agreement with specific serum IgE determinations and skin prick tests performed on the sample donor. Concordance tables were constructed to compare the HY-TEC BHR with skin prick tests and specific IgE tests for all of the activators used in the study. Similar comparisons were done using only the data from the two inhalant activators, Timothy grass and June grass pollen extracts. Table 1 shows the concordance with skin prick activators, and the Biomerica tracts. Table 1 shows the concordance with skin prick activators, Timothy grass and June grass pollen extracts were done using only the data from the two inhalant activators used in the study. Similar comparisons were made by other investigators using different assay methods.

The BHR method presented here is rapid, precise, specific, fully automated, and has potential uses in a number of clinical situations. We conclude that the many advantageous features of the HY-TEC BHR Assay will make it attractive to clinical investigators as a powerful adjunct for the diagnosis and treatment of allergy and hypersensitivity.

### Table 1. Clinical performance.

<table>
<thead>
<tr>
<th>Food and grass activators</th>
<th>Number of positives</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Concordance</th>
<th>Total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>HY-TEC BHR vs Skin prick tests</td>
<td>16</td>
<td>87.5%</td>
<td>85.0%</td>
<td>85.7%</td>
<td>56</td>
</tr>
<tr>
<td>Specific IgE</td>
<td>15</td>
<td>83.3%</td>
<td>86.8%</td>
<td>85.7%</td>
<td>56</td>
</tr>
<tr>
<td>Biomerica RIA</td>
<td>21</td>
<td>76.2%</td>
<td>88.6%</td>
<td>83.9%</td>
<td>56</td>
</tr>
<tr>
<td>Grass activators</td>
<td>HY-TEC BHR vs</td>
<td>Skin prick tests</td>
<td>10</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Specific IgE</td>
<td>11</td>
<td>90.9%</td>
<td>100%</td>
<td>93.8%</td>
<td>16</td>
</tr>
<tr>
<td>Biomerica RIA</td>
<td>11</td>
<td>90.9%</td>
<td>100%</td>
<td>93.8%</td>
<td>16</td>
</tr>
</tbody>
</table>

DNA microarray technology is revolutionizing the analysis of genetic information by enabling the high-throughput analysis of gene mutations and gene expression. This has been made possible by the development of technologies that can synthesize thousands of oligonucleotide probes in situ (1), and high-speed robotic “printing” of cDNA probes (2) on a glass chip coupled with fluorescent labeling and detection of hybridized nucleic acids.

The technologies involved in such analyses are out of the reach of low-budget research and clinical laboratories. Here we demonstrate the use of chemiluminescent detection in a simple and inexpensive method of detecting plasmid DNA and oligonucleotide arrays on nylon membranes and glass slides.

A new method for rapid chemiluminescent detection of DNA arrays on nylon membranes and glass slides was developed using an ultrasensitive chemiluminescent reagent, Lumigen<sup>TM</sup> APS-5 (Fig. 1A). The speed, sensitivity, and resolution of DNA spotted in low- and medium-density arrays was assessed with this reagent using several solid supports.

Lumigen APS-5 Chemiluminescent Substrate (Lumigen, Inc.) is supplied as an optimized formulation containing the substrate Lumigen APS-5 and lucigenin in pH 8.8 Tris buffer. DNA was spotted manually with fine-tipped pipet prepared by drawing out a Pasteur pipet over a flame. Higher density arrays were applied under a dissecting microscope. Nylon membranes from several commercial suppliers were evaluated, and polylysine-coated glass slides were from Sigma Chemical Co. The spotting was done in rows or in 1 x 1 or 2 x 2 cm grids with a 1-mm spot separation to provide arrays of 100 and 400 spots, respectively. Spot size varied with the pipet tip and was not controlled. Spot size was also influenced by the wicking property of the membranes used (see below). The nominal amount of DNA delivered per spot was calculated from the volume and concentration of stock DNA divided by the number of spots applied. The nominal amount of labeled plasmid DNA per spot was 0.5 pg or 10 pg for direct detection, and 1, 10, or 100 pg or 1 ng for detection by hybridization. In the cystic fibrosis (CF) assay, the amounts of oligonucleotides delivered per spot varied between experiments from 1 to 40 ng. The spotted plasmid DNA and oligonucleotides on the membrane and glass slides were UV cross-linked for 30 s at 120 mJ/cm<sup>2</sup> with a Hoefer UV cross-linker.

Digoxigenin (dig)-labeled pBR328 plasmid DNA was spotted and detected without hybridization, using anti-dig-alkaline phosphatase (AP) conjugate and Lumigen APS-5 (Lumigen). In a hybridization format, unlabeled pBR328 plasmid DNA was spotted, hybridized with a dig-labeled pBR328 probe according to standard protocols, and then.

### References

The three genotypes, Section 1, hybridization with the N/N genotype followed by binding with anti-fluorescein-AP and chemiluminescent detection revealed the N oligonucleotide probe; section 2, hybridization with the N/D genotype and mutant alleles of the CF genotypes, N/N, N/D, and D/D, specific sequences were selectively detected by hybridizing the arrays with fluorescein-labeled (anti-sense strand) PCR products of the N/N, N/D, and D/D genotypes, binding with anti-fluorescein-AP, and chemiluminescent detection.

The Lumigen APS-5 reagent provides highly sensitive and rapid room temperature detection of AP labels (3) by a unique cocatalyzed reaction involving AP and lucigenin, as shown in Fig. 1A. We reasoned that the efficient generation of chemiluminescence at room temperature would permit imaging without concern for evaporation. Furthermore, the speed of reaching peak intensity would minimize diffusion of the emitter and potential spreading of spots before images are obtained. Nevertheless, the ability to achieve adequate spatial resolution was uncertain because, in this reaction, the emitter is in solution rather than being fixed to the solid support, as are fluorescent labels. At the array densities tested, all spots were clearly detected within 1 min of the start of the experiment. Interestingly, image quality (spot size) was unaffected even after 2 h, indicating that the signal is relatively constant and that diffusion of the emitter is not an issue.

We observed substantial variability between different sources of nylon membrane in apparent spot size (data not shown), probably because of differences in membrane porosity and hydrophilicity. The membranes tested were Boehringer Mannheim positively charged membrane (BM), Amersham Hybond N (HN), and uncharged membranes MSI Magnagraph (MS) and Pall Biodyne A (BA). The order of apparent spot size was MS > BM > HN > BA. Comparative signal intensity was BM > HN. On the basis of these two criteria, quantitation was best achieved using the BM and HN membranes. Polysynme-coated glass slides provided acceptable results; however, uncoated glass slides did not provide adequate surface adhesion of DNA.

Feasibility has been demonstrated for a rapid, inexpensive, and robust method of chemiluminescent analysis of DNA arrays on nylon membranes or coated glass slides. The technique requires no expensive instrumentation and can be performed using commonly available materials. Key to the success of the method was the rapid detection at room temperature afforded by Lumigen APS.

Fig. 1. Reaction scheme for (A) and example of genotype detection (B) by the Lumigen APS-5 substrate.

(A) Reaction scheme showing a plausible mechanism for the production of chemiluminescence by Lumigen APS-5 substrate in the presence of alkaline phosphatase. (B) Detection of the CF genotypes in DNA arrays. Unlabeled oligonucleotide probes specific for the sense strand of the wild-type (N) and ΔF508 (Δ) alleles were each applied in medium-density arrays. The pattern of the spotting of the two probes consists of rows of four spots of the N probe and rows of two spots of the Δ probe, as revealed by chemiluminescent visualization. The arrays were hybridized with fluorescein-labeled (antisense primer) PCR products of the three genotypes. Section 1, hybridization with the N/N genotype followed by binding with anti-fluorescein-AP and chemiluminescent detection revealed the N oligonucleotide probe; section 2, hybridization with the N/Δ genotypic DNA and treatment as above revealed both the N and Δ oligonucleotide probes; section 3, hybridization with the Δ/Δ genotypic DNA detected only the Δ oligonucleotide probe.

bound and reacted with anti-dig-AP and APS-5. The signal was captured with either a charge-coupled device (CCD) camera or x-ray film.

For the CF oligonucleotide hybridization experiment, the spotted oligonucleotides specific for the wild-type (N) and mutant alleles of the ΔF508 mutation were hybridized with dig- or fluorescein-labeled PCR products of each of the CF genotypes, N/N, N/Δ and Δ/Δ, according to customary protocols.

Solutions of labeled pBR328 plasmid DNA at various concentrations were applied at various densities on nylon membrane and hybridized with the dig-labeled DNA of the same plasmid. Binding with anti-dig-AP followed by chemiluminescent detection produced images that were detectable as individually resolved spots in 1-min exposures of x-ray film for >2 h without change in the apparent size of the individual spots. Similar images were obtained using a cooled CCD camera system. DNA arrays with nominal amounts ranging from 10 to 0.5 pg/spot each produced sufficient signal intensities. Similar results were obtained when detecting unlabeled pBR328 DNA in a hybridization format. DNA on both membranes and glass slides was detectable in the picogram range with x-ray film exposures of ≤1 min.

APS-5 was also used in an assay for the differential detection of the CF genotypes in DNA arrays. Unlabeled oligonucleotides specific for the sense strand of the wild-type and ΔF508 alleles were each applied in alternate rows of an array (Fig. 1B). The wild-type (N) and mutation (Δ) specific sequences were selectively detected by hybridizing the arrays with fluorescein-labeled (anti-sense strand) PCR products of the N/N, N/Δ, and Δ/Δ genotypes, binding with anti-fluorescein-AP, and chemiluminescent detection.

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References