Laboratory Animal Allergy: Improving Occupational Safety through Allergen Exposure Monitoring

By Eva M King, MSc, PhD

Allergen exposure is a common cause of occupational asthma and other allergic diseases, particularly in vivaria and in laboratories that use animals on a regular basis. This had long been known to be a problem, but recent years have brought a significant increase in awareness of laboratory animal allergens (LAA), combined with an increased regulatory and AAALAC inspection focus on exposure monitoring and worker protection.

What Are Allergens?

Allergens are small proteins produced by a variety of sources, including dust mites, rodents, cockroaches, pollen, furry pets, molds, and foods. The allergens produced by mammalian laboratory animals have multiple sources (i.e., hair, dander, urine, saliva, and serum). In a laboratory setting, rodent urinary allergens are predominantly hazardous as contaminants on inhaled airborne particulates, typically 5-40μm in diameter. Laboratory workers become sensitized following prolonged exposure to airborne animal allergens, which are potent immunogens. Chronic exposure can cause wheezing and ultimately asthma symptoms. Direct contact of animals with the skin should also be avoided. Several studies have reported a high prevalence (20–40%) of sensitization in animal worker populations. Exposure response studies provide evidence that exposure to laboratory animal allergens may pose a considerable risk for sensitization even at very low exposure levels.[1,2,4]

Engineering controls, personal protective equipment (PPE), and safety protocols can significantly reduce occupational allergen exposures. However, the implementation and performance evaluation of any allergen avoid-

ance measures require reliable methods to quantify the actual exposures. This tech tip describes specific immunoassays used to evaluate animal allergen exposures, as well as airborne allergen sampling strategies using personal, task-oriented sampling; and exposure guidelines used in animal laboratory environments, to improve worker protection.

Laboratory Animal Allergens

The primary allergens of interest in animal facilities are proteins distributed to the environment via urine, dander, and/or saliva. In facilities with mice or rats, the main culprits are the urinary proteins and major allergens, Mus m 1 and Rat n 1. Depending on the animal facility, other allergens may also be important, such as the major cat allergens Fel d 1 and Fel d 4; dog allergen Can f 1, or guinea pig urinary proteins (GPUP).

While the individual proteins involved in laboratory animal allergy are diverse, all these allergens share certain common features: they tend to become and remain airborne easily, and are easily soluble in aqueous environments, which aids exposure. They therefore represent a high risk for allergic sensitization and allergic disease in exposed individuals.

Industrial hygienists are frequently tasked with monitoring animal facilities to investigate specific complaints, to establish risk assessment profiles associated with specific tasks or rooms, to evaluate efficacy of engineering controls designed to limit exposure, to monitor ongoing compliance with occupational exposure limits (OEL), and to monitor worker compliance with exposure control procedures. Ultimately, the aim is to prevent new sensitization of laboratory workers, and to reduce the risk of allergic symptoms in workers who are already sensitized to the animals. Currently, animal laboratories in pharma/biotech industry are more likely than academic or government facilities to have effective LAA exposure monitoring protocols in place. In the interest of worker protection, this discrepancy needs to be addressed.

Figure 1. 37mm Air Sampling Cassette and IOM Inhalable Dust Sampler.

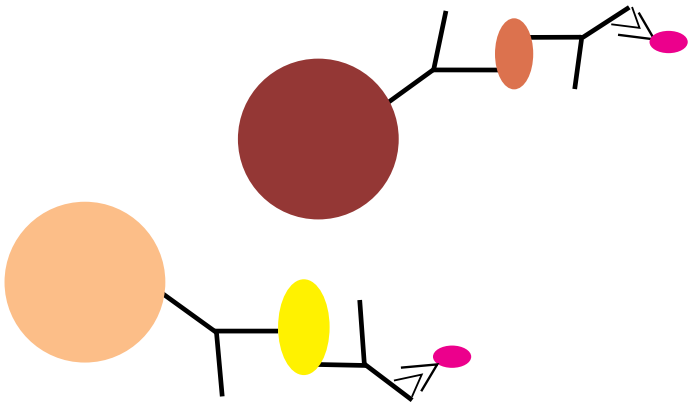

Figure 2. MARIA Bead Schematic: Allergen-specific antibodies covalently coupled to fluorescent microbeads of different internal color specifically capture animal allergens in the sample. Captured allergen is detected using biotinylated allergen-specific antibodies, and a streptavidin-coupled fluorophore.

LAA Sampling Procedures

Allergen exposure assessment in animal facilities is generally based on airborne allergen sampling of the work environment, or in the breathing zone of individual workers performing specific tasks. The sampling protocol most industrial hygienists follow involves 25mm IOM samplers or 37mm cassettes (Figure 1), attached to a calibrated portable sampling pump, running at 2 liters/minute. The sampling duration is dependent on the monitored task and goal, but typically ranges between 10–30 minutes. Room-based sampling may also be performed. Sampling media used in the sampling cassettes are usually glass fiber (GFA), polytetrafluoroethylene (PTFE), or mixed cellulose ester (MCE) filter membranes. Please note that the sampling method may vary based on a specific situation and question. Once sampling is completed, the samplers are capped, packed in individual bags to protect from cross-contamination and condensation, and then shipped on ice to the analytical lab. If samples are not analyzed within 2 days, samples should be stored frozen. In the laboratory, all air filter samples are extracted in PBS-T buffer (pH 7.4) for 2 hours at room temperature, and centrifuged. The resulting extract is then used for allergen analysis using immunoassay methods. Sampling of settled dust, or surface wipe sampling may also be useful in specific situations. However, current OEL targets are based on airborne exposures.

Allergen Detection Using Immunoassays

Analysis of airborne allergens is performed using state-of-the-art immunoassay methods that utilize antibodies that specifically and sensitively recognize the allergen in question. For airborne allergen sampling, the immunoassay method of choice is the MARIA (multiplex array for indoor allergens). MARIA is a microbead-based method that allows the simultaneous detection of multiple analytes in a single test. Internally labeled microbeads are covalently coupled with allergen-specific antibodies, which capture the allergen in

Allergen	ELISA (ng/ml)	MARIA(ng/ml)
Dust Mite: Der p 1	2	0.06
Dust Mite: Der f 1	2	0.06
Dust Mite: Mite Group 2	0.8	0.02
Cat: Fel d 1	**0.8**	**0.02**
Cat: Fel d 4	**0.08**	in development
Dog: Can f 1	**2**	**0.06**
Mouse: Mus m 1	**0.2**	**0.01**
Rat: Rat n 1	**0.8**	**0.02**
Guinea Pig Urinary Proteins	**2**	**0.49**
Cockroach: Bla g 2	2	0.98
Alternaria mold: Alt a 1	0.8	0.02
Aspergillus mold: Asp f 1	0.32	0.02
Peanut: Ara h 1	31.5	0.98
Peanut: Ara h 2	2	0.05
Peanut: Ara h 6	0.8	0.02
Birch Pollen: Bet v 1	2	0.05
Timothy Grass Pollen: Phl p 5	4	0.05

Table 1. Comparison of Assay Sensitivity, MARIA versus ELISA.

Sampling Duration	Sampling Volume (at 2 Liters/minute)	Lower Limit of Quantitation Mus m1	Lower Limit of Quantitation Rat n 1
10 min	20 Liters	1ng/m3	2ng/m3
30 min	60 Liters	0.33ng/m3	0.66ng/m3

Table 2. Scenarios of Detection Limits based on Total Sampling Volumes.

question to the bead surface. Following wash steps, a secondary, biotinylated allergen-specific antibody is added, followed by a streptavidin-coupled fluorophore (Figure 2). Results are generated in a Luminex instrument, which provides quantitative results for all bead types simultaneously. MARIA offers significantly improved sensitivity and reproducibility compared to the more traditional ELISA methods (Table 1).[3]

Assay Sensitivity and OEL targets

The current consensus OEL target applied by most industrial hygienists in pharma and biotech is 5 nanograms of allergen per cubic meter of air (5ng/m^3). The MARIA method enables exposure monitoring well below this OEL, even when using short-term sampling protocols (Table 2).

Conclusions

- Occupational exposure to laboratory animal allergens puts workers at risk of sensitization and allergic disease.
- Reducing allergen exposure through procedures, engineering controls, and PPE needs to be a high priority.
- Implementing and monitoring exposure controls requires sensitive and specific methods for detection of airborne allergens.
- Airborne sampling combined with MARIA allergen detection allows facility managers and industrial hygienists to manage and improve occupational health, and reduce worker morbidity and allergic sensitization.

The author declares employment in a company having a direct commercial interest in the subject matter discussed in the article.

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