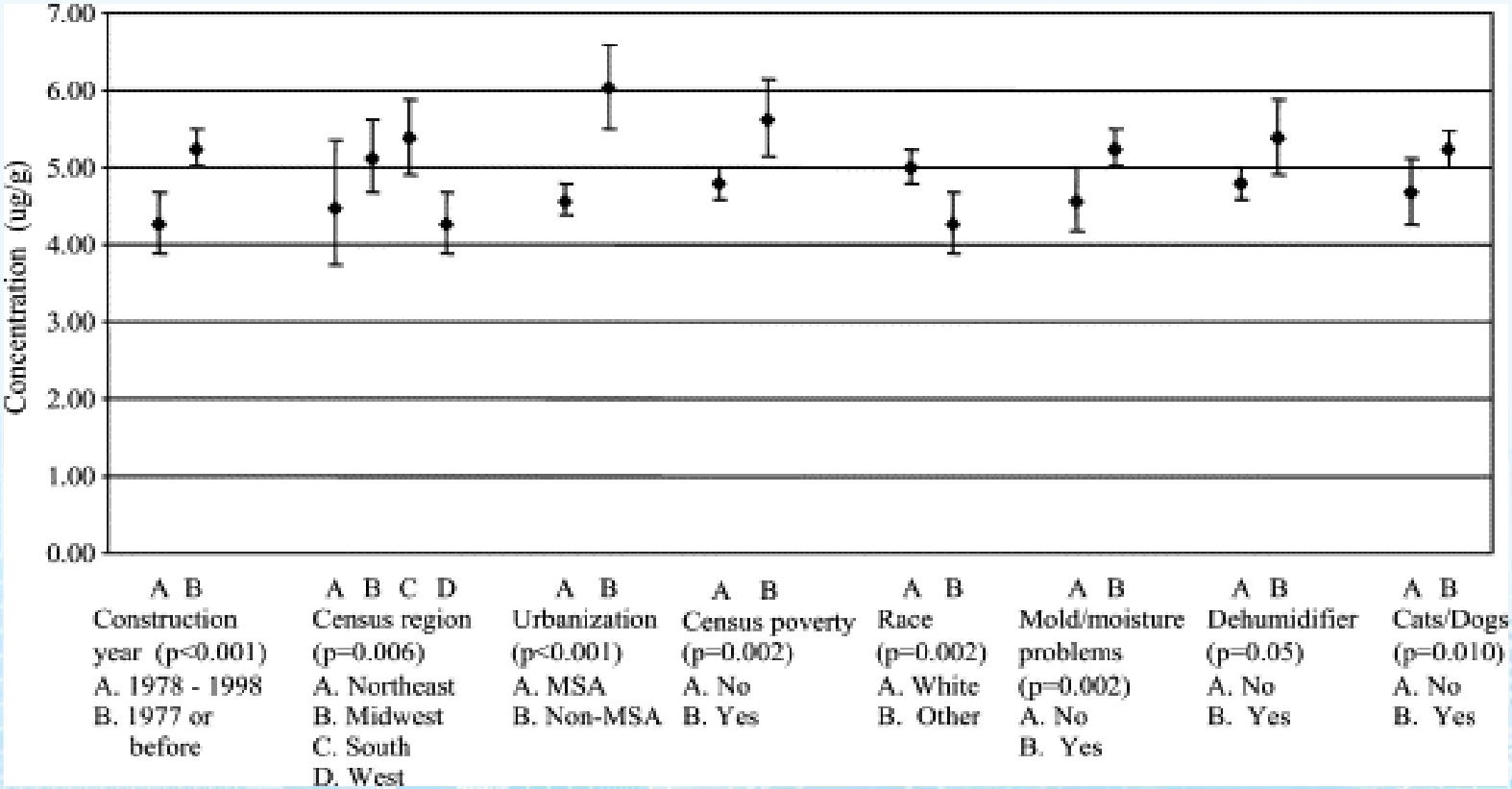
E. Grant Smith 1990

Airborne fungi in homes of children with asthma in U.S. Inner Cities

- Cultured indoor and outdoor fungi in
  - 7 urban communities
  - 414 homes of children with asthma
- Most prevalent species: *Alternaria, Aspergillus, Cladosporium, Penicillium*
- Risk factors for mold:
  - Dampness
  - Cockroach infestations
  - Cats

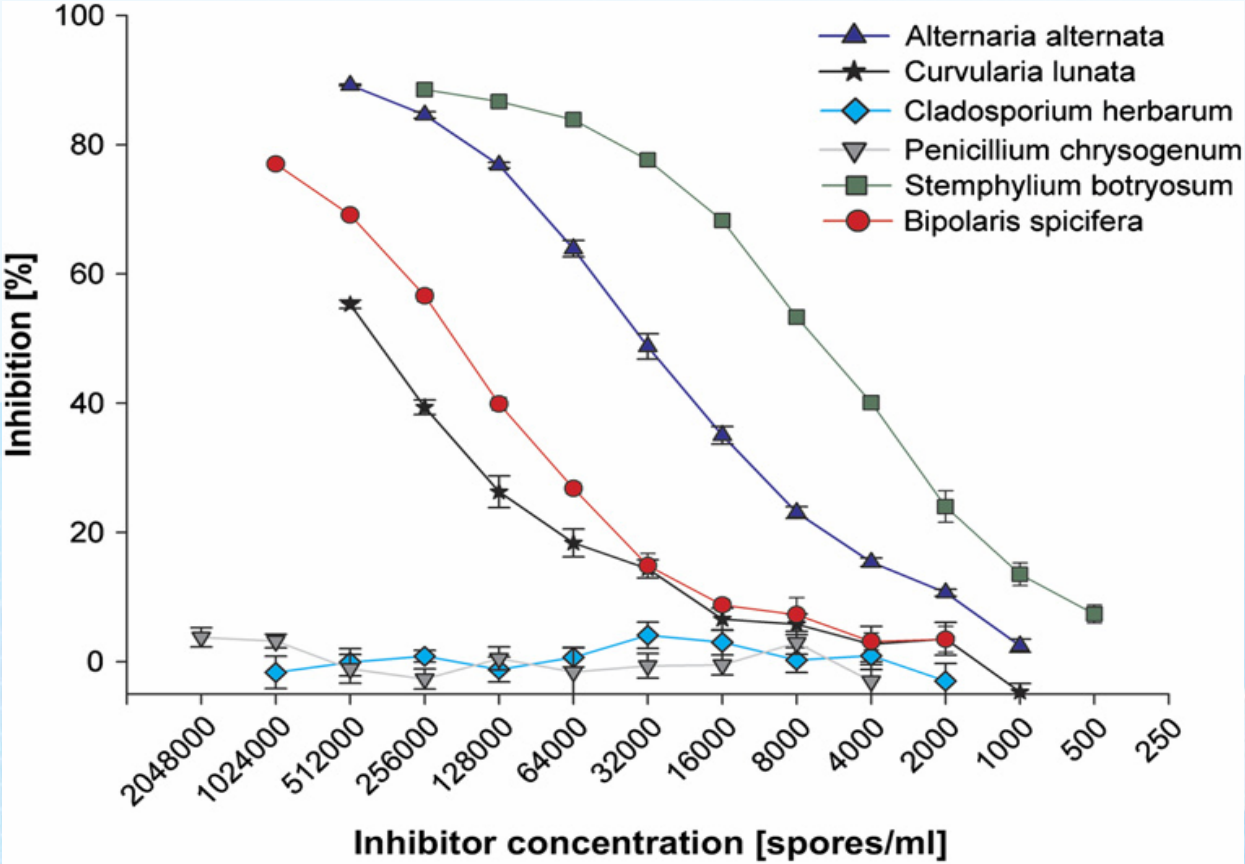
O'Connor et al, JACI 114:599, 2004

Alternaria antigens in dust from US homes: Data from the NSLAH



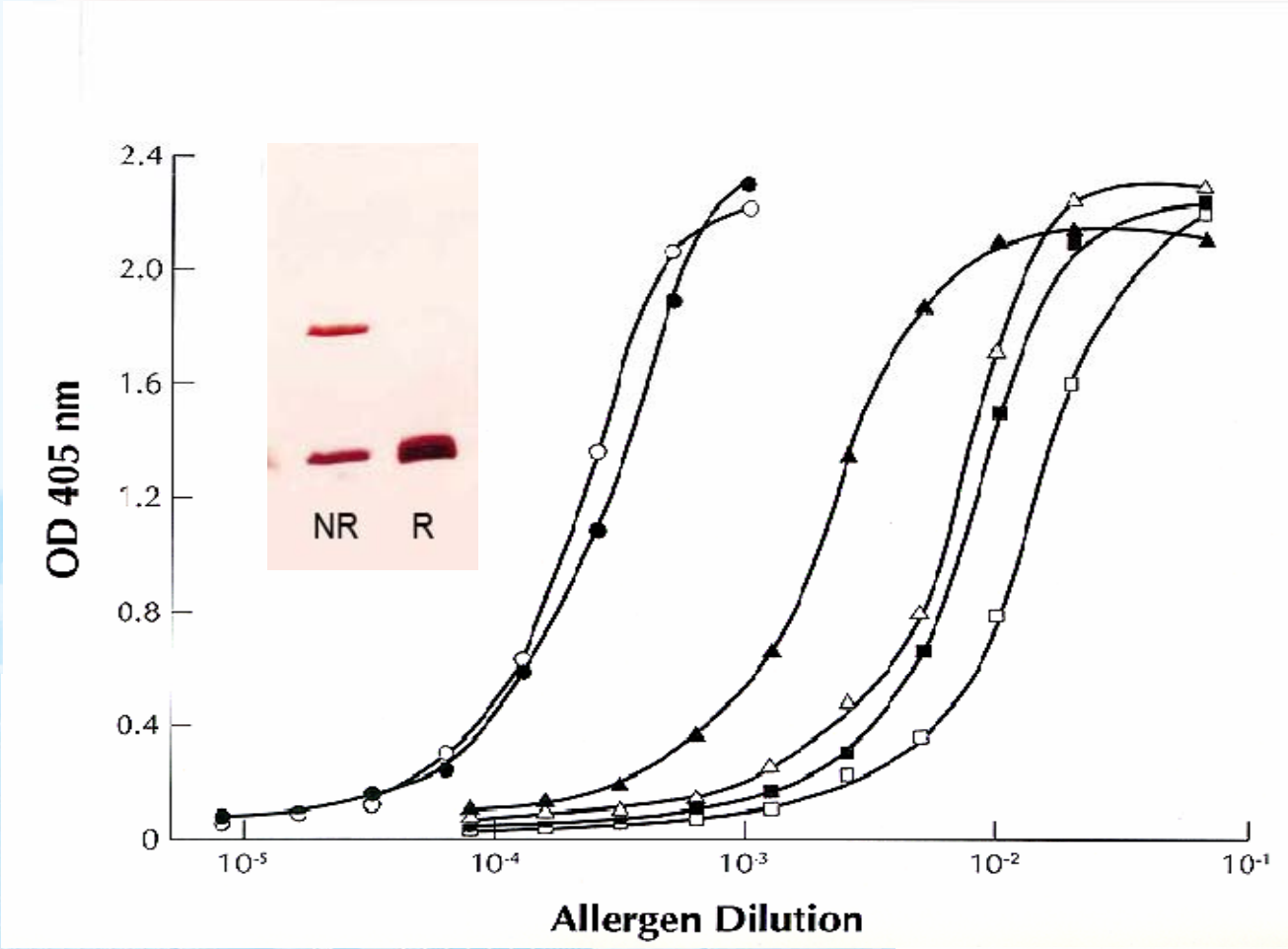
Salo et al., J Allergy Clin Immunol 2005;116:623-29.

Cross-reactivity of ELISA for *Alternaria alternata* using polyclonal antibodies



Schmechel et al., JACI 121: 763-8, 2008

Monoclonal ELISA for Alternaria Allergen, Alt a 1



Vailes et al, JACI 2001; 107:641-6

Health Effects of Indoor Molds

- **Stachybotrys - associated with idiopathic pulmonary hemorrhage**
  in infants (AIPH) in Cleveland ("toxic mold").

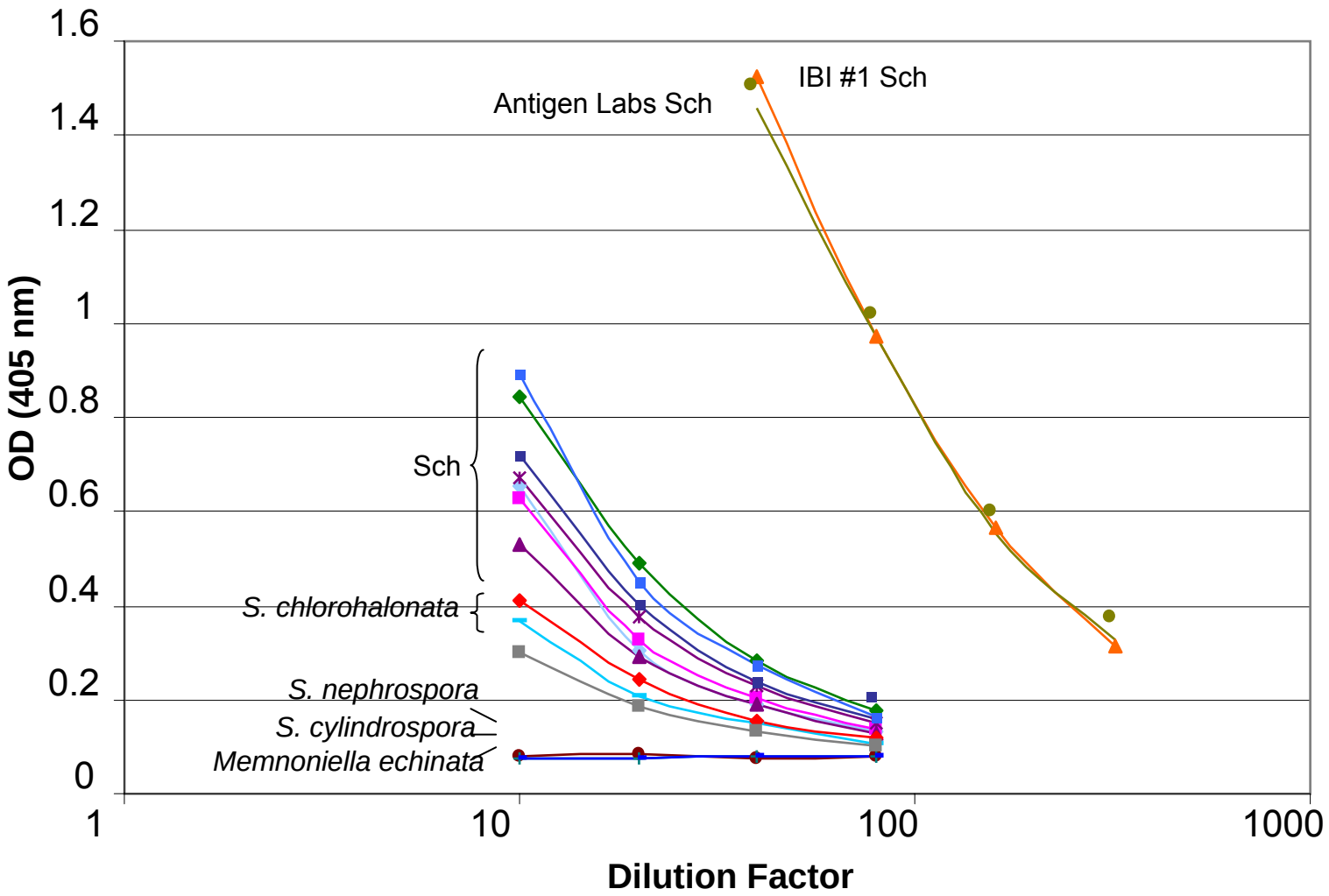
- **CDC associates Cleveland cases with von Willebrand disease (vWD)**
  – an inherited bleeding disorder, (MMWR 2004).

- **Position Statements: ACOEM 2002, AAAAI, 2006**

- **Guide for interpreting reports from inspections or investigations**
  of indoor mold. Horner et al, JACI 121: 592, 2008

- **"A new plague – mold litigation: How junk science and hysteria built an industry" Cliff Hutchinson and Robert Powell**
  US Chamber Institute for Legal Reform
  Center for Legal Policy at the Manhattan Institute, 2003

Specificity of ELISA for Sch34



Specificity of Monoclonal anti-*S. chartarum* Antibodies (SchX)

Mold Species

Monoclonal Antibody

	Stachybotrys chartarum	*Aspergillus versicolor*	*Aspergillus fumigatus*	*Penicillium chrysogenum*	*Penicillium expansum*	*Trichoderma harzianum*	*Chaetomium globosum*
3F10	**1.79**	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
3E11	**1.77**	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
2C9	**2.20**	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
10F2	**1.90**	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
9F6	**1.73**	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15

Values are OD (405 nm) in ELISA (assay background <0.09)

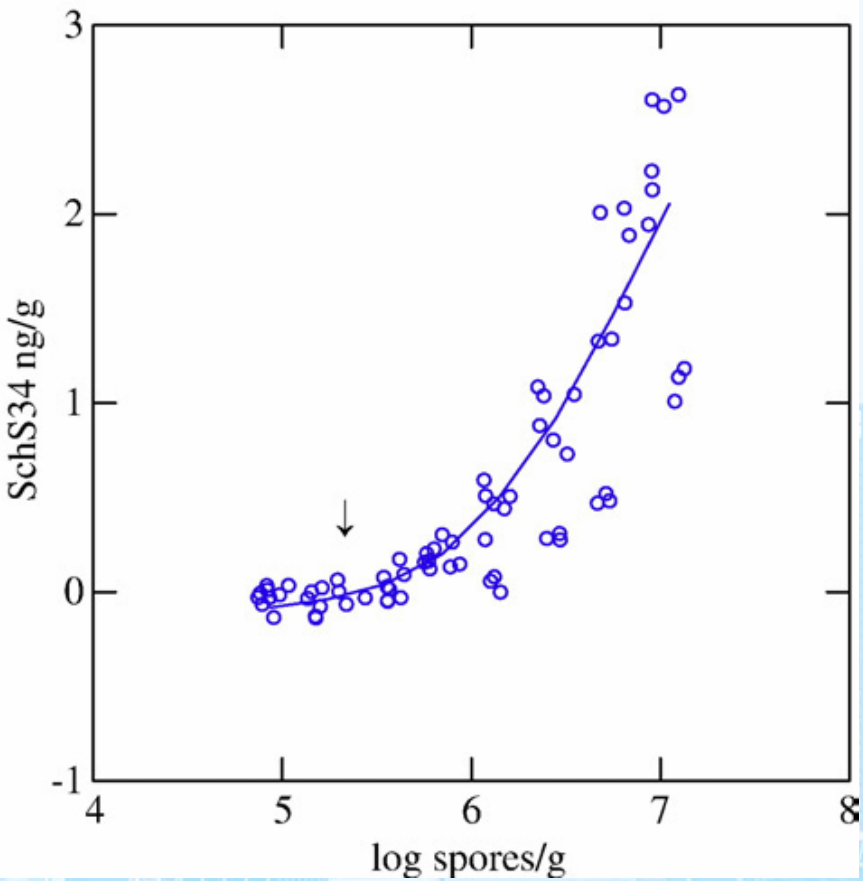
ELISA Assays for *S. chartarum* antigens

Source	Sch34 Conc. (ng/mL)	SchX Conc. (U/mL)
S.chartarum, EMPAT Challenge	73.1	3.0
S.chartarum, 32-47-19	6.2	85.4
S.chartarum, 32-43-05	6.3	36.1
S.chartarum, 32-43-03	5.3	64.1
S.chartarum, 29-63-12	9.7	70.9
S.chartarum, 29-58-26	7.2	11.5
S.chartarum, 44	0.4	3.8
S.chartarum, Antigen Labs	58.1	<1.0
S.chartarum, Ceiling tile	0.3	<1.0

Sch34: Xu et al, J Immunol Methods 332:121-8, 2008;

SchX: Smith et al, JACI 123: S172, 2009

Correlation between Sch34 antigen and spore levels in house dust samples



Xu et al., JIM 332: 121-128; 2008

Medical Effects of Mold Exposure

- Molds cause adverse health effects through
  - Allergic hypersensitivity responses
  - Infections
  - Toxicity (ingested mycotoxins)
- Little evidence for health effects of
  - Inhaled mycotoxins
  - Mycotoxin mediated immune dysfunction
- IgE ab measurements to mold allergens are for recommended diagnosis
- *Limited role for environmental assessments*

Position Statement: American Academy of Allergy, Asthma and Immunology, 2006

Key Points: Control of Environmental Factors that Affect Asthma

- **Exposure of patients who have asthma to allergens increases asthma symptoms and precipitates asthma exacerbations.**
- **Patients who have asthma should:**
  - **Reduce exposure to allergens to which they are sensitized and exposed.**
  - **Know that effective allergen avoidance requires a multifaceted, comprehensive approach**
  - **Consider allergen immunotherapy treatment**

Guidelines for the Diagnosis and Management of Asthma (EPR-3): US National Heart, Lung and Blood Institute: 2007

Supported by the following grants:

SBIR ES011920 & ES55545:
From the U.S. National Institute for Environmental Health Sciences

European Union **CREATE** Consortium
(G6RD-CT-2001-00582)

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Indoor Allergens:

Exposure Assessment and Intervention

Eva M. King, PhD
Senior Scientist
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Indoor Air Quality ASSOCIATION INC.

Excerpted from:
US National Heart, Lung and Blood Institute:
Guidelines for the Diagnosis and Management of Asthma (EPR-3), 2007

Assessment of Allergen Exposure in Home, Workplace, Schools, Public Buildings

- Collect "reservoir" dust samples from bedding, carpets, furnishings
- Collect air samples for animal allergens
- Send to lab for analysis
  (provides most accurate exposure measurement)
- Test on site using rapid "dipstick" tests
  - "yes/no" answer

**** 3rd Int'l Report on Indoor Allergens and Asthma JACI 1997; AAAAI Position Statement Feb, 1999; NHLBI Expert Panel Report 1997; JACI Suppl Mar, 2001;***

Standardized Dust Collection Device

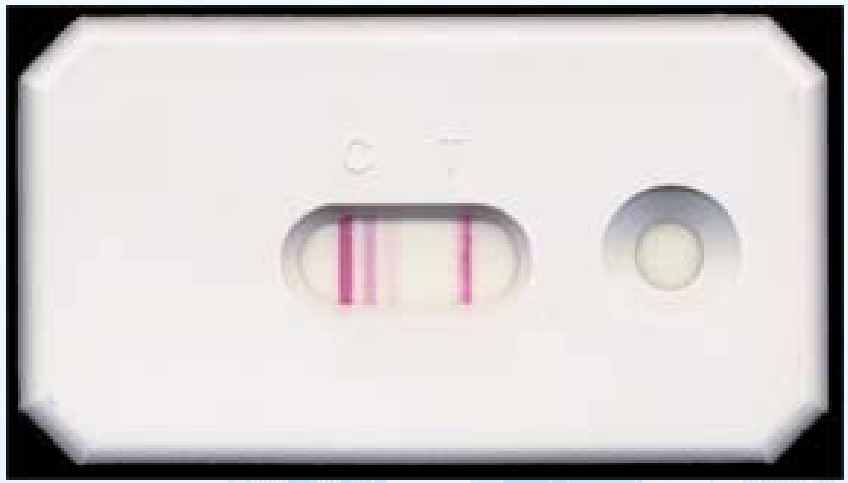
- Dust samples collected over defined area (0.25m^2) in 2 mins
- Disposable 40μm filters, easily collect indoor allergens, molds and endotoxin
- High correlation between subject- and technician-collected samples.
- In progress for ***IESO Standard***

DUSTREAM ™ dust collector

Tsay et al, Clin Exp Allergy 2002; Sercombe et al, Allergy 2005; Arbes et al Env Hlth Perspect, 2005

Improving health care delivery using a consumer test for mite allergen*

DUSTREAM ™ collector



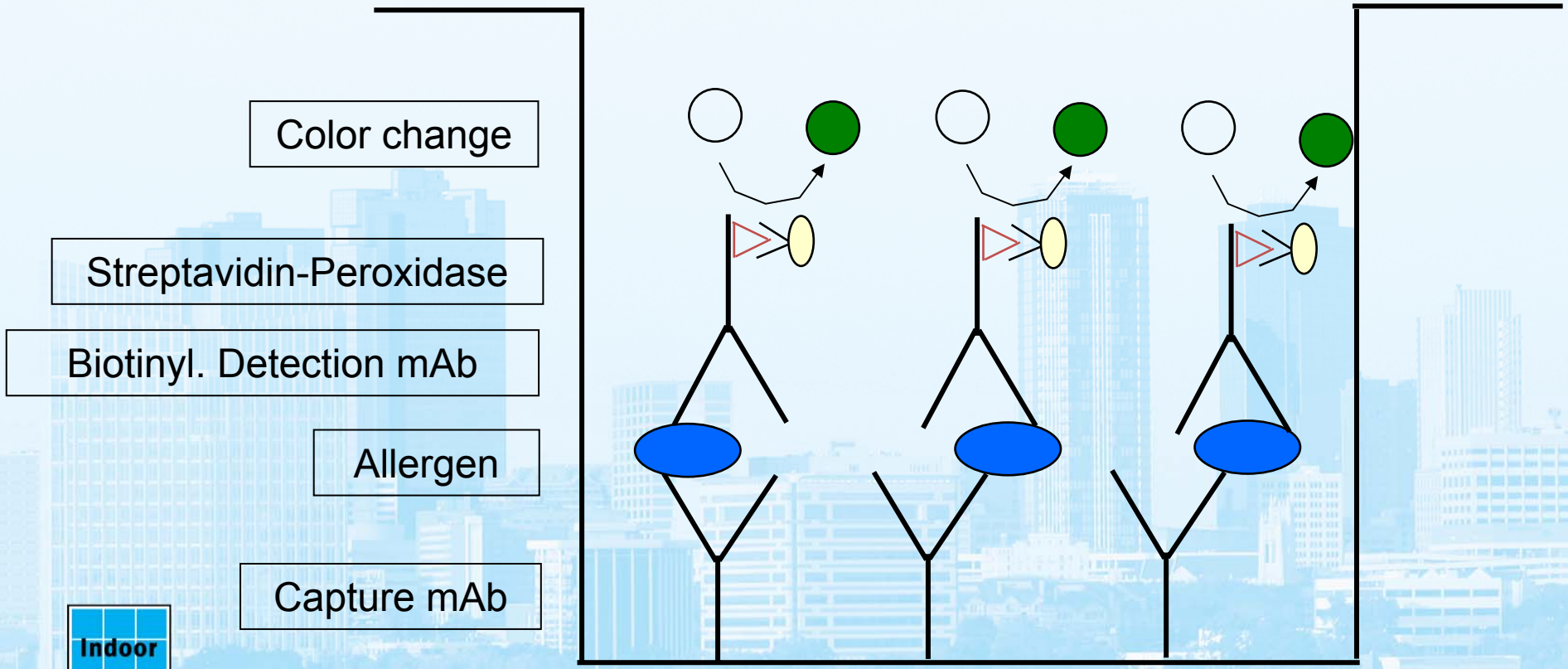
*** Detects mite Group 2 in 10 minutes**

Tsay et al, Clin Exp Allergy 2002; 32:1596-1601

Quantitative Exposure Assessment

ELISA

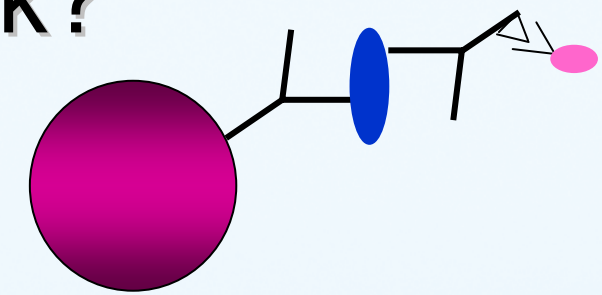
- Enzyme-Linked ImmunoSorbent Assay



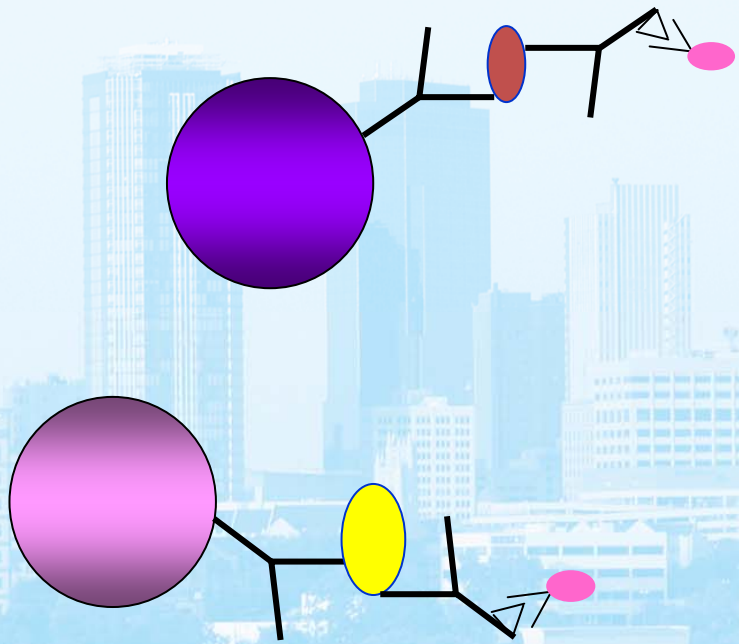
Multiplex ARray for Indoor Allergens (MARIA)

- Multiple allergens = One single test
- Quantitative data, improved standardization and efficiency
- Versatility – new assays can be added and custom combinations developed
- Suitable for allergens and molds

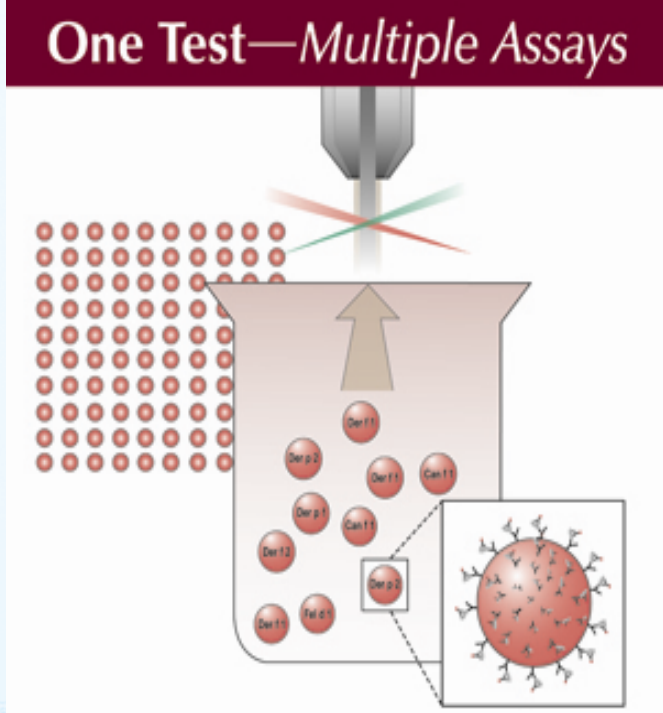
How does MARIA work?

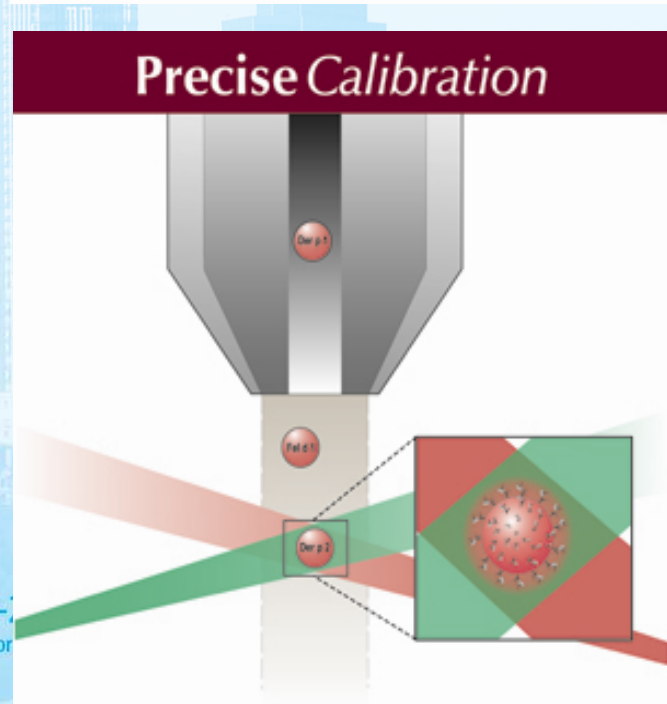


- 5.6μm polystyrene beads with internal fluorescent dyes (up to 100 colors)
- Beads coupled to capture mAb, incubate with allergen samples
- Detect allergen using biotinylated mAb and streptavidin-conjugated fluorophore



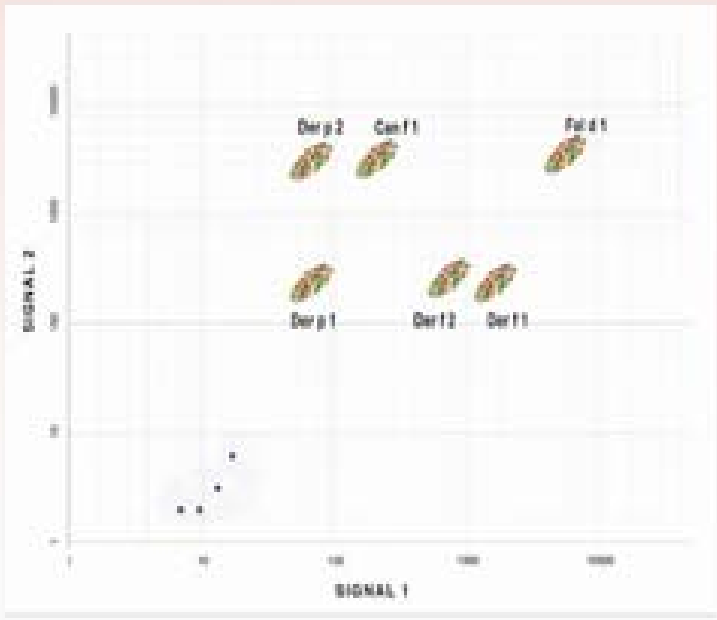
- Beads are drawn into the optical path of flow cytometer
- Red laser identifies internal bead color, i.e. beads bearing a specific allergen
- Green laser measures external signal intensity: quantifies the amount of allergen bound



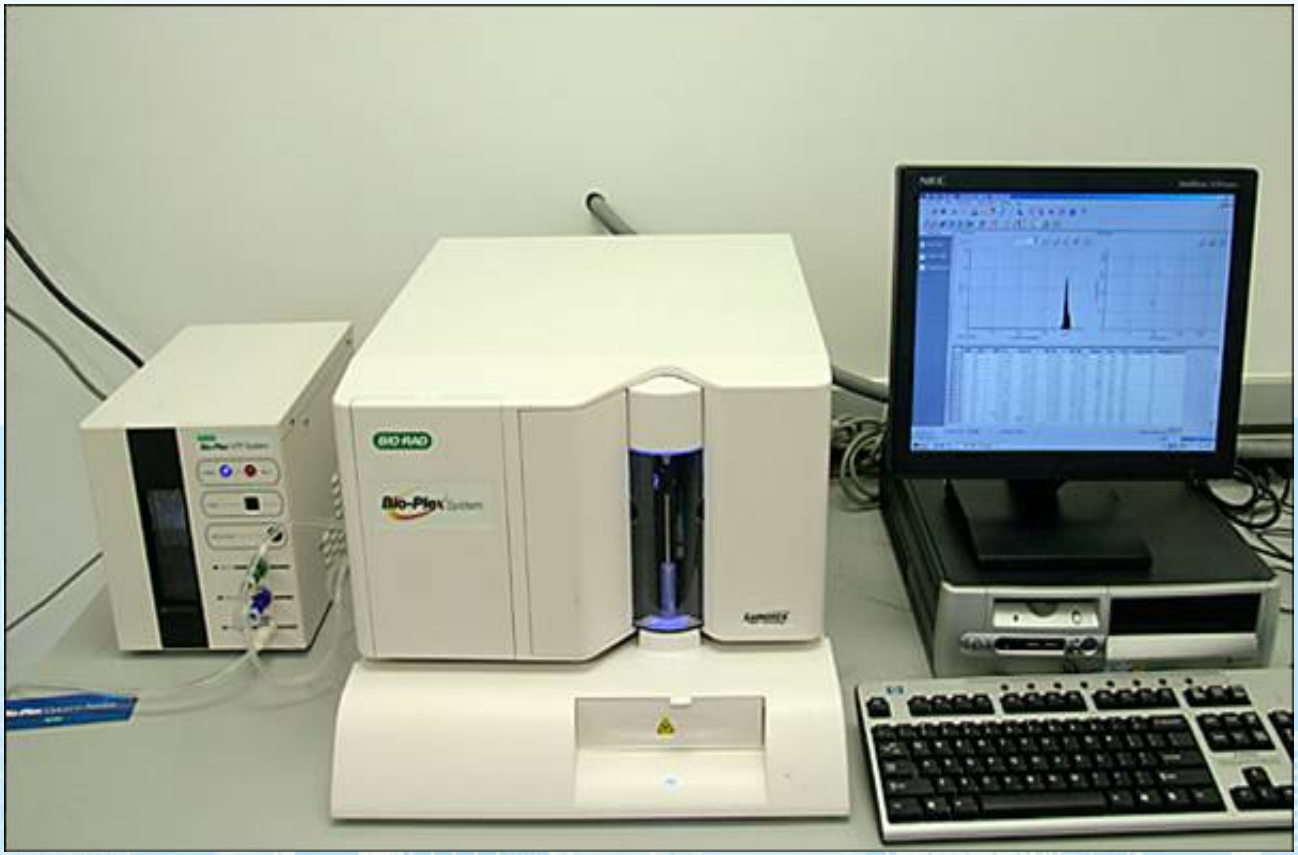


- 100 beads of each type are counted and mapped according to the analyte.
- The fluorescent signal is quantified to determine the allergen concentration

Quantitative Data



xMAP® Instrument (Bio-Plex)



Universal Allergen Standard

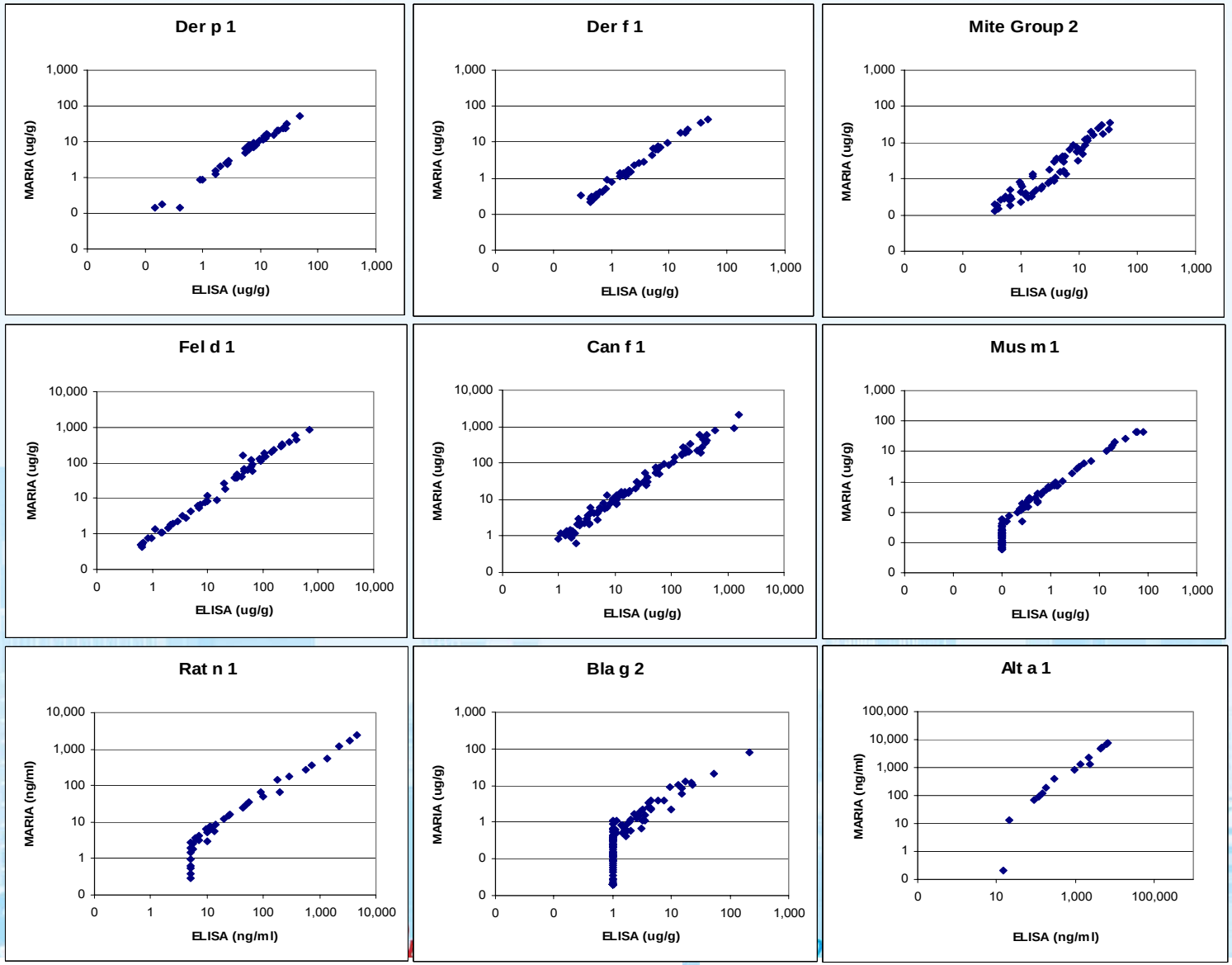
- Current ELISA standards are extracts

New Universal Allergen Standard:

- Modeled on EU CREATE Project
- Contains 8 purified natural allergens
- Protein concentrations determined by amino-acid analysis
- Can be used in ELISA and MARIA

Universal Allergen Standard	Concentration (ng/ml)
Der p 1	2500
Der f 1	2500
Der p 2	1000
Fel d 1	1000
Can f 1	2500
Mus m 1	250
Rat n 1	1000
Bla g 2	2500

MARIA versus ELISA



Earle *et al*, JACI 2007; 119:428-33

$R^2 = 0.90 - 0.99$, $p<0.001$

Assay Performance:

Lower Limit of Detection (LLOD)

Allergen	ELISA (ng/ml)	MARIA (ng/ml)
Der p 1	2	0.06
Der f 1	2	0.06
Mite Group 2	0.8	0.02
Fel d 1	0.8	0.02
Can f 1	2	0.06
Mus m 1	0.2	0.01
Rat n 1	0.8	0.02
Bla g 2	2	0.98
Alt a 1	0.8	0.02

Indoor

MARIA is up to 40-fold more sensitive than ELISA

Assay Performance

Example: 200 dust extracts for 8 allergens

	ELISA	MARIA
Samples per plate	18	23
Total number of plates	12 plates x 8 allergens = 96 plates	9 plates
Technician time	**16 days**	**3 days**

In ELISA, each additional allergen adds 2 work days.

In MARIA, all allergens are measured simultaneously.

Multi-Center Ring Trial of MARIA

NIH-funded third-party validation:

Study of Inter-/Intra-laboratory variability

- 151 samples
- tested for 8 allergens

  Der p 1, Der f 1, Mite Group 2, Fel d 1,
  Can f 1, Mus m 1, Rat n 1, Bla g 2
- by 10 laboratories
- on 3 separate occasions

  = *more than 36,000 data points*

Participating Centers

US

• Dr. Darryl Zeldin	NIEHS, Research Triangle Park, NC
• Dr. Detlef Schmechel	NIOSH, Morgantown, WV
• Dr. Thomas Platts-Mills	UVA, Charlottesville, VA
• Dr. Robert Hamilton	DACI Laboratory, JHU, Baltimore MD
• Dr. Donald Milton	University of Massachusetts, Lowell
• Dr. Peter Thorne	Dept Occ & Environ. Health, University of Iowa
• Dr. Eva M. King	IBI, Charlottesville VA (coordinating laboratory)

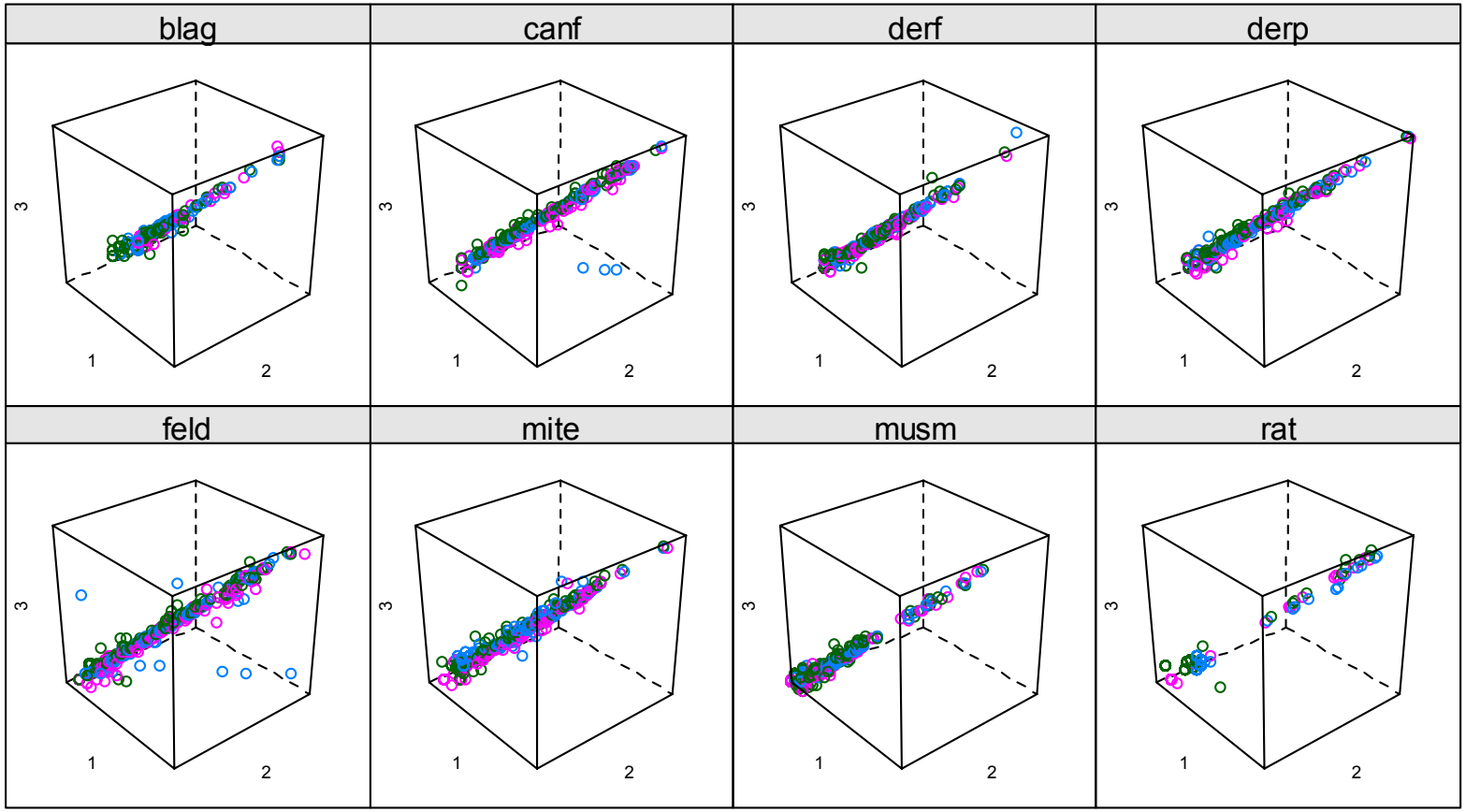
Europe

• Dr. Ronald van Ree	Academic Medical Center, Amsterdam
• Dr. B. Brunekreef / E. Krop	IRAS Div Occ & Env Health, Utrecht
• Dr. F. Ferreira / Dr. A. Hartl	Dept Molecular Biology, Univ. Salzburg/

Data Analysis:

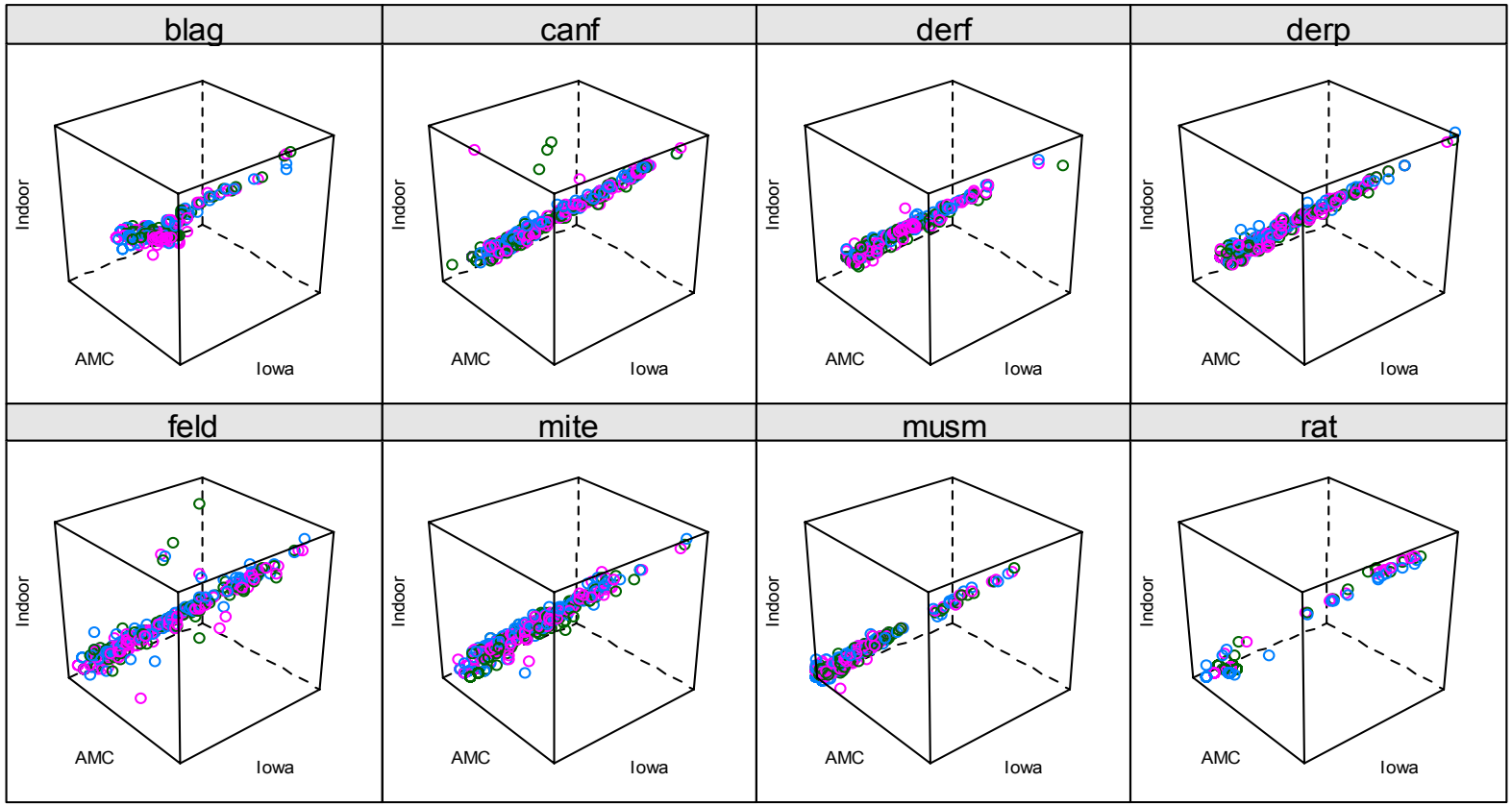
Dr. S. Arbes/Dr. A. Calatroni/Dr. H. Mitchell — Rho Inc., Chapel Hill, NC

Intra-Laboratory Variability



A. Calatroni, S. Arbes, H. Mitchell: Rho Inc.

Inter-Laboratory Variability



A. Calatroni, S. Arbes, H. Mitchell

Mean Correlation Coefficients

- Intra-lab variability (r):
  - Indoor: 0.98 **
  - Iowa: 0.97 **
  - AMC: 0.96 **
- Inter-lab variability (r):
  - AMC/Indoor: 0.95 **
  - AMC/Iowa: 0.96 **
  - Indoor/Iowa: 0.95 **

** = p<0.001

MCT Results Summary

- MARIA produces results that are reproducible within and between laboratories (r = >0.95)

- MARIA will improve standardization of allergen exposure assessment

External Quality Control

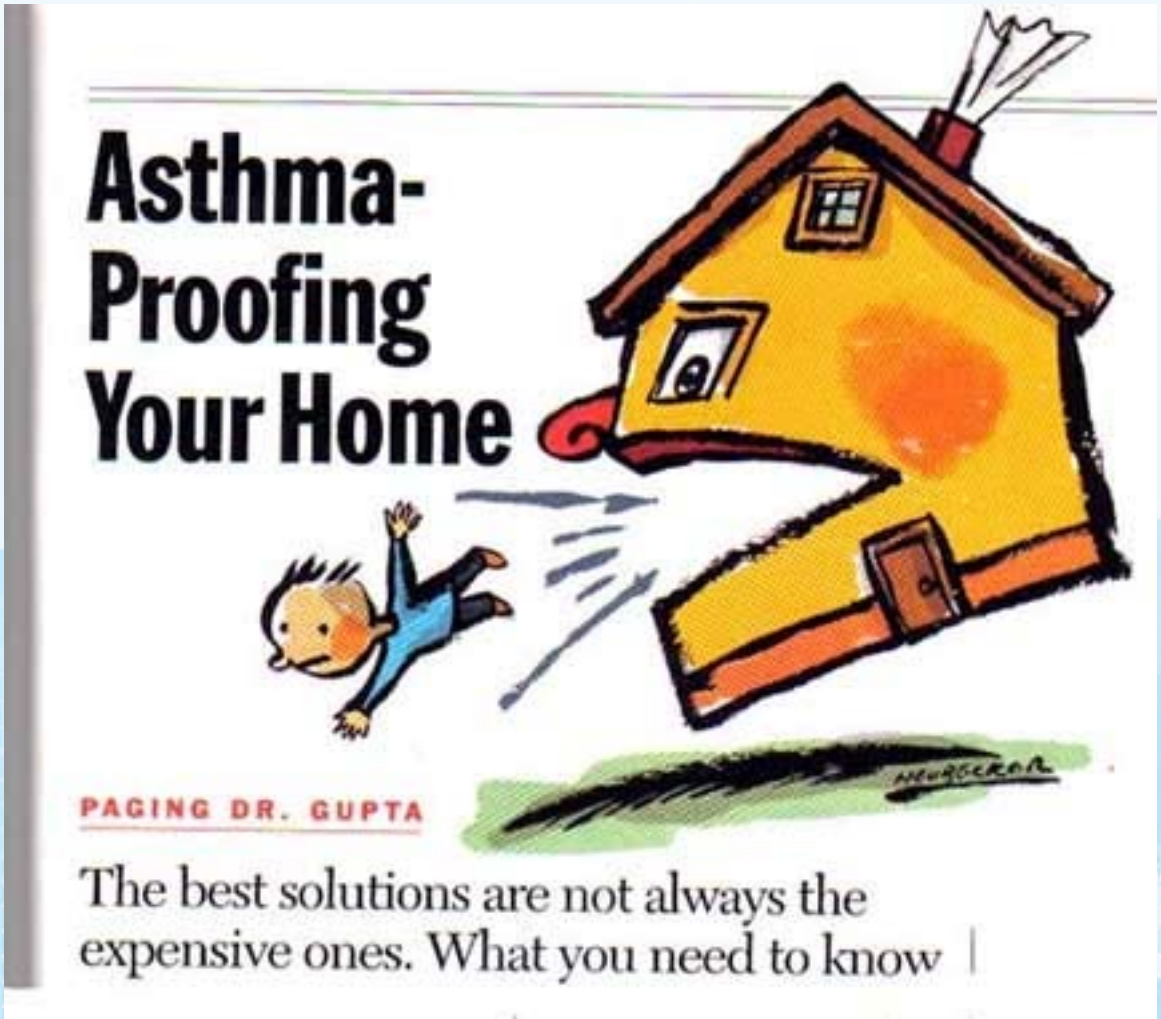
	Inbio Results		Spike Conc.		% Recovery	
Sample Type	Mus m 1 (ng/ml)	Rat n 1 (ng/ml)	Mus m 1 (ng/ml)	Rat n 1 (ng/ml)	Mus m 1 (ng/ml)	Rat n 1 (ng/ml)
QC Spike	4.38	19.26	5.06	22.00	86.6	87.5
QC Spike	4.31	18.17	5.06	22.00	85.2	82.6
QC Spike	3.48	14.75	5.06	22.00	68.8	67.0
QC Spike	0.05	0.20	0.05	0.22	100.0	90.9
QC Spike	0.04	0.19	0.05	0.22	75.5	86.4
QC Spike	4.40	21.92	5.06	22.00	87.0	99.6
QC Spike	4.70	20.43	5.06	22.00	92.9	92.9
QC Spike	4.24	19.53	5.06	22.00	83.8	88.8
QC Spike	1.09	4.81	1.01	4.40	107.9	109.3
QC Spike	0.83	3.72	1.01	4.40	82.2	84.5
QC Spike	1.01	4.93	1.01	4.40	100.0	112.0
QC Spike	0.05	0.21	0.05	0.22	100.0	95.5
QC Spike	0.05	0.24	0.05	0.22	100.0	109.1
Low Level Std	0.05	0.22	0.05	0.22	100.0	100.0
QC Blank	<0.01	<0.02				
QC Blank	<0.01	<0.02	*Mean Spike Recovery*		*90.7*	*93.3*

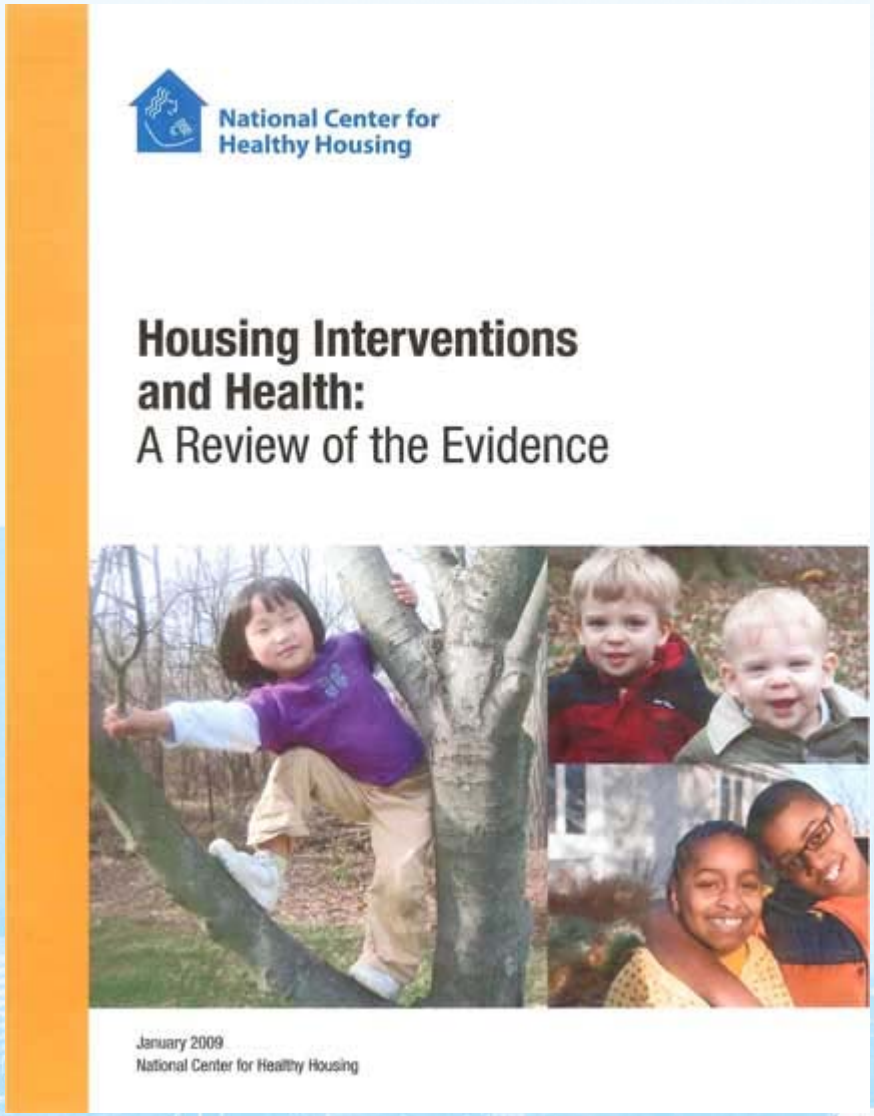
Allergen exposure assessment and indoor air quality

- Allergen exposure should be considered as part of IAQ investigations of allergic patients
- ELISA or MARIA™ technology provide quantitative data on exposure levels
- Rapid tests can be used for on-site screening by IAQ personnel
- MARIA™ technology is ideally suited for:
  - Research use (epidemiology, birth cohorts)
  - Monitoring allergen avoidance studies
  - Routine commercial analyses
  - Comprehensive indoor air quality assessments

Allergen Avoidance

Time Magazine, August 2004





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Effective Interventions

Multi-faceted in-home interventions, tailored to the individual, are effective in controlling asthma symptoms and morbidity:

- Home environmental assessment
- Education
- Mattress and pillow covers
- HEPA vacuums and HEPA filters
- No smoking
- Cockroach and rodent management
- Eliminate moisture intrusion
- Intensive household cleaning

Promising interventions

Further field evaluation needed:

- Moisture control through dehumidification
- Improved general/local exhaust ventilation
- Use of air cleaning devices
- Repeated dry-steam cleaning
- Repeated vacuuming

Basic research required:

- Carpet treatments/acaricides
- One-time professional cleaning

Ineffective Interventions

Each intervention in isolation from other interventions is not effective!

"Air cleaners" that produce high levels of ozone should not be used due to ozone exposure problems.

Proper management of allergic disease should include:

- Assessment of allergen exposure in the home
- Clinical assessment of allergic disease
- Targeted environmental control procedures

Exposure assessment:

- Collection of reservoir dust or air samples in the home
- Measurement of major allergens (**ELISA or MARIA)**

Conclusions

- Exposure to indoor allergens is an important risk factor for allergic disease
- Allergen avoidance is effective for improving health
- Environmental intervention requires a multi-faceted approach

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