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Research paper

A multi-center ring trial of allergen analysis using fluorescent multiplex array technology ☆

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ABSTRACT

Background: Consistent performance of allergen assays is essential to ensure reproducibility of exposure assessments for investigations of asthma and occupational allergic disease. This study evaluated intra- and inter-laboratory reproducibility of a fluorescent multiplex array, which simultaneously measures eight indoor allergens in a single reaction well.
Methods: A multi-center study was performed in nine laboratories in the US and Europe to determine the inter-laboratory variability of an 8-plex array for dust mite, cat, dog, rat, mouse and cockroach allergens. Aliquots of 151 dust extract samples were sent to participating centers and analyzed by each laboratory on three separate occasions. Agreement within and between laboratories was calculated by the concordance correlation coefficient (CCC).
Results: Results were obtained for over 32,000 individual allergen measurements. Levels covered a wide range for all allergens from below the lower limit of detection (LLOD = 0.1–9.8 ng/ml) to higher than 6800 ng/ml for all allergens except Mus m 1, which was up to 1700 ng/ml. Results were reproducible within as well as between laboratories. Within laboratories, 94% of CCC were $\geq$0.90, and 80% of intra-laboratory results fell within a 10% coefficient of variance (CV%). Results between laboratories also showed highly significant positive correlations for all allergens (~0.95, $p<0.001$). Overall means of results were comparable, and inter-laboratory CV% for all allergens except Rat n 1 ranged between 17.6% and 26.6%.

Abbreviations: MARIA, multiplex array for indoor allergens; ELISA, enzyme-linked immunosorbent assay; CV, coefficient of variation; CCC, concordance correlation coefficient; LLOD, lower limit of detection; PBS, phosphate buffered saline; NAEPP, National Asthma Education and Prevention Program.

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Conclusion: The data indicate that performance criteria for fluorescent multiplex array technology are reproducible within and between laboratories. Multiplex technology provides standardized and consistent allergen measurements that will streamline environmental exposure assessments in allergic disease.

1. Introduction

Exposure to dust mite, pet, rodent and cockroach allergens has been identified as an important risk factor for allergic sensitization and exacerbation of asthma (Platts-Mills et al., 1997). Allergen exposure assessments have played an essential role in multiple epidemiologic studies of asthma in the US, Europe and New Zealand (Eggleston et al., 1998; Phipatanakul et al., 2000; Arbes et al., 2003, 2004; Zock et al., 2006; Sears et al., 2003; Almqvist et al., 2003; Woodcock et al., 2004; Illi et al., 2006; Celedon et al., 2002).

Allergen measurements are routinely performed as part of indoor air quality investigations and occupational health monitoring (Curtin-Brosnan et al., 2010; Olmedo et al., 2011), and for standardization of allergenic products. Until recently, these measurements were made using enzyme-linked immunosorbent assay (ELISA). While ELISA has been used successfully for many years, separate tests are required for each allergen, and the process is time-consuming. Monitoring the performance of allergen assays is essential to ensure reproducibility of allergen measurements. Few prior data on the intra- and inter-laboratory variability of ELISA are available (Codina and Lockey, 2007; Pate et al., 2005). A proficiency testing study compared ELISA results for six indoor allergens between eight US laboratories and found significant differences between study sites, with CVs ranging between 61% and 93% (Pate et al., 2005). The study also included the dust handling and extraction process, and use of separate calibrators, which may have contributed to the high levels of variability observed.

Recently, fluorescent multiplex array technology has been developed that allows the simultaneous detection of multiple allergens in a single reaction well, with significantly increased sensitivity (Earle et al., 2007), which is increasingly being used for allergen detection both in homes, schools and occupational health settings (Permaul et al., 2012, Samadi et al., 2010; Wright et al., 2009). Fluorescent multiplex array technology is being extensively used in allergy and immunology research to measure cytokines, growth factors or respiratory viruses (Lalvani et al., 2008). Commercial kits are available for measurement of up to 50 cytokines and growth factors. While several studies investigate intra-laboratory performance of multiplex assays, or compare commercial multiplex kits between manufacturers or with other detection methods (Wong et al., 2008; Djoba Siawaya et al., 2008; Lewczuk et al., 2008; Johnson et al., 2007), few systematic studies of intra- and inter-laboratory performance of this fluorescent bead-based multiplex technology have been published (Fichorova et al., 2008). Systematic studies however are essential for the development of reliable methods and the direct comparison of results from different studies.

Here, we evaluate the precision and reproducibility involving intra- and inter-laboratory variance of a multiplex array for indoor allergens (MARIA) which simultaneously measures allergens of dust mites (Der p 1, Der f 1 and Mite Group 2), cat (Fel d 1), dog (Can f 1), rat (Rat n 1), mouse (Mus m 1) and German cockroach (Bla g 2). The objectives of this study were to conduct an international multi-center ring trial to assess the performance of MARIA technology, and document the intra- and inter-laboratory variability of allergen measurements.

2. Methods

2.1. Allergen measurements using fluorescent multiplex array for indoor allergens (MARIA)

The MARIA is based on xMAP® technology (Luminex Corp. Austin TX) which uses polystyrene microspheres that are internally labeled to create distinct sets of microspheres. Separate bead sets are covalently coupled with allergen-specific monoclonal antibodies, enabling the simultaneous capture and detection of multiple allergens in a single sample (Earle et al., 2007). The MARIA 8-plex used here allowed the simultaneous detection of allergens of dust mite (Der p 1, Der f 1, Mite Group 2), cat (Fel d 1), dog (Can f 1), mouse (Mus m 1), rat (Rat n 1) and German cockroach (Bla g 2). While results obtained by MARIA are comparable to ELISA within the dynamic range of the ELISA standard curves (Earle et al., 2007), MARIA is significantly more sensitive (Table A.1). The array uses a Universal Allergen Standard to quantify allergens (Filep et al., 2012; Chapman et al., 2008; van Ree et al., 2008; Earle et al., 2007).

2.2. Sample set

Since the intent of this study was to examine MARIA 8-plex assay performance alone, variability associated with sample processing (collection, sieving, extraction) was eliminated by providing pre-processed dust extracts to all study sites. Dust extracts from a bank of reservoir dust samples collected in households primarily in central Virginia were prepared at the coordinating center using established procedures (Vojta et al., 2002). In brief, 100 mg of fine dust was extracted in 2 ml of PBS-0.05% Tween 20, centrifuged and the resulting supernatant was used in this study. 151 samples were selected to create a set of specimens that covered a range of allergen concentrations including undetectable to very high for all eight tested analytes. As none of the available house dust samples contained detectable rat allergen, a number of samples were spiked using animal room bulk dust provided by Dr. Anne Renström (Karolinska Institute, Stockholm, Sweden). All samples were aliquoted into batches of 200 µl following thorough mixing, to create identical sets of 151 extract specimens for each of the participating laboratories. Samples were stored frozen at −20 °C until their use in the study.

2.3. Study design

Ten US and European laboratories with access to xMAP® instruments were recruited to participate in the study. Nine of the ten facilities completed the study and provided data