Simultaneous detection of total and allergen-specific IgE by using purified allergens in a fluorescent multiplex array

Eva-Maria King, PhD,[a] Lisa D. Vailes, MS,[a] Amy Tsay, MSc,[a] Shama M. Satinover, MS,[b] and Martin D. Chapman, PhD[a] *Charlottesville, Va*

Background: Testing serum samples for total and allergen-specific IgE requires separate testing for each antibody and allergen specificity.

Objective: To apply fluorescent suspension array technology to allow simultaneous detection of total and allergen-specific IgE in serum in a single quantitative test.

Methods: A 7-plex suspension array for the simultaneous detection of total IgE and IgE specific to Der p 1, Der p 2, Fel d 1, Can f 1, Bet v 1, and Phl p 5 was developed, using mAb or purified allergens covalently coupled to fluorescent microspheres. The multiplex array was validated by comparing total and allergen-specific IgE levels in serum from patients with allergy with results obtained by enzyme immunoassays.

Results: There was a highly significant correlation between total IgE levels measured by multiplex array and fluorescent enzyme immunoassay ($r = 0.97$; $P < .001$; $n = 63$). Total and allergen-specific IgE levels also correlated with enzyme-linked and fluorescent enzyme immunoassay results ($r = 0.44$-0.94; $n = 95$ or 106). The multiplex array was reproducible ($r = 0.86$-0.99; mean coefficient of variance percentage, 12% to 25%). The sample volume required for a 7-plex assay was <20 μL per sample, compared with >400 μL in current immunoassays.

Conclusion: The multiplex array is a high-throughput system that allows simultaneous quantification of allergen-specific and total IgE.

Clinical implications: Our results suggest that fluorescent multiplex technology will facilitate large-scale epidemiologic studies of allergic sensitization. The reduced serum volume is an advantage for pediatric studies. (J Allergy Clin Immunol 2007;120:1126-31.)

Key words: *Multiplex array, total IgE, allergen-specific IgE, enzyme immunoassay, allergic sensitization, allergy diagnostics, asthma*

Reliable methods for assessing IgE mediated sensitization to specific allergens are crucial for the investigation and diagnosis of allergic diseases. Allergy diagnosis is routinely performed by skin prick testing or using *in vitro* tests, such as RAST, ELISA, or fluorescent enzyme immunoassay (FEIA). Although skin testing is sensitive and convenient, *in vitro* testing has some advantages including precise quantitation, safety, lack of drug interference, and the possibility of long-term storage of specimens.[1] For some allergens such as foods, *in vitro* IgE testing strengthens the probability that the clinical diagnosis is correct.[2] Sampson[3] established 95% predictive decision points for food allergen-specific IgE, as measured using FEIA. All current methods for *in vitro* IgE analysis such as RAST, ELISA, and FEIA have a central limitation: tests for each antibody or allergen specificity have to be performed separately, which is time-consuming and associated with higher costs and an increased possibility of technical errors. The limitations of current *in vitro* procedures are an impediment for large studies of sensitization and for prospective studies to be performed over a period of several years. The use of significant amounts of serum is a further limitation, especially for pediatric studies.

In recent years, bead-based suspension arrays have been developed that enable the simultaneous detection of multiple analytes. The technology uses polystyrene beads that are internally labeled with a distinguishable fluorophore, allowing them to be identified by a laser-based scanner. Antigens, antibodies, or other molecules are covalently coupled to the surface of the beads. Combining different bead types with the respective different capture antigens allows the simultaneous detection of multiple analytes. Quantification of multiple cytokines in serum and culture supernatants is a common application of fluorescent multiplex array.[4,5] Other applications include monitoring environments for chemical and biological warfare agents[6] and detecting IgG Abs to toxins, bacterial serogroups, or capsular polysaccharides.[7-10]

There is good evidence that measuring IgE Abs to 2 to 4 major allergens from a given source is effective for diagnostic purposes.[11-13] In a pilot study, we found

From [a]Indoor Biotechnologies Inc and [b]the Asthma and Allergic Disease Center, University of Virginia.

Supported in part by Small Business Innovation Research contract ES55545 from the National Institute of Environmental Health Sciences.

Disclosure of potential conflict of interest: E.-M. King, L. D. Vailes, and A. Tsay have received grant support from a National Institute of Environmental Health Sciences Small Business Innovation Research contract and are employed by Indoor Biotechnologies Inc. M. D. Chapman owns stock in and is employed by Indoor Biotechnologies Inc and has received grant support from the National Institute of Environmental Health Sciences. S. M. Santiover has declared that she has no conflict of interest.

Received for publication March 27, 2007; revised June 6, 2007; accepted for publication June 28, 2007.

Available online September 10, 2007.

Reprint requests: Eva-Maria King, PhD, Indoor Biotechnologies Inc, 1216 Harris Street, Charlottesville, VA 22903. E-mail: eking@inbio.com.

0091-6749/$32.00

doi:10.1016/j.jaci.2007.06.043

Abbreviations used
CV: Coefficient of variance
FEIA: Fluorescent enzyme immunoassay

a good correlation between IgE measurements by streptavidin CAP and a multiplex array for recombinant cockroach allergens.[14] The aim of the current study was to develop and validate a multiplex fluorescent suspension array that allows the simultaneous detection of total IgE and a panel of allergen-specific IgE Abs directed to indoor and outdoor allergens.

METHODS

Human sera

Human sera originated from studies of allergic sensitization performed previously at the University of Virginia[15-17] (n = 62) or were kindly provided by Dr Ronald van Ree from the European Union Development of certified reference materials for allergenic products and validation of methods for their quantification Allergen Standardization Project (n = 33)[18] and by Dr Enrique Fernandez-Caldas of Laboratorios LETI, Spain[19] (n = 63). Collection of sera was approved by the institutional review board for the respective institution. Sera from 95 patients with allergy were screened by ELISA and multiplex array for total serum IgE as well as IgE Ab to allergens of dust mite (natural [n]Der p 1 and nDer p 2), cat (nFel d 1), dog (nCan f 1), birch pollen (recombinant [r]Bet v 1a), and timothy grass pollen (rPhl p 5a). Sera were also analyzed by FEIA (ImmunoCAP; Phadia, Uppsala, Sweden) for total IgE (n = 63) and for dust mite–specific IgE (n = 106).

ELISA procedures

Quantification of allergen-specific IgE by chimeric ELISA. Microtiter plates (Nunc Maxisorp, Rochester, NY) were coated with allergen-specific capture antibody at 100 ng/well at 4°C overnight: mAb 5H8 anti–Der p 1, mAb DpX anti–Mite Group 2, mAb 6F9 anti–Fel d 1, mAb 6E9 anti–Can f 1, mAb 3B4 anti–Bet v1, and mAb 1D11C8 anti–Phl p 5. Plates were washed and blocked for 1 hour with 1% BSA PBS 0.05% Tween-20, pH 7.4, followed by 1-hour incubation with purified allergen at 80 to 500 ng/mL (nDer p 1, nDer p 2, nFel d 1, nCan f 1, rBet v 1a, or rPhl p 5a). Diluted serum samples were added and incubated for 1 hour. Bound IgE was detected with biotinylated goat antihuman IgE and streptavidin-peroxidase. The optical density was measured at 405 nm.

Allergen-specific IgE was quantified by using a chimeric mouse anti–Der p 2/human Fcε Ab (2B12-IgE), as reported previously.[20,21] Allergen-specific IgE levels were determined from the 2B12-IgE standard curve using a 4-parameter curve fit (KC4 software, version 3.4, BIOTEK Instruments, Winooski, Vt). The chimeric antibody was previously substandardized against World Health Organization IgE Standard 75/502. Results were expressed as IU IgE/mL.

Measurement of dust mite–specific IgE using FEIA. Dust mite–specific IgE was measured by *Dermatophagoides pteronyssinus* (d1) CAP using ImmunoCAP 250 (Phadia, Uppsala, Sweden). IgE Abs to purified dust mite allergens were analyzed by streptavidin CAP using biotinylated Der p 1 and Der p 2.[22]

Measurement of total serum IgE. Total IgE levels were determined by ELISA and by FEIA using the Phadia ImmunoCAP 250.[23] For ELISA, microtiter plates were coated with monoclonal anti-IgE (CIA/E/4.15, kindly provided by Dr Andrew Saxon, University of California–Los Angeles) and incubated with diluted serum samples, and bound IgE was detected by using biotinylated goat–anti-IgE and streptavidin-peroxidase. A standard curve was included on each plate using the chimeric mouse-human 2B12-IgE. Total IgE levels were interpolated from the standard curve by using a 4-parameter curve fit (KC4 software, version 3.4), and results were expressed as IU IgE/mL.

Multiplex fluorescent suspension array

Coupling of microspheres. Fluorescent microspheres (1.25×10^6 each set in 100 μL) were centrifuged at 10,000*g*/min for 2 minutes. Beads were resuspended in 80 μL activation buffer and mixed with 0.5 mg N-hydroxy-sulfosuccinimide (Pierce, Rockford, Ill) followed by 0.5 mg 1-ethyl-3-(3-dimethylaminopropyl-)carbodiimide-HCl (Sigma-Aldrich, St Louis, Mo) for 20 minutes at room temperature to activate carboxyl groups at the bead surface. After washing with PBS, bead sets for allergen-specific IgE were covalently coupled with purified allergens at optimized concentrations (250 μg nDer p 1, 250 μg nDer p 2, 200 μg nCan f 1, 50 μg nFel d 1, 2.5 μg rBet v 1a, and 0.1 μg rPhl p 5a). The bead set for total IgE was coupled to 400 μg antihuman IgE capture mAb 4.15. Coupling procedures were performed in 500 μL PBS at room temperature for 2 hours.

Beads were blocked with 500 μL blocking buffer for 30 minutes before resuspension in storage buffer at a concentration of 10,000 beads/μL. Exposure of the beads to light was kept to a minimum. All activation and coupling procedures were performed in amber tubes (USA Scientific, Ocala, Fla) in the dark, and coupled beads were stored in amber tubes at 4°C.

Multiplex procedure. Assays were performed on 96-well filter plates (Millipore, Molsheim, France) at room temperature in the dark. Beads for the detection of allergen-specific and total IgE were added to each well in a total volume of 50 μL using 2000 beads per well for each analyte in assay buffer (PBS, 0.02% Tween-20, 1% BSA). Fifty microliters of serum (diluted at 1:4 and 1:400 in assay buffer) was added to the beads and mixed thoroughly. After 60 minutes of incubation and a washing step, beads were incubated for 60 minutes with 50 μL biotinylated antihuman IgE at 1:1000. After washing, 50 μL streptavidin-R-phycoerythrin at 20 μg/mL (Invitrogen, Eugene, Ore) was added and incubated for 30 minutes. After resuspension in 100 μL assay buffer, fluorescence was measured by using the Bio-Plex System and software (Bio-Plex Manager, version 4.0; Bio-Rad, Hercules, Calif). A minimum of 100 beads per region was analyzed to determine the median fluorescent intensity.

The IgE standard curve ranged from 600 to 0.03 IU/mL and was developed using beads coupled to nDer p 2 (for allergen-specific IgE measurement) or IgE capture mAb 4.15 (for total IgE measurement) and dilutions of chimeric 2B12-IgE. A 5-parameter logistic curve fit was applied to the standard curve, and sample concentrations were interpolated from the curve. The limit of detection for each assay was defined as twice the fluorescent signal of the blank control samples.

Statistical analysis

IgE levels were not normally distributed following logarithmic transformations, as determined by Kolmogorov-Smirnov tests, and were therefore analyzed by nonparametric tests. The relationship between variables was analyzed by Spearman rank correlation. All statistical tests were 2-tailed. Data were analyzed with SPSS for Windows (version 10.0; SPSS Inc, Chicago, Ill).

RESULTS

Array for total IgE

To establish the best coupling concentration of IgE capture mAb in the array for detection of total IgE, activated microspheres were covalently coupled with

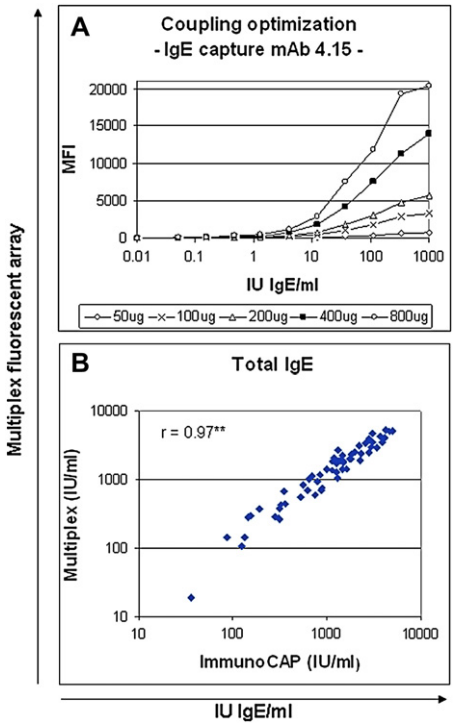

FIG 1. A, Optimization of coupling concentration of IgE capture mAb for detection of total IgE. **B,** Comparison of total serum IgE levels measured by ImmunoCAP and multiplex; n = 63; $^{**}P < .001$. *MFI,* Median fluorescent intensity.

capture mAb at concentrations of 50 to 800 μg per coupling batch (1.25×10^6 beads). A 2B12-IgE standard curve ranging from 0.05 to 1000 IU IgE/mL was prepared on each of the coupled bead sets. A coupling concentration of 400 μg was found optimal on the basis of signal intensity and dynamic range of the standard curve (Fig 1, *A*). After optimization, total serum IgE was measured in 63 sera from patients with allergy by using 7-plex suspension array and FEIA (ImmunoCAP). There was a highly significant correlation between results obtained using both methods ($r = 0.97$; $P < .001$; Fig 1, *B*). The mean coefficient of variance (CV) was 17.2%. Total IgE levels in the sera ranged from <2 IU/mL to >5000 IU/mL.

Total IgE levels measured in 95 sera by multiplex array were also compared with ELISA and were highly correlated ($r = 0.94$, $P < .001$). The CV between methods was 33%, and total IgE levels in the sample set ranged from <2 to >10,000 IU/mL.

Allergen-specific IgE measurement by 7-plex suspension array and ELISA

Bead sets for the detection of allergen-specific IgE were coupled using 0.1 to 250 μg purified allergen. Selected sera from patients with allergy were tested for IgE binding to the beads. Quantitative allergen-specific IgE measurements were interpolated from a standard curve on the basis of chimeric 2B12-IgE, and the optimal allergen coupling concentration was determined.

Allergen-specific IgE levels in 95 serum samples were measured by multiplex and ELISA (Fig 2). Specific IgE levels in the sera covered a range between <0.5 and >100 IU/mL for each analyte. IgE levels specific to dust mite allergens showed highly significant positive correlations between ELISA and 7-plex array ($r = 0.80$, Der p 1, and $r = 0.88$, Der p 2; $P < .001$).

There was also a good correlation between detection methods for specific IgE to Fel d 1 ($r = 0.85$; $P < .001$). The correlation coefficient for Can f 1–specific IgE was weaker ($r = 0.44$; $P < .01$). The samples used in this study were derived from previous studies that focused on allergic sensitivity to dust mite, pollen, or cat allergen. The total number of samples in the sample set that were positive for Can f 1–specific IgE in both detection methods was substantially lower (n = 9) than for the other allergen specificities, which most likely explains the weaker correlations.

IgE specific to pollen allergens showed significant positive correlations between ELISA and multiplex array: Bet v 1, $r = 0.73$ ($P < .01$), and Phl p 5, $r = 0.76$ ($P < .01$). CVs between the methods were Der p 1, 39%; Der p 2, 29%; Fel d 1, 42%; Can f 1, 22%; Bet v 1, 39%; and Phl p 5, 61%.

Interassay reproducibility of the multiplex array was determined by repeated measurement of the entire sample set on 2 different days. Correlations between multiplex assays were very good for all analytes, with $r = 0.86$ to 0.99 ($P < .001$) and CV% between 12% and 25%.

Parallelism of results with respect to the standard curve was tested measuring dilution recovery of 8 serum samples for all analytes. Sera were measured at dilutions of 1:5, 1:10, and 1:20, and results were compared. Mean CVs of results between the different dilutions were as follows: total IgE, 17.8%; and specific IgE to Der p 1, 10.1%; Der p 2, 16.7%; Fel d 1, 10.3%; Can f 1, 11.2%; Bet v1, 8.2%; and Phl p 5, 12.2%.

Assay requirements differed significantly between the 2 methods. The total assay duration of the 7-plex assay for 95 samples was 6 hours as opposed to 35 hours in ELISA. The 7-plex assay also required significantly less serum sample volume than ELISA (7-plex, <20 μL; ELISA, 420 μL).

Dust mite–specific IgE measured by multiplex and FEIA

When comparing allergen-specific IgE levels measured by multiplex array and FEIA (ImmunoCAP), it must be taken into account that the beads used in the multiplex array are coupled with purified allergens, whereas FEIA uses mite allergen extracts. Der p 1 and Der p 2 represent the major allergens with more than 80% of patients allergic to dust mite reacting to these 2 allergens. Significant correlations between combined Der p 1 and Der p 2 specific IgE measurements by chimeric ELISA and *D pteronyssinus* ImmunoCAP were reported previously.[24] We compared combined multiplex results for Der p 1 and Der p 2 with results obtained by *D pteronyssinus* ImmunoCAP (d1 CAP) and found a significant positive correlation ($r = 0.83$; $P < .01$; n = 106; Fig 3, *A*).

Multiplex results from the same 106 serum samples were compared with results obtained by streptavidin CAP

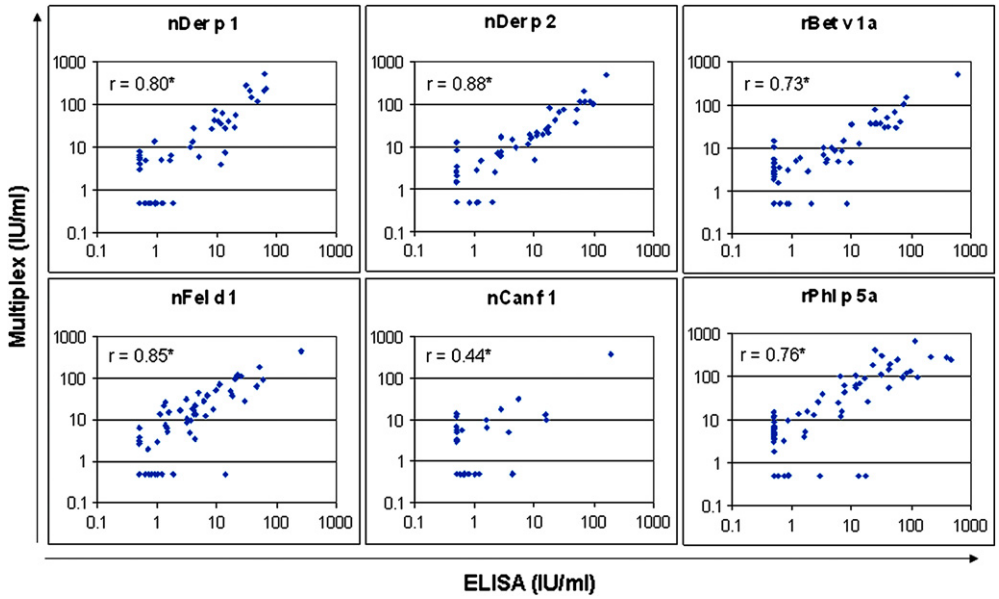

FIG 2. Specific IgE responses to indoor and outdoor allergens. Comparison between ELISA and multiplex, n = 95. *$P < .01$.

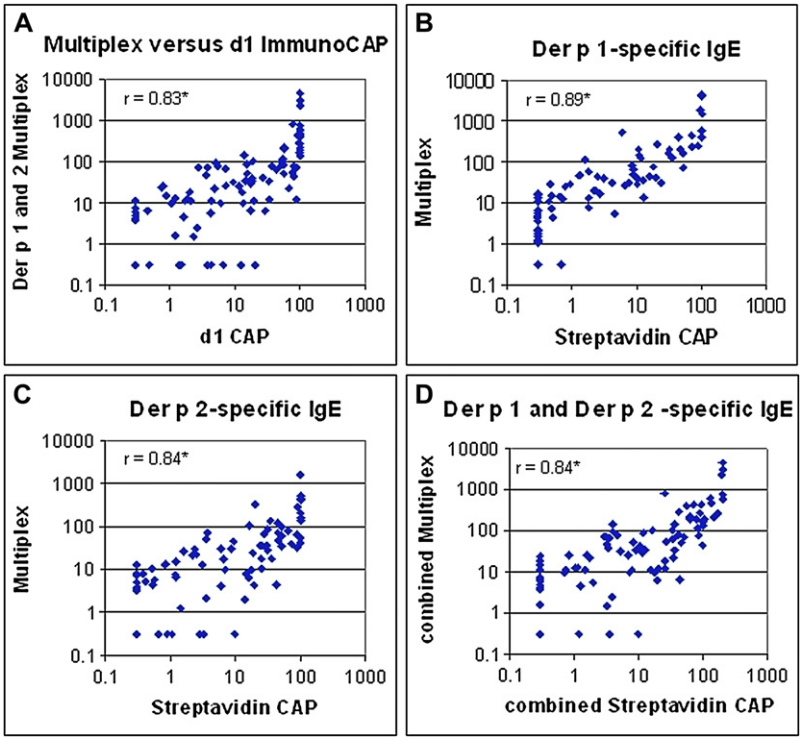

FIG 3. A-D, Comparison of dust mite-specific IgE levels measured by different detection methods: multiplex, *D. pteronyssinus* (d1 CAP) and streptavidin ImmunoCAP, n = 106. *$P < .01$.

using biotinylated Der p 1 and Der p 2. This method enables a different comparison between the assay techniques for measuring allergen-specific IgE. There were significant positive correlations between the methods for both allergens (Der p 1, $r = 0.89$, $P < .001$; Der p 2, $r = 0.84$, $P < .001$; Fig 3, *B-D*).

DISCUSSION

The study presented here used fluorescent multiplex suspension array technology to develop a method enabling the simultaneous detection of total and allergen-specific

IgE. The benefits of multiplex technology over ELISA methods for various analytes have recently been reviewed.[5,25,26] Speed, the ability to measure different antibody concentrations with minimum sample volume, and more desirable reaction kinetics and enhanced dynamic range are major benefits of multiplex technology versus ELISA. Indeed, our study confirmed these benefits for serum IgE measurements. The dynamic range of the multiplex array for total and allergen-specific IgE detection typically ranged between 0.3 and 200 IU IgE/mL as opposed to 1.5 to 25 IU IgE/mL for ELISA, with an associated 5-fold increase in sensitivity. Furthermore, the multiplex array enabled all 7 analytes to be measured in a total serum volume of less than 20 μL and in 6 hours. Both total and allergen-specific IgE were measured in serum at the same time. Multiplex results showed highly significant correlations with ELISA and total IgE ImmunoCAP. Results for dust mite–specific IgE Abs also correlated significantly between multiplex and *D pteronyssinus* CAP as well as Der p 1 and Der p 2 linked streptavidin CAP.

For all allergen-specific analytes, there were, however, a number of samples that differed in their classification as positive (above 0.5 IU IgE/mL) or negative (below 0.5 IU/mL) responders, indicating intrinsic differences between the methods. This may be because methods are based on different allergen coupling mechanisms (covalent vs noncovalent binding) and different solid phase matrices. As a general trend, more samples were classified as positive in multiplex and negative in ELISA or streptavidin CAP than vice versa. In comparison between multiplex and ELISA, 2% to 21% of samples were negative in ELISA and positive in the suspension array, whereas 4% to 9.5% were positive in ELISA and negative in multiplex. A possible explanation for this observation may therefore be the increased sensitivity of the multiplex assay in comparison with the ELISA. A similar effect was observed in comparison between multiplex and combined dust mite–specific IgE Abs measured by streptavidin CAP, where 2% of samples were positive in streptavidin CAP and negative in multiplex, whereas 8.5% were positive in multiplex and negative in streptavidin CAP. All of these samples had relatively low IgE Ab levels (<15 IU/mL). The actual levels of allergen-specific IgE measured by the 2 methods tended to be higher in the suspension array than in the respective ELISA or CAP. The extent of this intermethod variation differed between analytes.

In the comparison between dust mite–specific multiplex and *D pteronyssinus* CAP results, 93% of patients were diagnosed by combined Der p 1 and Der p 2 specific IgE measurements. Our study therefore confirms findings by Pittner et al,[24] who investigated component-based diagnosis of house dust mite allergy with purified mite allergens as opposed to mite extracts, and found that >95% of patients could be diagnosed by measuring their IgE Abs to the major allergens Der p 1 and Der p 2 alone. Use of purified allergens rather than allergen extract–based testing has recently been described using static microarrays,[27,28] in which a fixed set of analytes is arranged on a glass slide. The static microarray was less sensitive than CAP but was suitable for the diagnosis of patients with allergy for a range of allergens.

During ongoing expansion of the allergen-specific IgE panel, we have found that some allergens are easier to incorporate into the multiplex than others. Recent studies indicate that Der f 1, Bla g 1, Bla g 2, and Asp f 1 specific IgE measurements can be incorporated into the system to produce an 11-plex array.[29] Other allergens such as Phl p 1 and Amb a 1 showed IgE reactivity that did not correlate well with ELISA results. These difficulties may be caused in part by the proximity of IgE epitopes to coupling sites on the beads or to structural alterations of the allergen during the covalent coupling procedure. We expect that use of different coupling chemistries will alleviate these difficulties.

In summary, our results show that multiplex fluorescent array technology allows the simultaneous measurement of total and allergen-specific IgE. The method described here is applicable to large-scale studies of allergic sensitization, such as birth cohorts and population surveys. The array uses minimal amounts of serum, which is valuable for pediatric studies. Multiplex technology for IgE detection is envisioned as a research tool, which requires further validation before it could be used in clinical IgE testing. We have recently developed a multiplex array for measuring environmental allergens.[25] This allows allergen sensitization and exposure to be measured using the same technology and should be useful for studies of gene-environment interactions.

We thank Dr Ronald van Ree and the EU CREATE Allergen Standardization Project for providing sera from patients with pollen allergy and Dr Enrique Fernandez-Caldas of Laboratorios LETI, Madrid, Spain, for sera from patients with mite allergy. We also thank Dr Oliver Cromwell of Allergopharma, Reinbek, Germany, for access to Phl p 5 reagents.

REFERENCES

1. Hamilton RG, Adkinson NF. *In vitro* assays for the diagnosis of IgE-mediated disorders. J Allergy Clin Immunol 2004;114:213-25.
2. Bernstein JA, Bernstein IL, Bucchini L, Goldman LR, Hamilton RG, Lehrer S, et al. Clinical and laboratory investigation of allergy to genetically modified foods. Environ Health Perspect 2003;111:1114-21.
3. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001;107:891-6.
4. Prabhakar U, Eirikis E, Reddy M, Silvestro E, Spitz S, Pendley C II, et al. Validation and comparative analysis of a multiplexed assay for the simultaneous quantitative measurement of Th1/Th2 cytokines in human serum and human peripheral blood mononuclear cell culture supernatants. J Immunol Methods 2004;29:7-38.
5. Kellar KL, Kalwar RR, Dubois KA, Crouse D, Chafin WD, Kane BE. Multiplexed fluorescent bead-based immunoassays for quantitation of human cytokines in serum and culture supernatants. Cytometry 2001;4:27-36.
6. McBride MT, Gammon S, Pitesky M, O'Brien TW, Smith T, Aldrich J, et al. Multiplexed liquid arrays for simultaneous detection of simulants of biological warfare agents. Anal Chem 2003;75:1924-30.
7. Biagini RE, Schlottmann SA, Sammons DL, Smith JP, Snawder JC, Striley CAF, et al. Method for simultaneous measurement of antibodies to 23 pneumococcal capsular polysaccharides. Clin Diagn Lab Immunol 2003;10:244-50.

8. Biagini RE, Sammons DL, Smith JP, MacKenzie BA, Striley CAF, Semonova V, et al. Comparison of a multiplexed fluorescent microsphere immunoassay and an enzyme-linked immunosorbent assay for measurement of human immunoglobulin G antibodies to anthrax toxins. Clin Diagn Lab Immunol 2004;11:50-5.
9. Biagini RE, Sammons DL, Smith JP, MacKenzie BA, Striley CA, Robertson SA, et al. Simultaneous measurement of specific IgG responses to five select agents. Anal Bioanal Chem 2005;382:1027-34.
10. Lal G, Balmer P, Joseph H, Dawson M, Borrow R. Development and evaluation of a tetraplex flow cytometric assay for quantitation of serum antibodies to Neisseria meningitides serogroups A, C, Y and W-135. Clin Diagn Lab Immunol 2004;11:272-9.
11. Scheiner O, Kraft D. Basic and practical aspects of recombinant allergens. Allergy 1995;50:384-91.
12. Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomés A. Recombinant allergens for diagnosis and therapy of allergic disease. J Allergy Clin Immunol 2000;106:409-18.
13. Kazemi-Shirazi L, Niederberger V, Linhart B, Lidholm J, Kraft D, Valenta R. Recombinant marker allergens: diagnostic gatekeepers for the treatment of allergy. Int Arch Allergy Immunol 2002;127:259-68.
14. Satinover SM, Reefer AJ, Pomes A, Chapman MD, Platts-Mills TAE, Woodfolk JA. Specific IgE and IgG antibody-binding patterns to recombinant cockroach allergens. J Allergy Clin Immunol 2005;115:803-9.
15. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TAE. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989;83:875-82.
16. Call RS, Smith TF, Morris E, Chapman MD, Platts-Mills TAE. Risk factors for asthma in inner city children. J Pediatr 1992;121:862-6.
17. Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TAE. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. Am Rev Respir Dis 1993;147:573-8.
18. van Ree R, CREATE Partnership. The CREATE project: EU support for the improvement of allergen standardization in Europe. Allergy 2004;59:571-4.
19. Boquete M, Iraola V, Fernandez-Caldas E, Arenas Villaroel L, Carballada FJ, Gonzalez de la Cuesta C, et al. House dust mite species and allergen levels Galicia, Spain: a cross-sectional, multicenter, comparative study. J Investig Allergol Clin Immunol 2006;16:169-76.
20. Schuurman J, Perdok GJ, Lourens TE, Parren PWHI, Chapman MD, Aalberse RC. Production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE. J Allergy Clin Immunol 1997;99:545-50.
21. Trombone APF, Tobias KRC, Ferriani VPL, Schuurman J, Aalberse RC, Smith AM, et al. Use of a chimeric ELISA to investigate immunoglobulin E antibody responses to Der p 1 and Der p 2 in mite-allergic patients with asthma, wheezing and/or rhinitis. Clin Exp Allergy 2002;32:1323-8.
22. Erwin AE, Custis NJ, Satinover SM, Perzanowski MS, Woodfolk JA, Crane J, et al. Quantitative measurement of IgE antibodies to purified allergens using streptavidin linked to a high-capacity solid phase. J Allergy Clin Immunol 2005;115:1029-35.
23. Ichikawa K, Iwasaki E, Baba M, Chapman MD. High prevalence of sensitization to cat allergen among Japanese children with asthma, living without cats. Clin Exp Allergy 1999;29:754-61.
24. Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F, et al. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. Clin Exp Allergy 2004;34:597-603.
25. Earle CD, 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;119:428-33.
26. Pickering JW, Martins TB, Schroder MC, Hill HR. Comparison of a multiplex flow cytometric assay with enzyme-linked immunosorbent assay for quantitation of antibodies to tetanus, diphtheria and *Haemophilus influenzae* type b. Clin Diagn Lab Immunol 2002;9:872-6.
27. Hiller R, Laffer S, Harwanegge G, Huber M, Schmidt WM, Twardosz A, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB J 2002;16:414-6.
28. Wohrl S, Vigl K, Zehetmayer S, Hiller R, Jarisch R, Prinz M, et al. The performance of a component-based allergen-microarray in clinical practice. Allergy 2006;61:633-9.
29. King EM, Earle CD, Tsay A, Vailes LD, Satinover SM, Chapman MD. Simultaneous detection of total and allergen-specific IgE using fluorescent multiplex array technology [abstract]. J Allergy Clin Immunol 2007;119:S265.