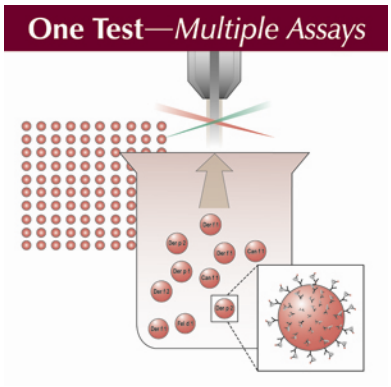
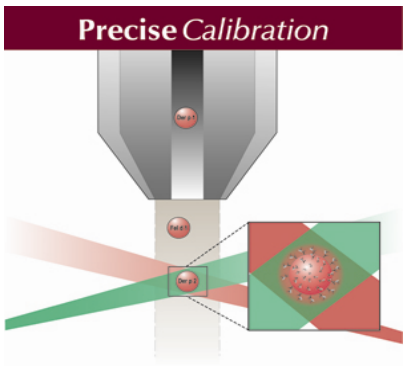
Fluorescent Multiplex Array for Indoor Allergens:
MARIA™

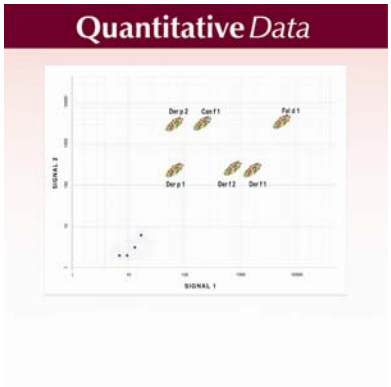
Reliable methods for assessing exposure to specific allergens are crucial for the investigation of allergic diseases. Aerobiological techniques (pollen or mold spore counts) are widely used for assessing outdoor allergen exposure, whereas enzyme immunoassay (ELISA) of dust or air samples has been the gold standard for assessing exposure to indoor allergens. The use of ELISA based detection systems has been influential in understanding the health effects of environmental allergen exposure. However, ELISA tests for each allergen have to be performed separately, which is time consuming, costly and prone to technical errors. This is especially true for large population surveys, or allergen avoidance studies, which may require exposure assessments on hundreds or thousands of samples. The limitations of ELISA procedures are an impediment for large studies of exposure assessment and for prospective studies to be carried out over several years.

MARIA™ – The next generation of allergen detection systems

Indoor Biotechnologies has developed a Multiplex ARray for Indoor Allergens (MARIA™) that measures the eight most important indoor allergens at once in a single test. The array simultaneously measures the mite allergens Der p 1, Der f 1 and Mite Group 2, animal allergens of cat (Fel d 1), dog (Can f 1), rat (Rat n 1) and mouse (Mus n 1) as well as cockroach (Bla g 2). MARIA™ provides improved assay performance (increased sensitivity, accuracy and precision) in a high throughput system with substantial time and cost savings.







How does multiplexing work?

- Fluorescent multiplex arrays using Luminex xMAP® technology are based on polystyrene microspheres of 5.6 µm in diameter, which are labeled with internal fluorescent dyes. One-hundred bead sets can be distinguished by their internal dye ratio.
- Microspheres are covalently coupled with capture monoclonal antibodies.
- During the assay, beads bearing the desired capture antibodies are combined and incubated with allergen sample. Bound allergens are detected using biotinylated mAb and a streptavidin-conjugated fluorophore.
- During measurement, microspheres are drawn into the optical path of the Luminex xMAP® instrument, where a red laser identifies beads by their internal color as bearing a specific allergen. A green laser quantifies the intensity of the external fluorescent signal, i.e. the amount of streptavidin-conjugated fluorophore, and thereby quantifies the amount of allergen bound.
- A minimum of 100 beads are counted for each allergen to obtain a highly reproducible result.

Advantages of Fluorescent Multiplex Array Technology

- Simultaneous measurement of multiple analytes = significantly reduced assay-time
- High-throughput: 35 samples per plate = 280 allergen data points
- Improved standardization – Assay conditions are the same for each allergen.
  All measurements are made using a single Universal Allergen Standard.
- Increased sensitivity and dynamic range of the standard curve:
  - Fewer sample dilutions may be required = more samples per plate
  - Results represent the median of at least 100 separate measurements per analyte = Increased reproducibility, less variation

Assay Requirements compared

Example: **Allergen 8-plex on 100 dust samples**

	ELISA	**MARIA™**
Samples per plate	18	35
Plates required	6 plates * 8 = 48	3
Sample volume required	160µL	10µL
Total assay duration	**6-8 days**	**8 hours**

Reference: Earle C, King EM, Tsay A, Pittman K, Saric B, Vailes LD *et al*. High throughput fluorescent multiplex array for indoor allergen exposure assessment. J Allergy Clin Immunol. 2007, 119: 428-433.