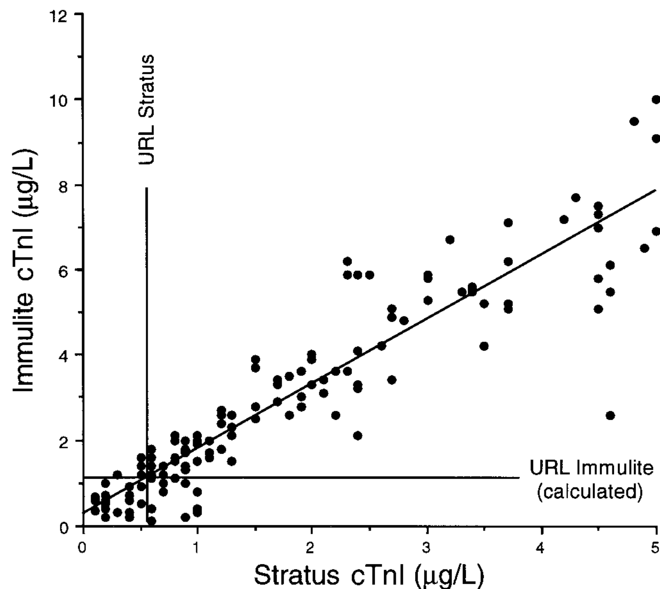

Fig. 1. Correlation of Immulite cTnI and Stratus cTnI assays, using patients with Stratus cTnI <5 μg/L.

Data points below the detection limit of the Immulite or Stratus were not included.

limit (15 and 22 below the limits for the Stratus and Immulite assays, respectively) and 42 (12%) had both results below the respective detection limits. Regression analysis was performed on the 268 remaining samples: Immulite cTnI = 1.84 (Stratus cTnI) − 1.1 μg/L; $r = 0.977$; $S_{y|x} = 4.7$ μg/L. The high slope might result from a lack of standardization between cTnI assays *(2)* and/or a difference in the reactivities of the antibodies used to the various circulating forms of the protein *(3–6)*. We routinely used an upper reference limit (URL) of 0.6 μg/L for the Stratus cTnI assay. To estimate the corresponding value for the Immulite cTnI assay, we established the relationship between the two assays in 137 samples with Stratus cTnI values <5 μg/L: Immulite cTnI = 1.51 (Stratus cTnI) + 0.27; $r = 0.924$. The estimated Immulite URL (based on the regression) corresponding to a Stratus value of 0.6 μg/L was 1.18 μg/L (Fig. 1). Using these cutoffs, we studied 80 patients admitted to the intensive care unit (37 with acute myocardial infarction, 22 with unstable angina, and 21 with chest pain). Heparinized samples were collected on admission. The Stratus (0.6 μg/L) and estimated Immulite (1.18 μg/L) URLs gave specificities of 93% [95% confidence interval (CI), 69.6–98.8%] and 95% (95% CI, 76.2–99.9%), respectively, for acute coronary syndrome (vs 21 chest pain patients) and sensitivities of 95% (95% CI, 85.9–98.9%) and 92% (95% CI, 81.3–97.2%), respectively.

In conclusion, the data presented here demonstrated acceptable analytical performance for the Immulite cTnI assay. Furthermore, there was excellent clinical concordance between the DPC Immulite and Dade Stratus cTnI assays. Additional evaluations will be necessary to define the URL.

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Detection of Multiple Allergen-specific IgEs on Microarrays by Immunoassay with Rolling Circle Amplification, *Steve Wiltshire,[1] Shawn O'Malley,[1] Jeremy Lambert,[1] Kari Kukanskis,[1] David Edgar,[2] Stephen F. Kingsmore,[1] and Barry Schweitzer[1*]* ([1] Molecular Staging Inc., 66 High Street, Guilford, CT 06437; [2] Regional Immunology Service, Microbiology Building, Royal Hospitals Trust, Belfast BT12 6BN, Northern Ireland; * author for correspondence: fax 203-453-2732, e-mail barrys@molecularstaging.com)

First described in 1967, the radio allergo sorbent test (RAST) has been the standard technique for measuring allergen-specific IgE antibodies in serum *(1)*. An updated version of the RAST test, termed CAP (Pharmacia), has been introduced *(2)*. In clinical practice, CAP results must be interpreted with care. The diagnostic performance of CAP varies in an allergen-specific manner, and CAP scores do not always correlate with clinical severity *(3, 4)*. CAP sensitivity, specificity, and positive predictive values agree well with skin prick tests (SPTs) for house dust mites and grasses, but poorly with tests for cat dander and peanuts *(5)*.

Microarray technology potentially offers advantages in diagnostic applications such as allergy testing because the amount of reagent required, and thus the cost per assay, is greatly reduced *(6)*. This approach has been difficult to reduce to practice, however, because the extremely small volumes (~0.5–5 nL) of sample used to create spots on these microarrays require extremely sensitive methods of analyte detection *(7)*.

We have used rolling circle amplification (RCA) *(8)* for the detection of antibody bound to antigen *(9)*. In this "immunoRCA", the 5′ end of a RCA primer is attached to an antibody; thus, in the presence of circular DNA, DNA

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polymerase, and nucleotides, the rolling circle reaction produces a concatamer of circular DNA sequence copies that remain attached to the antibody. The amplified DNA can be detected by hybridization of complementary oligonucleotide probes. ImmunoRCA, therefore, represents a novel approach for signal amplification of antibody-antigen recognition events on microarrays.

ImmunoRCA can detect IgE in a format using high-density microarrays of anti-human IgE printed on glass slides by a pin-tool type microarraying robot *(9)*. Here, we describe the production of microarrays of multiple allergens and demonstrate the utility of these microarrays in combination with immunoRCA to simultaneously detect allergen-specific IgEs for multiple allergens in patient samples.

We studied a population of 30 patients attending an allergy outpatient clinic (14 males, 16 females; age range, 2–47 years). A standard clinical questionnaire was used, which sought symptoms related to inhaled allergens and exposure to nuts.

Skin prick testing was performed with grass pollen (25 g/L), *Dermatophagoides pteronyssinus* (12 g/L), cat fur (10^6 QAU/L; Bencard), and peanuts (10 HEP; ALK Soluprick SQ; ALK-Abello A/S). A 10 g/L histamine solution was used as a positive control, with normal saline as a negative control. A standard skin prick technique was used, with weal diameter measured at 15 min. A weal diameter >3 mm was regarded as positive.

Allergen-specific IgE was detected in undiluted sera by use of the AutoCAP system (Pharmacia). Results were expressed as class 0–6. Total serum IgE was also measured by the AutoCAP system.

For allergen microarrays, extracts of cat hair, house dust mites (*D. farinae* and *D. pteronyssinus*), and peanuts (ALK-Abello) were passed over PD-10 columns (Pharmacia) to remove low-molecular weight components and then concentrated by ultrafiltration on Centricon YM-3 filters (Millipore). Spotting of the extracts onto activated glass slides was accomplished using a pin-tool type microarrayer (GeneMachines) as described previously *(9)*. Arrays were blocked with protease-free bovine serum albumin (20 g/L), air-dried, and stored under nitrogen at 4 °C until use.

The immunoRCA conjugate consisted of monoclonal anti-human IgE antibody (PharMingen), activated with the heterobifunctional cross-linking agent *N*-[γ-maleimidobutyryloxy] succinimide ester, conjugated to a 40mer thiolated oligonucleotide primer, and purified as described previously *(9)*.

In the immunoRCA method, 10 μL of human serum was added to each array and incubated for 30 min at 37 °C in a humidity chamber. After the arrays were washed twice in phosphate-buffered saline with Tween 20 (0.5 mL/L), the mouse monoclonal anti-IgE antibody DNA conjugate and its complementary circular DNA were applied to each array and incubated at 37 °C for 30 min. RCA was carried out at 37 °C for 30 min, using T7 native DNA polymerase as described previously *(9)*. The RCA product was detected by hybridization with a complementary oligonucleotide labeled with the fluorophore Cy3. Slides were scanned in a General Scanning Luminomics 5000 microarray scanner at a 10-μm resolution with a laser setting of 75 and a photomultiplier tube setting of 65. Mean pixel fluorescence intensity was quantified using the fixed-circle method in the QuantArray software.

In a scanning image of an allergen microarray incubated with serum from a patient with multiple allergies (Fig. 1A), positive signals could be seen from spots of peanut, cat dander, and mite allergens. Signals could also be seen from spots of an oligonucleotide that served as the primer for the circular DNA used in the RCA reaction. The sequence of this primer was the same as the one conjugated to the anti-IgE antibody; consequently, these spots served as positive controls for the RCA reaction on the microarray. Aliquots of IgE were also spotted onto the array as positive controls for the DNA-conjugated anti-IgE. In Fig. 1A, signals from these spots can be seen at the center of the bottom of the image.

Experiments were carried out to examine the performance characteristics of the microarray-based allergen-specific IgE assay. In one experiment, a serum sample from a patient with a CAP score of 6 for peanut IgE was serially diluted into peanut IgE-negative serum and assayed on allergen microarrays. A signal from peanut-specific IgE was observed up to a 1000-fold dilution; importantly, the dilution–response curve was linear (r = 0.87) over this range (data not shown). In another experiment, serum from a patient with peanut allergy was mixed with different sera from a panel that included multiple births, first-trimester pregnancy, third-trimester pregnancy, increased triglycerides, anti-nucleoprotein antibodies, hemolyzed blood, rubella, Epstein-Barr virus, increased IgM, toxoplasmosis IgG, syphilis, dialysis, increased cholesterol, and increased liver enzymes to test for assay interferences. None of the 14 different potentially interfering specimens had a significant effect on the peanut-specific IgE signal. Conversely, none of the interfering samples gave rise to a peanut-specific IgE signal when mixed with serum from a patient who was not allergic to peanuts. Finally, high serum IgE did not appear to interfere with the immunoRCA microarray assay; for example, a patient with angioedema and a total serum IgE of 432 kIU/L was negative for all allergens on the microarray. Additional work will be needed to better define these and other performance characteristics of the allergen microarray/ImmunoRCA assay.

ImmunoRCA on allergen microarrays was compared with the Pharmacia CAP test in 30 patients for the diagnosis of IgE-mediated allergy to several allergens, including two species of house dust mite, cat dander, and peanuts, to assess whether the new microarray-based test system has similar or better clinical relevance than CAP. Allergy diagnosis was based on clinical history and SPTs. The results shown in Table 1 indicate that immunoRCA was more sensitive than CAP for peanuts and cat dander, but not house dust mites. The increase in sensitivity afforded by immunoRCA was most pronounced for pea-

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nut allergen. Fig. 1B shows a microarray image from a patient with a positive SPT to peanuts but who tested negative by CAP; with immunoRCA detection, positive signals from peanut spots can be seen. ImmunoRCA was more specific than CAP for all allergens, and the specificity of the new test was always >90%. Fig. 1C shows a clearly negative immunoRCA assay for a patient allergic to eggs who had a CAP score of 3 for peanut-specific IgE but a negative SPT for peanuts.

Although only a small group of allergens was examined for a relatively small group of patients, the data obtained to date indicate that immunoRCA on microarrays provides an allergen-specific IgE assay with good clinical accuracy. A striking feature of this preliminary data is the good correlation between immunoRCA and skin prick testing. One factor behind the observed clinical accuracy is that the allergens used for microarray production are the same type of preparations used for SPTs; the use of these expensive reagents is economically feasible in the microarray product format because only subnanoliter amounts are used per assay. Further gains in sensitivity for detection of allergen-specific IgE may be feasible because the anti-IgE antibody used in the immunoRCA detection scheme has not yet been optimized.

In addition to clinical accuracy, a desirable feature in this new diagnostic test is an automated, high-through-

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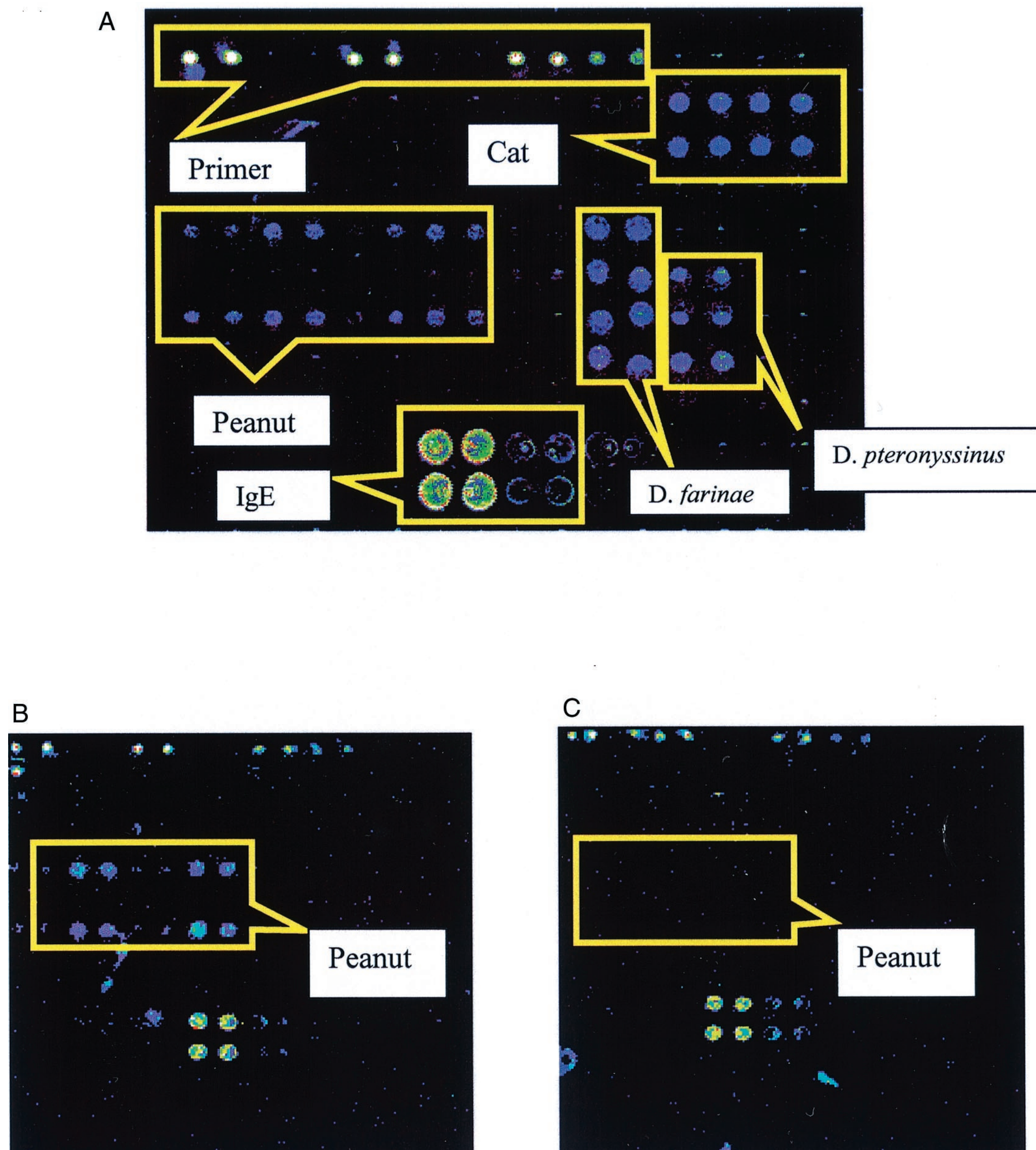

Fig. 1. ImmunoRCA microarray detection of allergen-specific IgEs in patient sera.

(*A*), scanning image of a microarray incubated with serum from a multiple-allergy patient. (*B*), scanning image of a microarray incubated with serum from a patient with a positive SPT for peanut allergy but who was negative for peanut allergy by CAP. (*C*), scanning image of a microarray incubated with serum from a patient with a negative SPT for peanut allergy but who was positive for peanut allergy by CAP.

Table 1. Sensitivity, specificity, and positive predictive value (PPV) of CAP and ImmunoRCA.[a]

Assay	Mites			Cat dander			Peanuts		
	Sensitivity, %	Specificity, %	PPV %	Sensitivity, %	Specificity, %	PPV, %	Sensitivity, %	Specificity, %	PPV, %
CAP	100	71	67	73	78	79	74	79	82
RCA	83	92	91	79	100	100	88	100	100

[a] CAP and RCA results were compared with SPTs performed as described in the text. Nine patients had a positive SPT to dust miles (21 negative), 10 patients had a positive SPT to cat dander (20 negative), and 14 patients had a positive SPT to peanuts (16 negative). A CAP score ≥1 was considered positive. An immunoRCA result was considered positive if fluorescence from microarray spots for a particular allergen was greater than the background signal from microarray spots of an anti-prostate-specific antigen antibody. An immunoRCA result was considered positive for mites if either *D. farinae* or *D. pteronyssinus* spots showed a positive signal.

put, low-cost format because most allergen-specific IgE testing currently is performed in regional reference laboratories. To that end, the immunoRCA microarray assay has been adapted to glass slides with 16 microwells, each separated by a Teflon mask. Microarrays of 100–400 spots can be printed in each microwell; consequently, it is possible to carry out ~4000 assays per slide. Each of these wells can be used to assay different patient samples or negative or positive controls. Internal control features are included on each array to permit more rigorous standardization of results for each patient or each allergen than is currently possible with CAP; this feature allows for more meaningful serial testing of allergic patients. Finally, semi-automation of the immunoRCA assays on allergen microarrays in this multiwell format has been implemented in our laboratory on an Beckman BioMek liquid-handling robot.

The microarray-based immunoRCA assay is applicable to other multiplexed antibody assays. For example, certain immunological reactions are caused by specific IgG_4 rather than IgE *(10)*. The use of an anti-human IgG_4 conjugated to a DNA primer complementary to a DNA circle that is different in sequence from the one coupled to the anti-human IgE antibody would allow the simultaneous measurement of allergen-specific IgG_4 and IgE. Such an assay would potentially be of use during allergen desensitization therapy or for monitoring response to anti-IgE therapy *(11)*. The enormous multiplexing capabilities of immunoRCA on microarrays, both spatial (i.e., the ability to detect multiple analytes on the array) and colorimetric (i.e., the ability to detect and differentiate multiple antibody types binding to each analyte), would potentially be useful for other clinical diagnostic tests involving detection of multiple specific antibodies, such as autoantibodies in suspected systemic autoimmune disorders, inflammatory arthritis, organ-specific autoimmune disorders, or in histocompatibility testing. Additional applications include infectious disease diagnostics with measurement of strain- and species-specific IgM and IgG, as well as in vitro testing of functional antibody responses in patients with suspected primary and secondary immunodeficiency diseases.

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Macroprolactin and the Roche Elecsys Prolactin Assay: Characteristics of the Reaction and Detection by Precipitation with Polyethylene Glycol, *Michael Fahie-Wilson,[1*] Penelope Brunsden,[1] John Surrey,[2] and Anthony Everitt[2]* ([1] Department of Clinical Chemistry, Southend Hospital, Prittlewell Chase, Westcliff-on-Sea, Essex SSO ORY, United Kingdom; [2] Department of Clinical Chemistry, Basildon Hospital, Nether Mayne, Basildon, Essex SS16 5NL, United Kingdom; * author for correspondence: fax 44-1702-221059, e-mail mike.fahie-wilson@hospital.southend.nhs.uk)

Prolactin occasionally is present in serum in a macromolecular complex. The most common form is macroprolactin, an antibody-antigen complex of prolactin (PRL) and immunoglobulin G with a molecular mass of 150–170 kDa *(1–4)*. The PRL component remains reactive (to various degrees) in immunoassays for PRL *(5–7)*, and macroprolactin is cleared more slowly than PRL from the circulation, leading to apparent hyperprolactinemia *(8)*.

Macroprolactin is bioactive in vitro but has minimal bioactivity in vivo, probably because of the failure of the high-molecular mass complex to cross the capillary mem-

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