Performance Characteristics of a Fluorescent Multiplex Array for the Simultaneous Detection of Total and Allergen-specific IgE.

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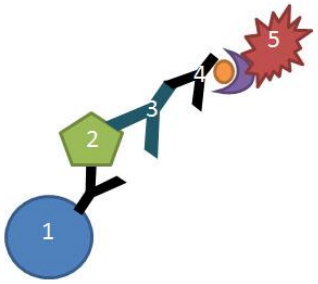
Background: Current *in vitro* methods for total and allergen-specific IgE quantification, such as RAST, ELISA and FEIA require separate testing for each analyte. This represents a potential impediment for sensitization studies, and particularly for pediatric studies, where available serum sample volumes are limited. Fluorescent microsphere array technology provides a flexible platform for the detection of several analytes in one sample, reducing the sample volume requirement significantly when multiple analyses are desired. We previously demonstrated proof-of-principle of simultaneous IgE detection using xMAP® technology.

Here, we present an improved array for the detection of total and allergen-specific IgE and compare results with Total and Allergen-Specific (Streptavidin) ImmunoCAP.

Methods: Polyclonal or monoclonal antibodies were covalently coupled to fluorescent microspheres to develop a suspension array for detection of total IgE and allergen-specific IgE against Der p 1, Der f 1, Mite Group 2 (Der p 2 or Der f 2), Fel d 1, Can f 1, Mus m 1, Rat n 1, Bet v 1, Phl p 5, and Alt a 1.
Intra- and Inter-assay reproducibility and parallelism were evaluated to determine IgE multiplex array performance. The array was validated by comparing total and allergen-specific IgE levels in plasma or serum with respective IgE concentration using Total or Streptavidin-ImmunoCAP.



Results: The multiplex array produced reproducible results: Mean Intra-Assay CV: <20%; Mean Inter-Assay CV: <15%. Results within linear range of standard curve demonstrated good parallelism (mean CV: <30%). Comparison of total and allergen-specific IgE measurements between fluorescent multiplex array and Total and Streptavidin-ImmunoCAP correlated closely (mean r = 0.83, range 0.49 –0.95) (see Table 1 and Figure 1). Assay sensitivity was <0.70 IU/ml. The required total sample volume for a 12-plex IgE assay was 30µl.

Conclusions: The multiplex array is a high-throughput assay platform for simultaneous quantification of allergen-specific and total IgE that produces reproducible and accurate results. The array uses only a fraction of the serum sample volume required in other methods, which is particularly valuable for pediatric studies. Our results suggest that fluorescent multiplex technology may facilitate future studies of allergic sensitization. The multiplex platform can be adapted for measurement of IgE antibodies in animal models.

References:

- King EM *et al*. Simultaneous detection of total and allergen-specific IgE by using purified allergens in a fluorescent multiplex array. JACI 2007; 120:1126-31
- Lohman MJ *et al*. Simultaneous Detection of Total and Allergen-Specific IgE in Human Plasma Using Multiplex Array. AAAAI 2013, San Antonio, poster presentation #245, on Feb 24, 2013

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Table 1: Performance characteristics of the IgE multiplex array

	Repeatability		Parallelism	Comparison IgE Array vs. ImmunoCAP			
Assay	**Intra-Assay CV% ***	**Inter-assay CV% ****	**Mean CV% of Dilutions *****	**Mean CV% †**	**(r) ‡**	**Inter-Method Concordance (%)°**	**N**
Total IgE	4.4	16.0	32.2	37.7	0.76	92.4	79
Der p 1	19.0	11.9	19.0	31.8	0.87	89.1	55
Der f 1	22.6	15.5	23.9	51.7	0.74	62.7	67
Der p 2	26.7	10.9	24.0	31.6	0.92	79.2	53
Der f 2	5.2	12.1	21.6	31.9	0.93	83.6	51
Fel d 1	7.9	10.5	40.0	35.1	0.82	90.0	70
Can f 1	11.3	10.1	34.5	57.4	0.90	46.0	63
Mus m 1	13.8	15.9	30.6	66.2	0.85	61.0	37
Rat n 1	33.4	9.6	20.7	56.3	0.49	63.4	29
Bet v 1	21.8	12.5	24.6	20.5	0.89	95.0	40
Phl p 5	11.0	11.1	34.3	23.0	0.95	85.4	48
Alt a 1	15.5	15.6	25.4	73.4	0.84	40.4	52
Average	**16.1**	**12.6**	**27.6**	**43.1**	**0.83**	**74.0**	

* Within-assay triplicates (MFI)

** Thrice-repeated samples (IU/mL)

*** Mean CV% of serial 5-fold dilutions (IU/mL)

† Mean CV% of IgE concentration for samples analyzed using IgE array and SA-CAP (IU/mL)

‡ Pearson correlation coefficient of data analyzed by IgE array vs. SA-CAP

° Inter-method agreement of sample data using IgE array or Streptavidin-CAP

Figure 1:

Comparison of IgE measurements performed using Total or Streptavidin-ImmunoCAP or Multiplex Array for Total IgE (A) or allergen-specific IgE against Der p 1 (B), Der f 1 (C), Der p 2 (D), Der f 2 (E), Fel d 1 (F), Can f 1 (G), Rat n 1 (H), Mus m 1 (I), Bet v 1 (J), Phl p 5 (K), and Alt a 1 (L).