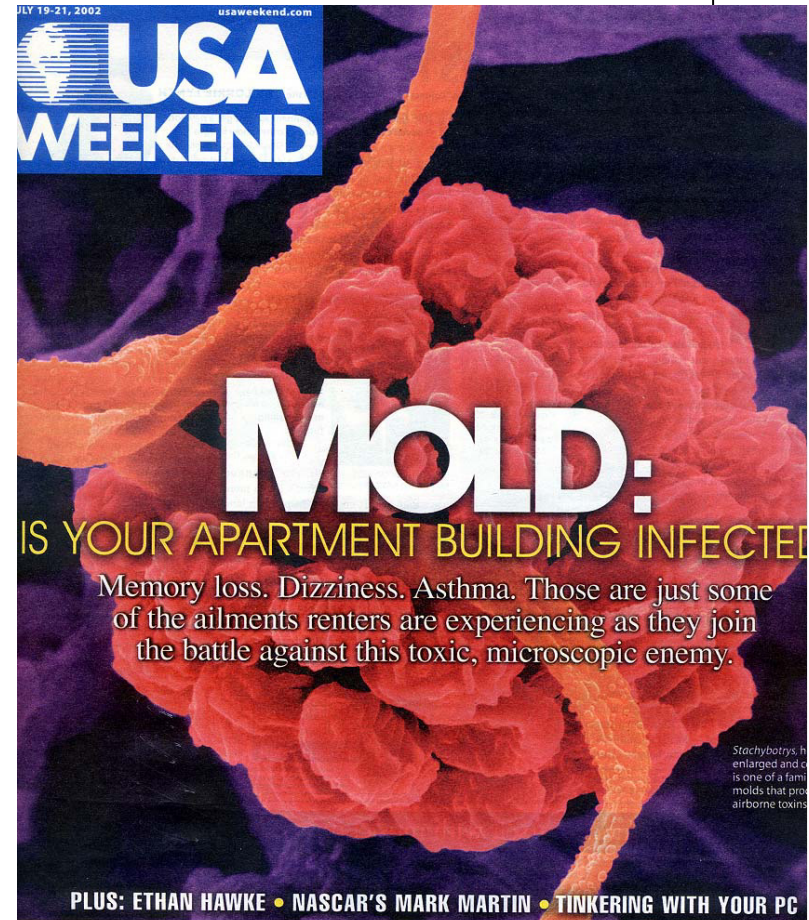


# Challenges associated with mold:

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



# ***Fungi associated with respiratory disease***



## Disease

## Fungal allergens

Allergic rhinitis/  
Asthma:

Alternaria, Aspergillus,  
Cladosporium, Penicillium,  
Trichophyton

Allergic fungal  
sinusitis:

Bipolaris, Curvularia

ABPA/Cystic fibrosis: Aspergillus sp.

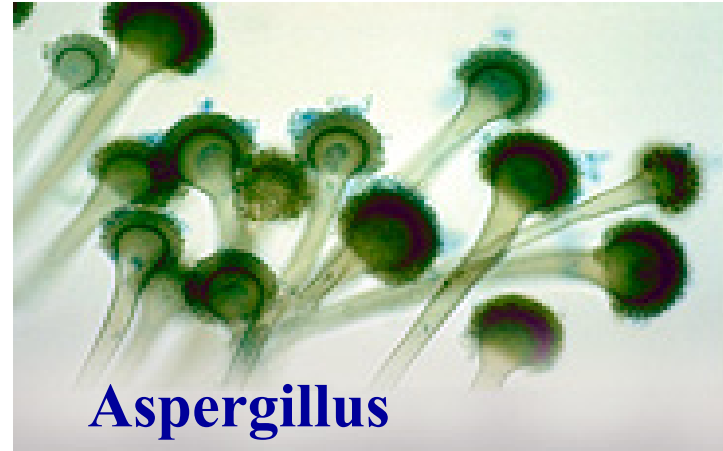
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***Horner et al, Allergy 53:1114, 1998; Bush & Portnoy JACI 107:2430, 2001.***

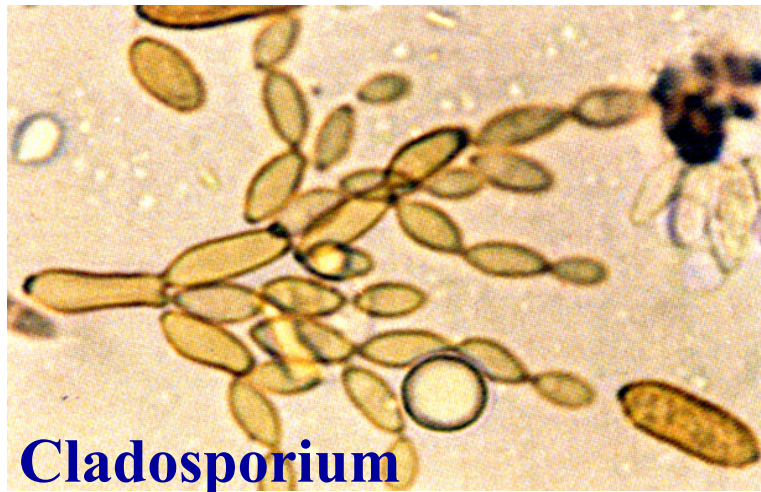
# *Fungi associated with asthma*



**Alternaria**



**Aspergillus**



**Cladosporium**



**Penicillium**

# *Alternaria alternata*



- A risk factor for
  - childhood asthma
  - severe asthma attacks
  - (Odds ratios 5-6.8)
- Occurs in arid climates
  - Australian desert
  - US Midwest & Arizona
- Major Allergens: Alt a 1, Alt a 2



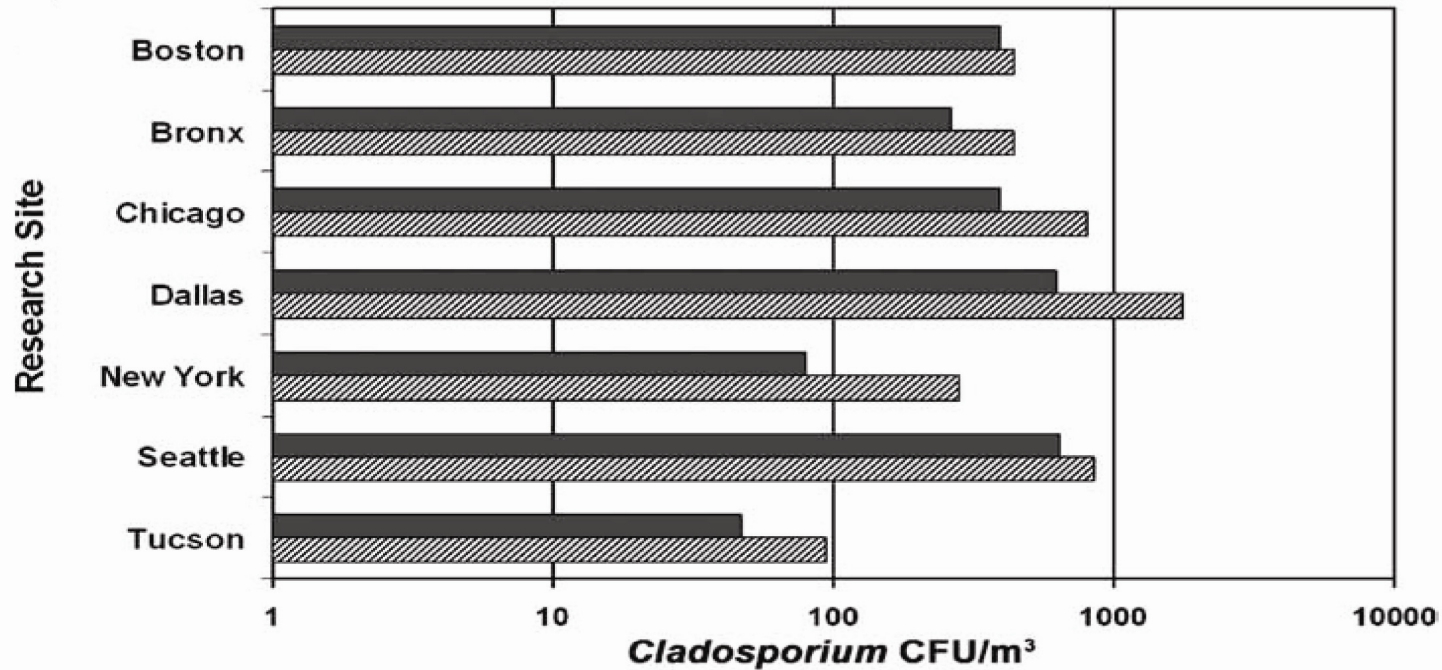
*A. alternata* spores 2x50  $\mu$ m



# 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

# Indoor and outdoor airborne *Cladosporium* levels in US cities



# ***Biologic functions of cloned fungal allergens***



## Allergens\*

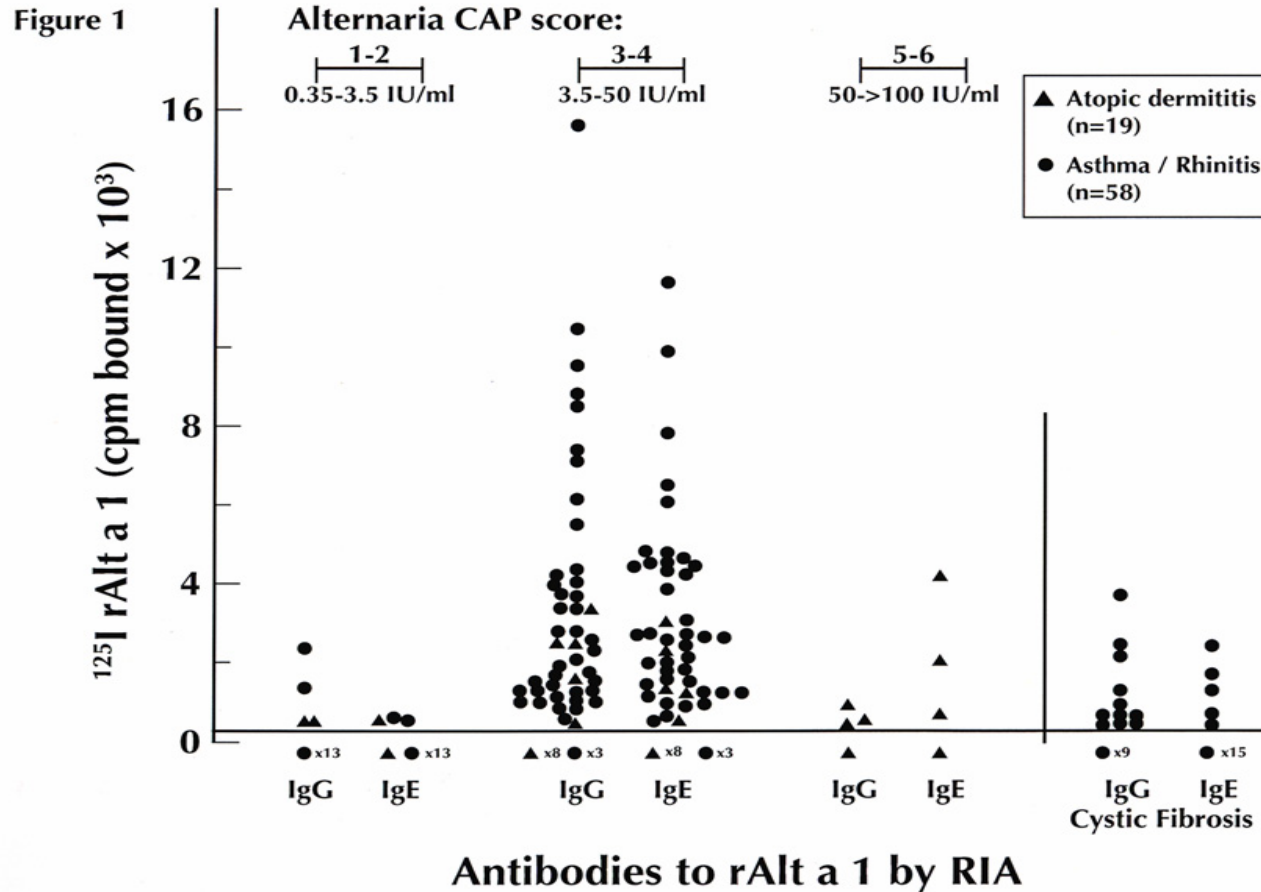
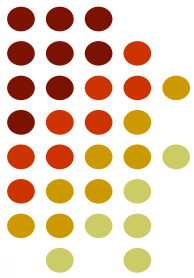
- ✓ Enzymes:
  - Serine Proteases      Asp f 13, Pen ch 13, Tri t 4
  - Ribosomal Proteases      Cla h 4, Alt a 6, Asp f 8
  - Enolase      Alt a 11, Cla h 6, Asp f 8
  
- ✓ Heat shock proteins      Alt a 3, Asp f 12
  
- ✓ Cytotoxin      Asp f 1
  
- ✓ Unknown      Alt a 1, Cla h 1, Tri t 1

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WHO/IUIS Allergen Nomenclature Committee; [www.allergen.org](http://www.allergen.org)

\*Principally from *Aspergillus*, *Alternaria*, *Cladosporium* and *Trichophyton* Species

# IgE and IgG ab to rAlt a 1 in patients with asthma or atopic dermatitis





# Assessment of exposure to fungal allergens



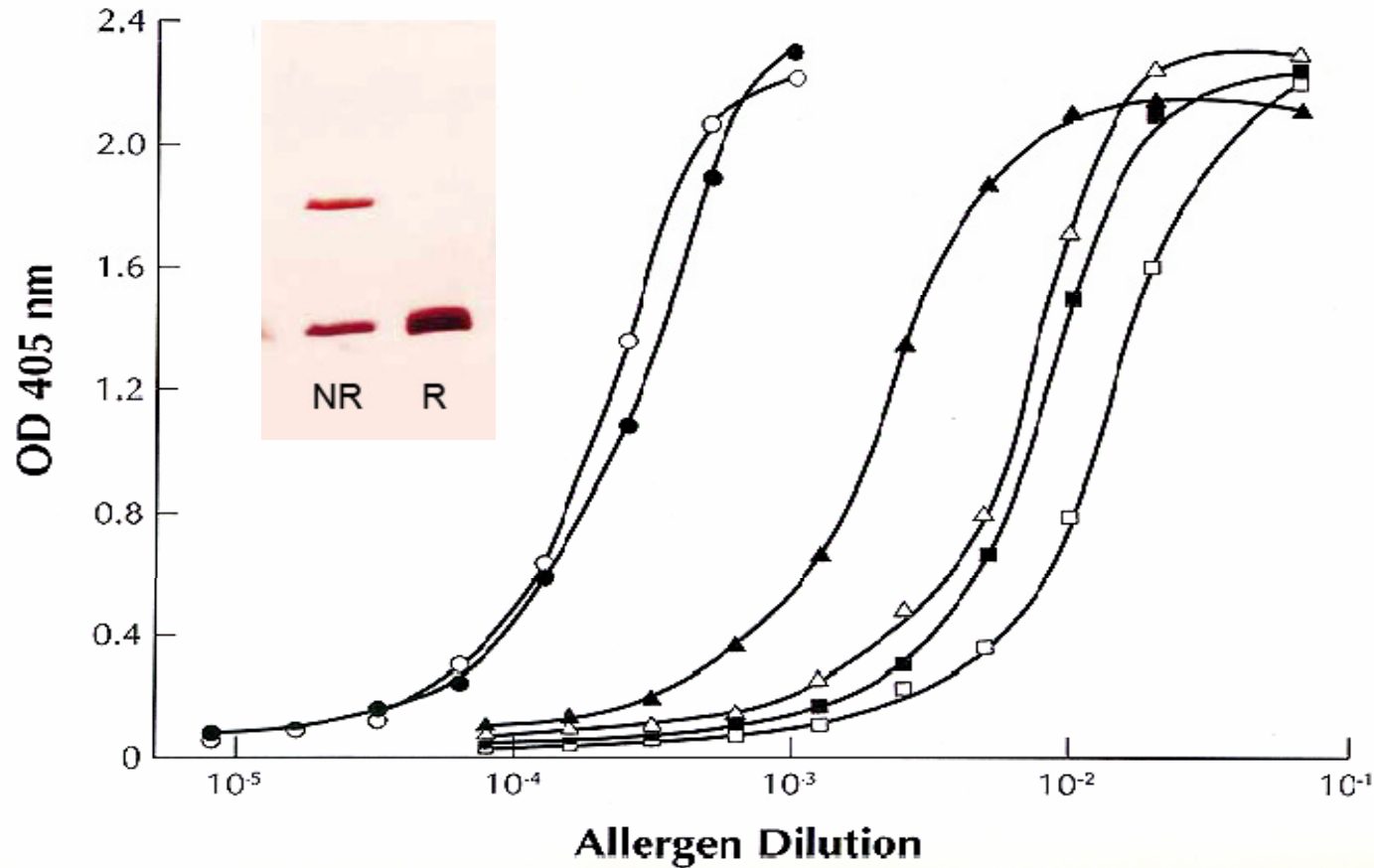
- Spore counts and cultures
- Polyclonal ELISA “total antigen load”
- Monoclonal ELISA for specific allergens
- $\beta$ -glucans; ergosterol; extracellular polysaccharides

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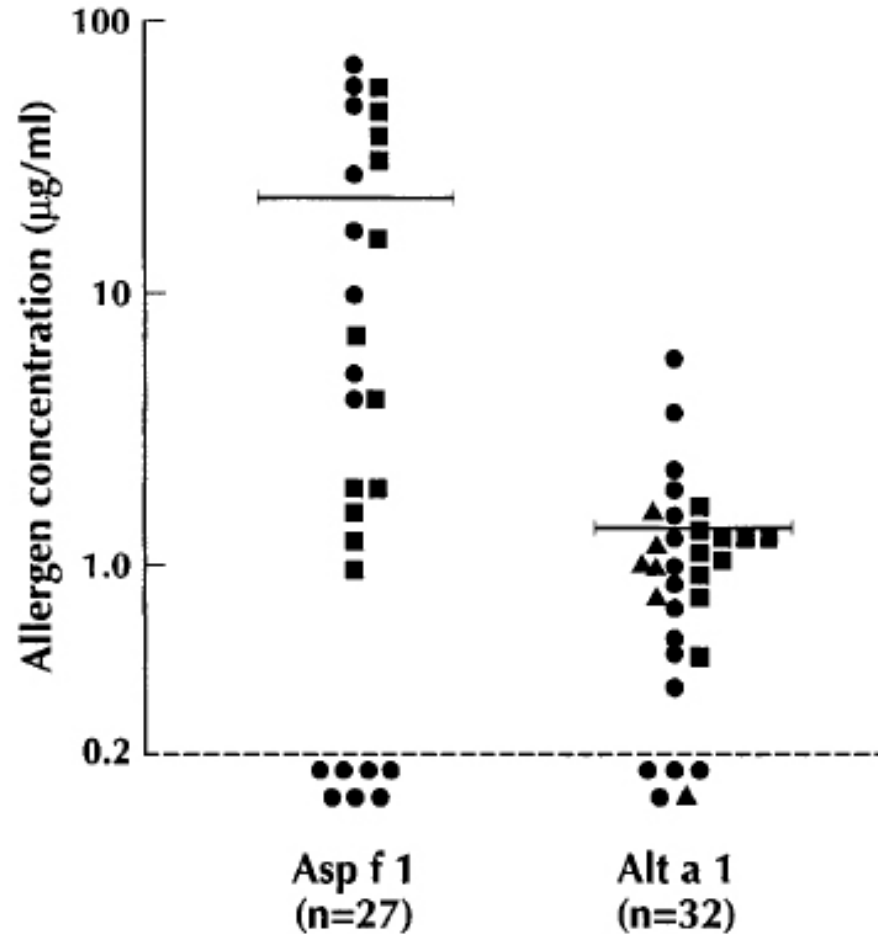
Douwes et al JACI, 1999; Barnes et al Ann Allergy 2001;  
Bush & Portnoy JACI Suppl.2001



# Monoclonal immunoassay for Alt a 1



# Comparison of allergen levels in diagnostic allergenic products





## Stachybotrys

- Implicated in a cluster of cases of lung hemorrhage (Cleveland, OH, 1993-4)
- Not found to be causative by CDC (2000)
- ~30% of *Stachybotrys* strains produce toxins
- Spores given to nasal passages and lungs of mice and rats cause inflammation and bleeding

# Stachybotrys – indoor exposure insufficient to cause toxic effects in humans



- Dose for “no effect” in rats = 3 million spores/kg.
- Comparable exposure level in humans based on breathing rates:
  - One month old infant = 2 million spores/m<sup>3</sup> air
  - 15 year old boy = 6 million spores/m<sup>3</sup> air
  - Adult male = 15 million spores/m<sup>3</sup> air

Robbins et al, App Occ Env Hyg 15:773-84, 2000;  
ACOEM Position Statement, 2002

# The Growing Hazard of Mold Litigation.....



- “A new plague – mold litigation: How junk science and hysteria built an industry”

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

**Association not confirmed by CDC, may represent cases of von Willebrand disease (vWD) – an inherited bleeding disorder, (MMWR 2004).**

Hutchinson and Powell, US Chamber Institute for Legal Reform  
Center for Legal Policy at the Manhattan Institute, July 2003



# Mold – the next Asbestos?

- The trial lawyer/remediator complex
  - 10,000 mold-related lawsuits pending in U.S.
  - Multi-million dollar damages awarded against insurance companies and contractors.
    - *Ballard case*, \$32 million awarded in Texas.
  - Mold remediation companies are largely unregulated.
-

# Testing for Stachybotrys and mold toxins



- Rapid test for Stachybotrys spores
- ELISA for Tricothecenes toxins (Stachybotrys and Aspergillus spp.)

***EnviroLogix Inc., Portland, ME***





# Remediation of fungal allergens

- Remove contaminated materials
- Thorough household cleaning
- Reduce humidity (< 45% RH)
- Reduce spore infiltration (AC)
- Use air cleaners with HEPA filters
- Wear personal protection masks

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*Bush and Portnoy, JACI 107:S430-40, 2001.*

# Allergen removal procedures that are ineffective or unproven



- Ionizers, electrostatic devices, air cleaners
- Wet steam cleaning
- HEPA filtration units (mite allergens)
- Air duct cleaning
- Special shampoos, diets, or breeds (animal allergens)
- Simply vacuuming....

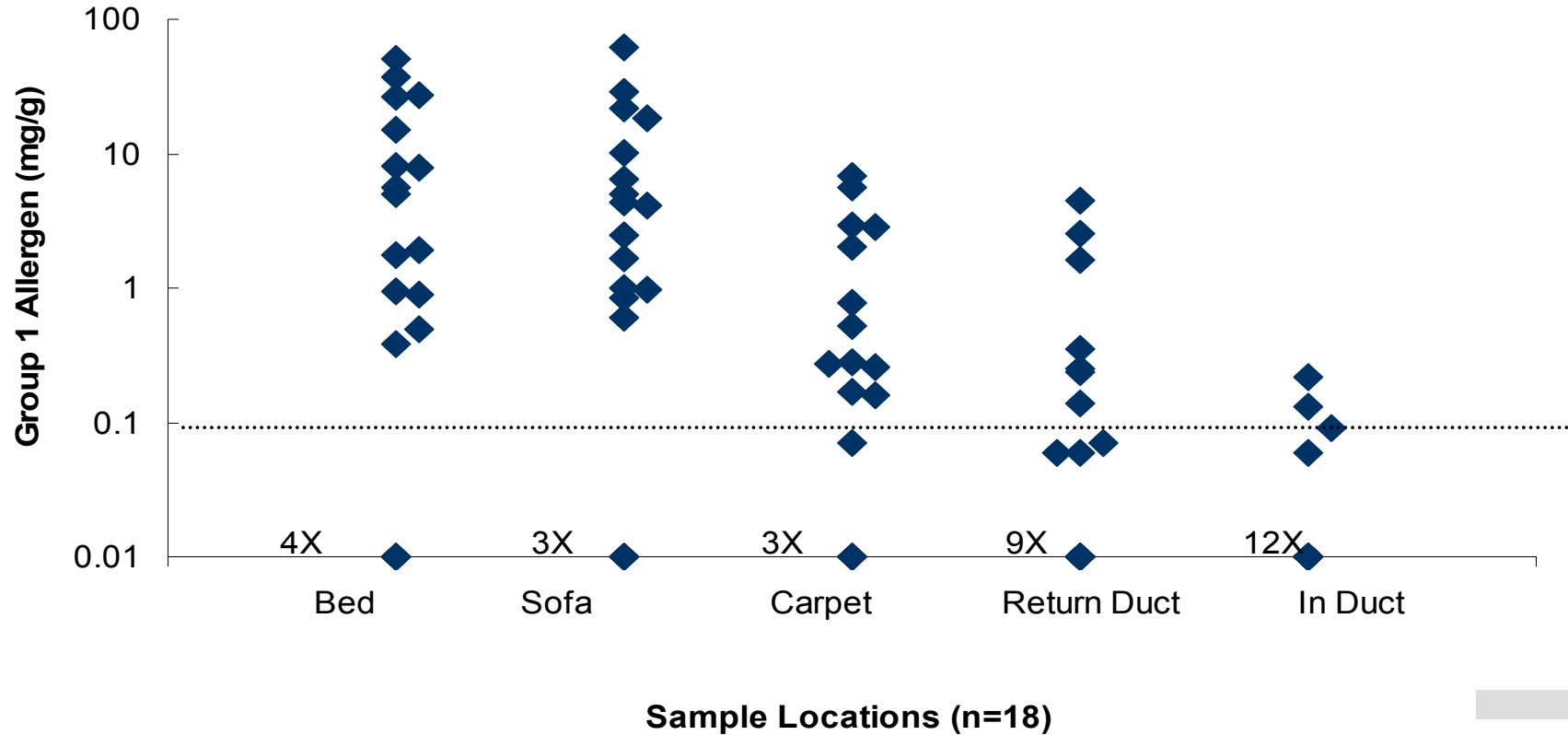


# Allergen Levels in Air Ducts

- Most air ducts (74%) did not contain detectable mite allergen ( $<0.1\mu\text{g/g}$ )
- Animal allergens accumulated at high levels in return ducts (up to  $300\mu\text{g/g}$ ). These levels were reduced by 85% in the supply ducts
- Air duct cleaning:
  - would not be recommended for mite allergens
  - would be of limited value for animal allergens

Dust samples from return ducts, supply ducts, bed, sofa and carpet were collected from 24 homes and tested by ELISA

# Comparison of mite allergen levels at five sites in the home

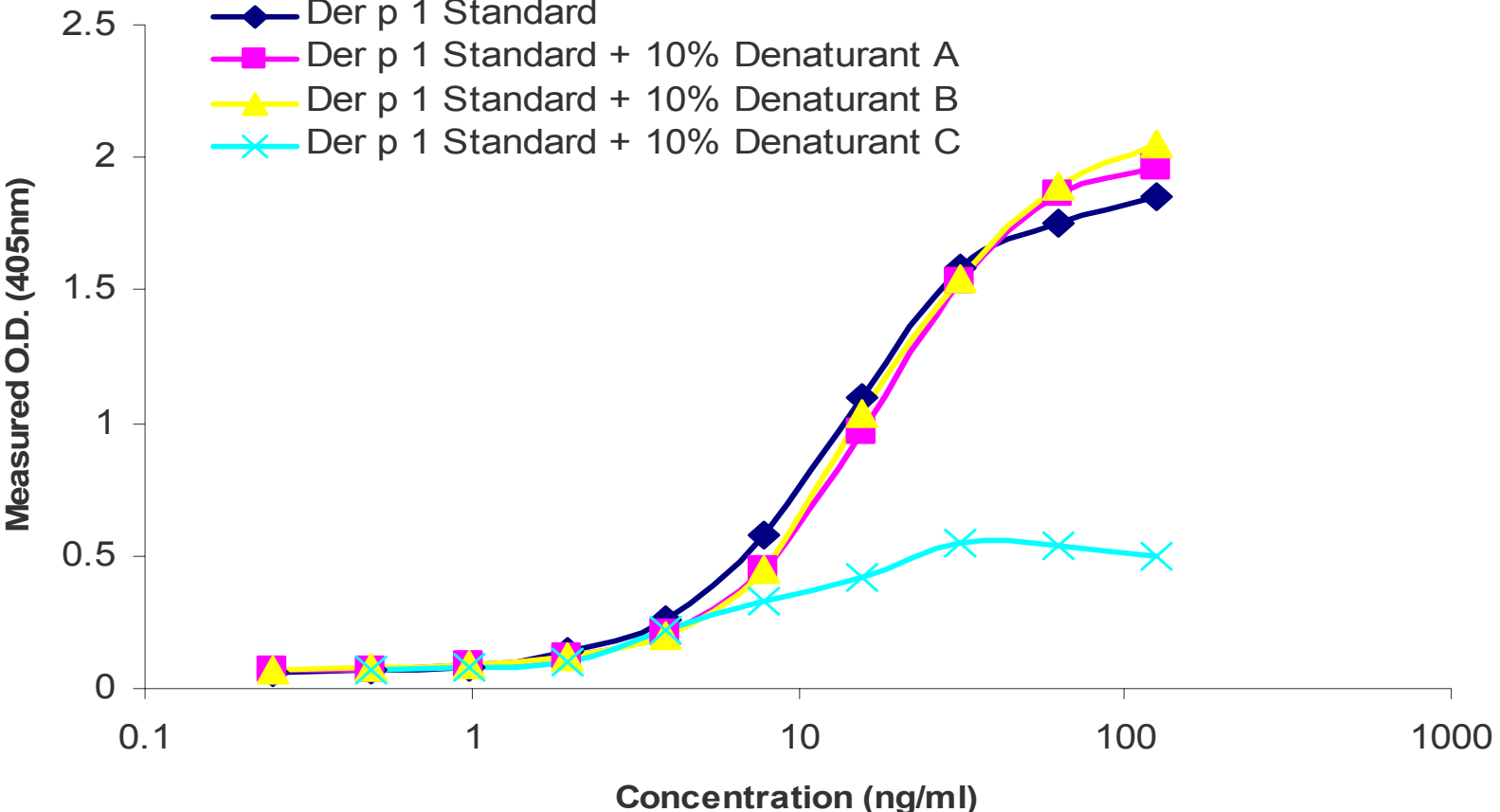


# Essential Steps in Allergen Remediation



- Development and production of target compounds/products/devices
  - Assessment of efficacy
    - Laboratory studies
    - Trials in homes (establish biological effects and effects on allergen levels)
  - Controlled trials of clinical efficacy
-

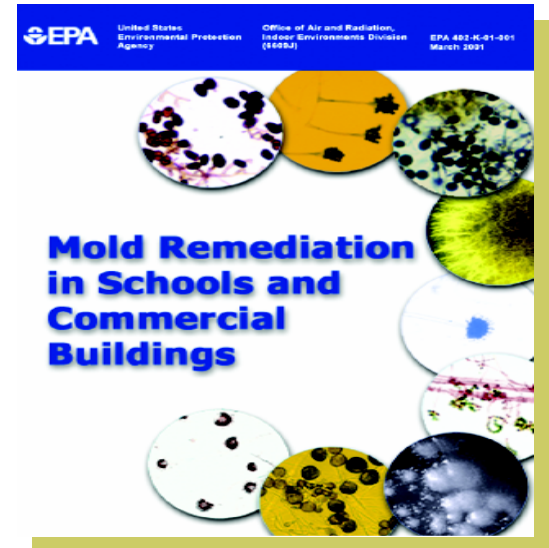
# Assessing denaturant effects on Der p 1 ELISA



# Mold Remediation in Schools and Commercial Buildings



- Non-regulatory guidance
- Aimed at building managers, professional remediators, *anyone* dealing with mold issues
- Health effects, prevention, moisture control, clean-up, protection of occupants, sampling and more
- [www.epa.gov/iaq/molds](http://www.epa.gov/iaq/molds)



# Resources

- [www.epa.gov/iaq](http://www.epa.gov/iaq)
- EPA Publications/Resources
  - *Asthma Triggers* video
  - *Clear Your Home of Asthma Triggers* brochure
  - Speaker's Kit
  - *IAQ Tools for Schools & Managing Asthma in the School Environment*
  - *Mold Remediation in Schools & Commercial Bldgs*
- Public Service Announcements
  - [www.epapsa.com](http://www.epapsa.com) to order broadcast or VHS





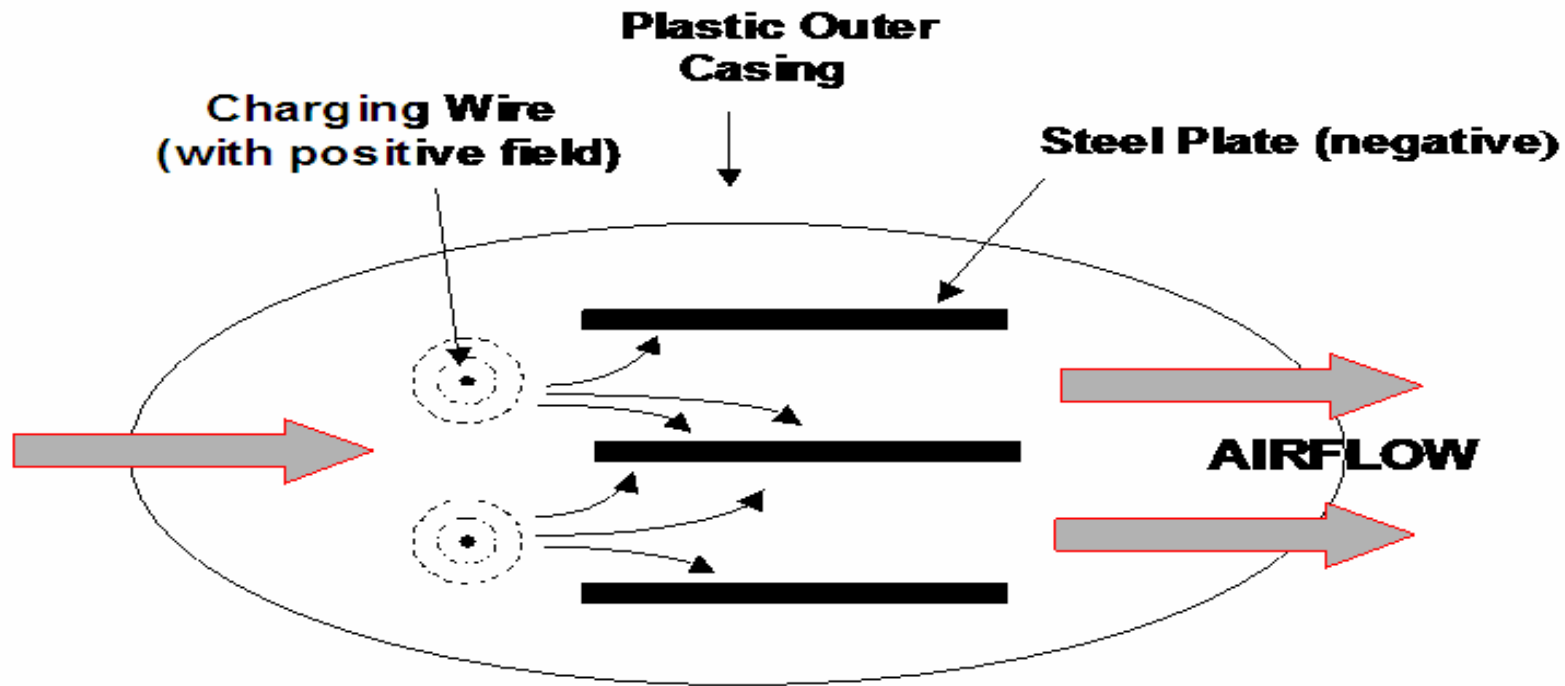
# Ion Charging Device - a new tool for monitoring airborne allergen and molds



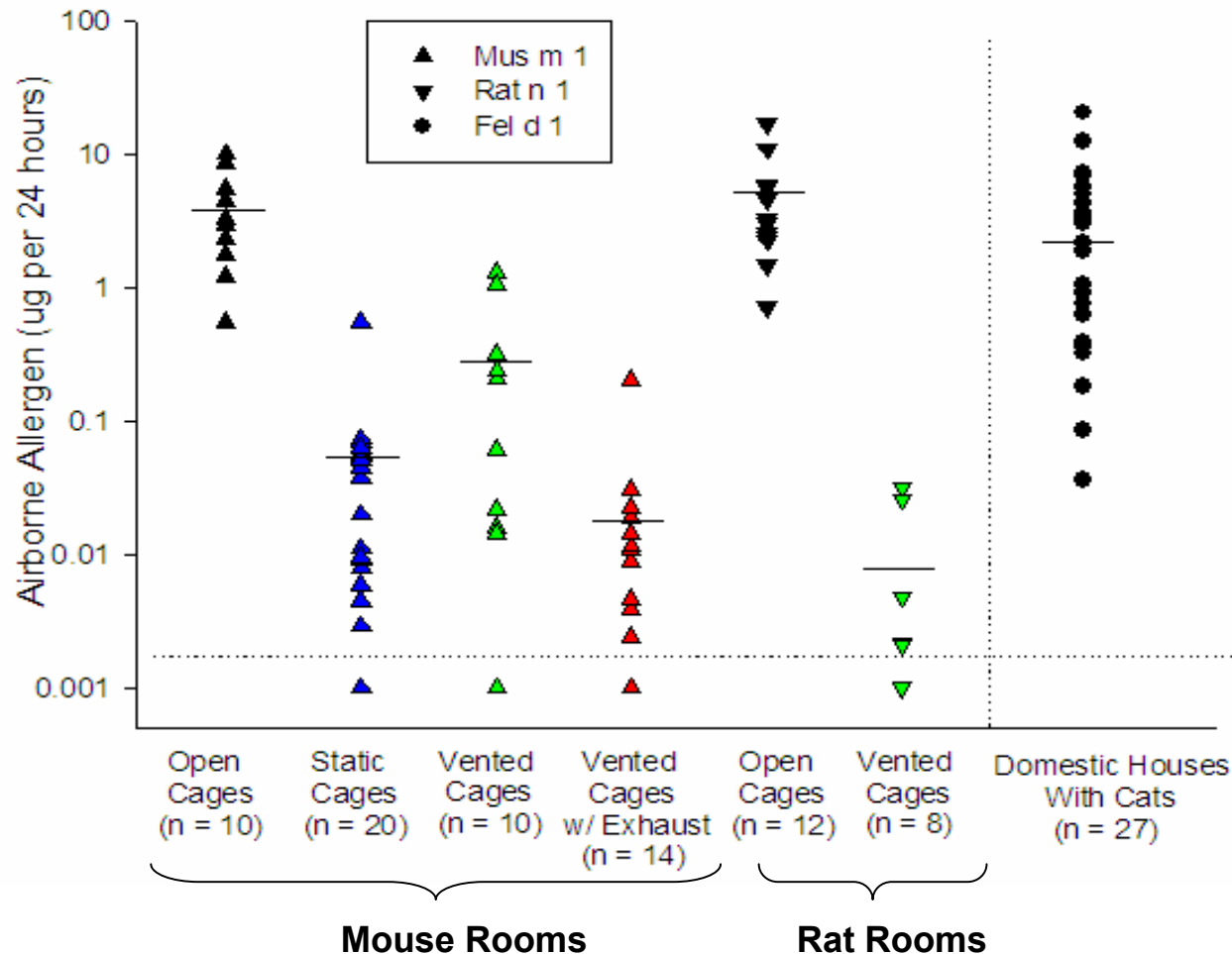
- Silent airflow - designed for home use
- Allergen collects on 3 stainless steel blades
- Collects Fel d 1 and Can f 1 at 0.5-8 $\mu$ g/24hrs
- Detects mite allergen with disturbance ( $\sim$ 1 $\mu$ g)



# Cross-sectional view through an Ion-Charging Device (ICD)



# Airborne Allergens in Animal Rooms and Domestic Houses

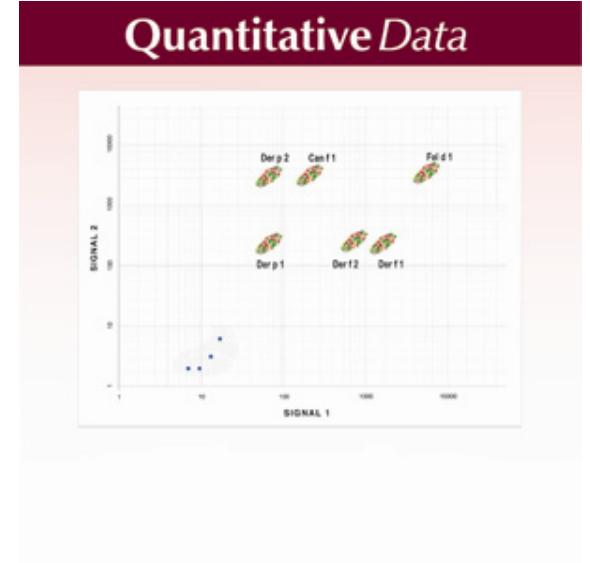
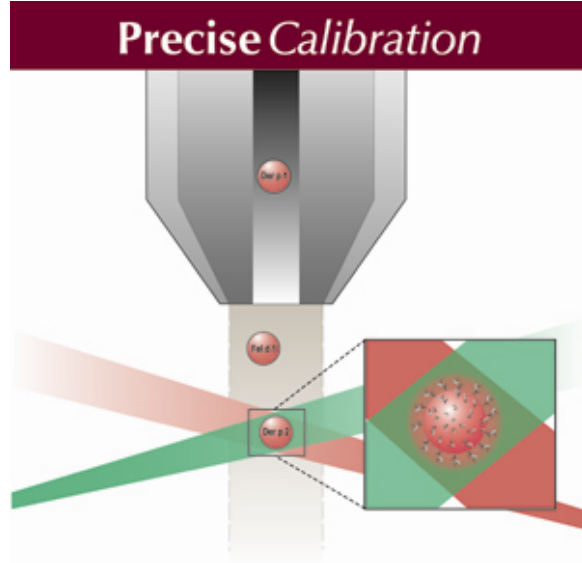
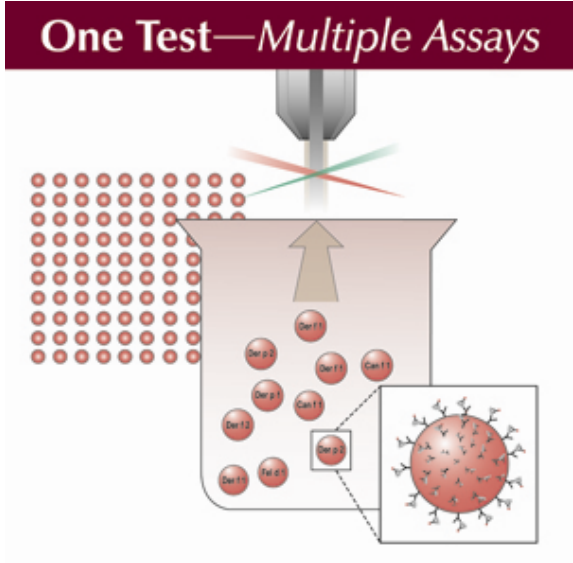




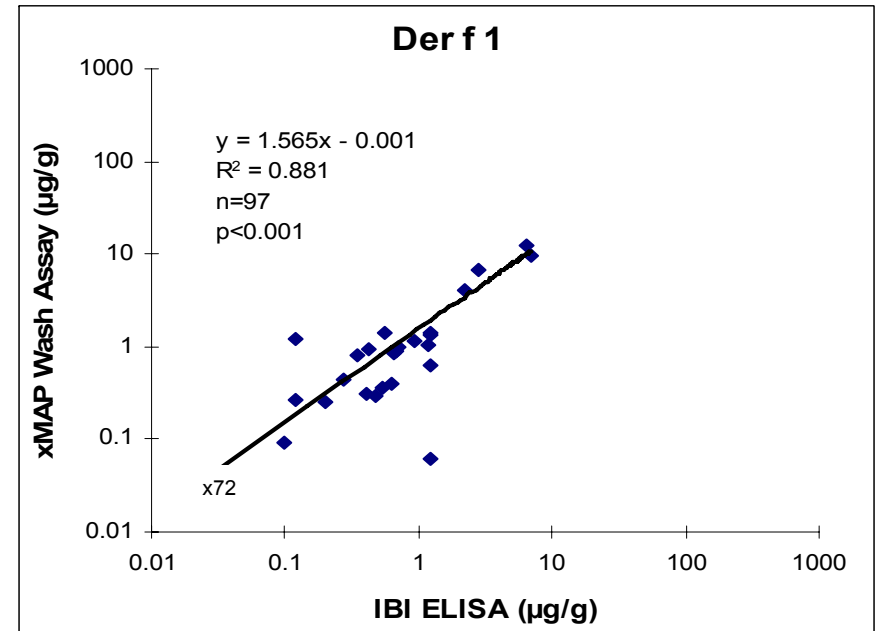
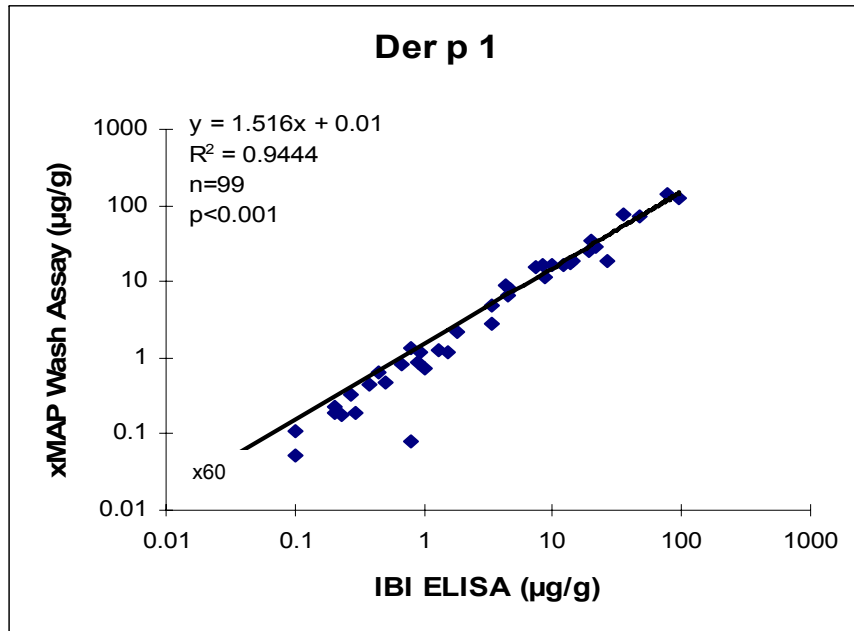
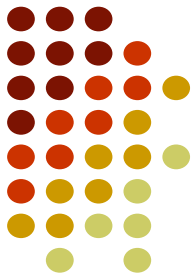
# Multiplex Systems for Detecting Allergen or IgE Antibodies, or Molds (?)

- Static or flat microarrays
- Suspension arrays
- Lateral flow tests
  
- Capable of measuring multiple analytes in a single test.

# Multiplex arrays for indoor allergens and molds

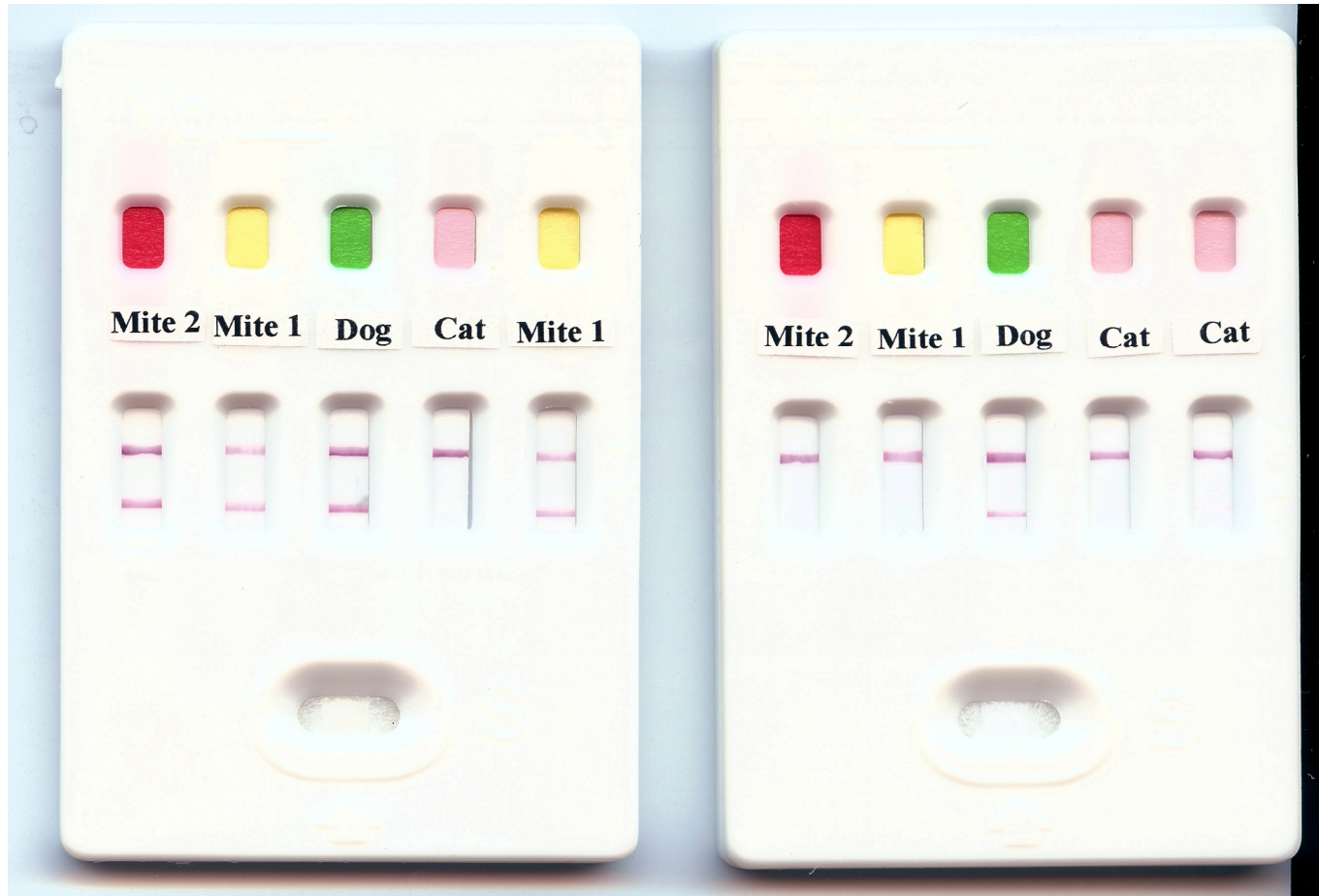


# Correlation between ELISA and multiplex array: mite Group 1 allergens





# *Multi-allergen environmental screening test*



# 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