What is MARIA® Technology?

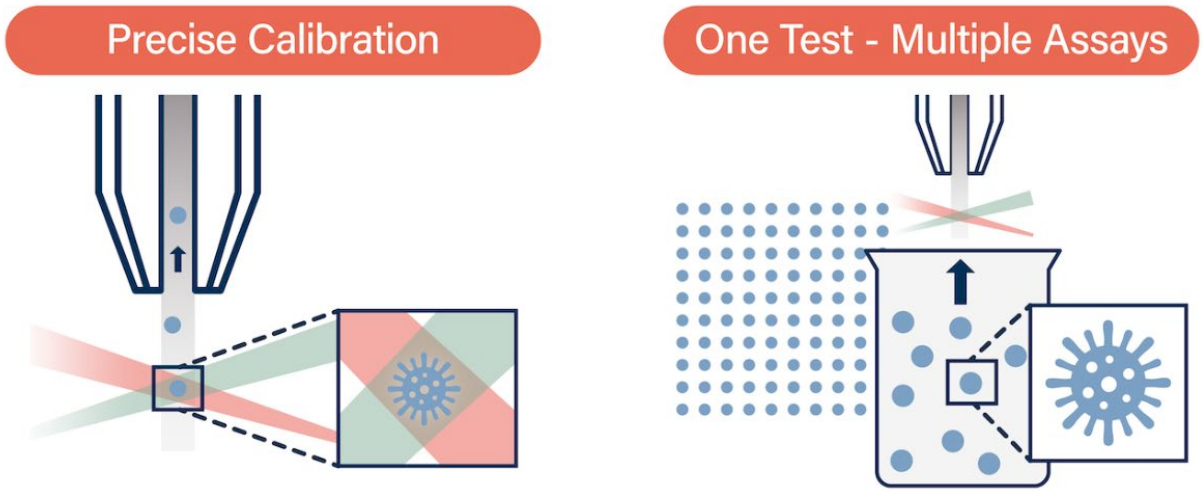
The Multiplex Array for Indoor Allergens (MARIA®) combines InBio's proprietary panels of monoclonal antibodies with Luminex xMAP® multiplexing technology. MARIA® uses polystyrene microspheres that are internally dyed with distinct fluorophores to create as many as 100 uniquely coded bead sets. Capture antibodies are covalently coupled to different bead sets and then used to develop quantitative immunoassays using biotinylated detector antibodies and a reporting fluorophore.

Different bead sets can be combined to measure multiple allergens in a single test, offering a significant advantage compared to other test methods. With the capability to measure up to 15 common environmental/inhaled allergens simultaneously, MARIA® has become the preferred method for multiple allergen testing. InBio offers MARIA® as a testing kit or service.

InBio is recognized as the world leader in assessment of environmental exposure to allergens. With over 25 years of experience, InBio's laboratory, located in Charlottesville, VA, USA, and Cardiff, Wales, tests nearly one thousand environmental dust and air samples each year, from homes, schools, offices, and other commercial buildings. Considering the volume of samples tested and variety of potential allergens, MARIA® is the ideal technology for this type of testing. On average, greater than 90% of samples tested at InBio contain at least one allergen at a level above the detectable limit.

Dust is the most common type of environmental sample tested for allergens, but MARIA® can be used for other types of samples and allergens as well. MARIA® for Foods has recently been developed, measuring 17 of the most important food allergens. The detection panels for environmental and food allergens are continuously being expanded to include other allergens.

Multiplex allergen detection using MARIA®



MARIA® offers additional advantages compared to ELISA and other immunoassays, such as time savings achieved by analyzing multiple allergens at once, as well as improved sensitivity, accuracy, and reproducibility.

Comparisons of Assay Performance between ELISA and MARIA®

a: Comparison of required Assay Time
Example: 200 dust extracts to be analyzed for 8 allergens

	ELISA	MARIA®
Samples per plate	18	20
Number of plates required	12 x 8 = 96	10
Total technician time	**16 days**	**3 days**

b: Sensitivity: Lower Limit of Detection (LLOD)
MARIA® is up to 40-fold more sensitive than ELISA

Allergen	**ELISA** (ng/mL)	**MARIA®** (ng/mL)
Dust mite, Der p 1	2.0	0.06
Dust mite, Der f 1	2.0	0.06
Dust mite, Mite Group 2	0.8	0.02
Cat, Fel d 1	0.8	0.02
Dog, Can f 1	2.0	0.06
Mouse, Mus m 1	0.2	0.01
Rat, Rat n 1	0.8	0.02
Cockroach, Bla g 2	2.0	0.98
A. alternata mold, Alt a 1	0.8	0.02
Birch pollen, Bet v 1	2.0	0.20
Timothy grass pollen, Phl p 5	4.0	0.20

MARIA® has been extensively validated in an international, multi-center ring trial, demonstrating that results are reproducible both within and between laboratories. A multitude of applications can be pursued with MARIA®, including validation of allergen-reducing cleaning products and detection of environmental allergens in homes, offices, or schools.

Allergen Testing in the Home, Office, and School

Allergic individuals, or those experiencing asthma-like symptoms or signs of allergic sensitization, such as rhinitis or eczema, can benefit from having their home, office, or school tested for allergens. Environmental allergen testing is also recommended for undiagnosed individuals presenting symptoms that are allergy-related (e.g. sneezing, itchy/watery eyes, or shortness of breath in the presence of cats). This type of testing is an important part of exposure monitoring and allergen avoidance program.

Sources of inhalable indoor allergens in the United States include dust mite, cat, dog, mouse, rat, cockroach, and mold. To assess exposure to these indoor allergens, dust or air samples are collected from the environment and analyzed for allergen content using MARIA®. Guidelines have been established for exposure to the major allergens from dust mite, cat, dog, and cockroach. The guidelines include the following categories:

Common indoor spore-forming mold, *Aspergillus*.

Low- Not sufficient to cause allergic symptoms
Significant- Risk for sensitization and bronchial hyperactivity
High- Risk for acute asthmatic attack

Exposure Guidelines for Dust Mite, Cat, Dog, and Cockroach Allergens.

	MITE Group 1 (Der p 1/Der f 1)	CAT (Fel d 1)/DOG (Can f 1)	COCKROACH (Bla g 2)
Low	<2µg Mite Group 1/g dust	<0.2µg Fel d 1 or Can f 1/g dust	<0.20µg Bla g 2/g dust
Significant	2-10µg Mite Group 1/g dust	8-20µg Fel d 1 or Can f 1/g dust	0.20-0.4µg Bla g 2/g dust
High	>10µg Mite Group 1/g dust	1-8µg Fel d 1 or Can f 1/g dust	>1µg Bla g 2/g dust

Note: *Fel d 1 and Can f 1 levels in the 'High' exposure range are lower than the 'Significant' range due to the potential for desensitization and reduced risk at higher exposures (variable, depending on the individual).*

Improvements in the sensitivity of allergen assays, such as MARIA®, now allow for quantification of allergens at limits of detection as low as 0.001µg/g. To put this into perspective, the guideline for low level exposure to dust mite allergens is <2µg/g dust, a two thousandfold difference.

Minimizing exposure to allergens and remediating the environment are critical in asthma and allergy treatment. Typical approaches to remediation include thorough cleaning using products that have been tested for allergen reducing capabilities and use of appliances (such as a vacuum cleaner with HEPA filter) that have been validated for allergen removal effectiveness. Other important practices may include integrated pest management, and education to help implement allergy care practices that minimize allergen exposure. It is recommended to consult a physician to discuss treatment options and allergen control measures.

Additional MARIA® Resources

What can we learn from multiplex allergen testing? (pdf)
Presented at the Indoor Air Quality Association annual meeting, Tampa, Florida, February 2010.

International Multi-Center Study of MARIA® (pdf)
Presented at the European Academy of Allergy and Clinical Immunology (EAACI) annual meeting, Warsaw, Poland, June 2009

Quality Control procedures using MARIA® (pdf)
Presented at the EAACI annual meeting, London, UK, June 2010.

Earle et al. High throughput fluorescent multiplex array for indoor allergen exposure assessment. J Allergy Clin Immunol, 2007; 119:428-33 (pdf).