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An automated microfluidic-based immunoassay cartridge for allergen screening and other multiplexed assays

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ABSTRACT

A microfluidic cartridge and system for multiplexed immunoassays is described. The passive microfluidic cartridge was composed of three layers of injection molded plastic sealed together using a thermal staking technique. Using this platform technology, a specific immunoglobulin E (IgE) panel assay was constructed. Allergen extract targets, positive and negative controls, and IgE calibration standards were immobilized within the cartridge as a microarray. A computer-controlled solenoid array provided the necessary actuation force for pumping reagents within the cartridge to perform an automated, chemiluminescent indirect immunoassay. A 20-target allergen extract panel was demonstrated on the device with a total analysis time of 27 min. Allergen screening results showed 84% agreement for 3 house dust mites ($N = 300$) compared with a commercial test and 80% agreement overall ($N = 978$). Average coefficients of variation ($N = 80$) were measured as 20.5% for low/medium levels and 20.4% for medium/high levels. The average limit of detection ($N = 160$) was measured at 0.535 AU, and cutoff levels of 1.0 AU were estimated at less than 1 IU/ml (2.4 ng/ml). Such a system has potential applications in decentralized allergen screening as well as in other near-patient diagnostic immunoassays where multiplexed analysis, ease of use, and short analysis time are critical.

For more than 30 years, in vitro testing of allergen-specific immunoglobulin E (IgE)[1] has been used along with, or as an alternative to, invasive skin prick testing [1–3]. Such tests allow allergists to accurately monitor immunotherapy techniques as well as to screen adults, infants, and small children for atopic allergic sensitivities. Clinically, the most common in vitro technique today is the ImmunoCAP System (PhaDia, Uppsala, Sweden) [4], but other tests are becoming more widely used, including the CLA Allergy Test (Hitachi Chemical Diagnostics, Mountain View, CA, USA) [5,6], AlaSTAT and IMMULITE (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) [7,8], and HY-TEC (Hycor Biomedical, Garden Grove, CA, USA) [9,10], and a variety of multiple allergen-coated nitrocellulose strip tests [11]. Of these, several tests are available as quantitative assays (e.g., ImmunoCAP, IMMULITE, HY-TEC), where specific IgE response is quantified using a nonspecific IgE calibration curve and results are expressed as international units (IU) per milliliter (IU/ml, where 1 IU is equivalent to 2.4 ng). Because currently there are no specific IgE standards available, it has been noted that specific IgE results can vary from test to test due mainly to differences in allergen source materials [12]. Other tests (e.g., CLA) are available as semiquantitative, and results are reported on a continuous scale using arbitrary units. In both cases, results are typically separated into class scores.

Recently, there has been increased interest in the use of microarrays for the analysis of specific IgE levels in serum. The advantages of microarrays for allergen screening are the ability to provide a multitarget screening technique (50+) while using minimal serum (25–100 μl). This is even more critical for allergen screening in small children, where large blood draws are difficult and serum supply is limited. Schweitzer's group used microarrays and rolling circle amplification to detect specific IgE [13,14]. Signal amplification using streptavidin–horseradish peroxidase (HRP) conjugate and a fluorescent substrate was later employed in a seven-allergen panel using a microarray [15]. Cutoff levels achieved on the microarray were 1 kIU/L. A house dust mite and food extract microarray was also demonstrated using a streptavidin–cyanine-3 (Cy3) conjugate with clinical limits of detection (LODs) near 1 kIU/L [16]. Recombinant proteins have also been demonstrated on microarrays for grass and tree pollen specific IgE detection using a

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[1] *Abbreviations used:* IgE, immunoglobulin E; IU, international units; HRP, horseradish peroxidase; Cy3, cyanine-3; LOD, limit of detection; RT-PCR, reverse transcription polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; CCD, charge-coupled device; ABS, acrylonitrile, butadiene, and styrene; CV, coefficient of variation; AU, arbitrary units; SD, standard deviations.

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fluorescently labeled anti-IgE conjugate for detection [17]. Typical incubation times for specific IgE detection on microarrays is 60 min per step. Therefore, total reaction times for these assays were between 2 and 3 h. Additional time is required for washing the arrays between steps and scanning the arrays in a fluorescent scanner. Chemiluminescence detection has also been reported in microarray analysis of specific IgE using a flow cell [18]. Total analysis times were reduced to 1 h plus washing by shortening incubation times to 30 min. In the case of chemiluminescence, LODs for both allergen extracts and recombinant proteins were similar to those for the other fluorescence-based methods. A high-sensitivity colorimetric-based microarray was used to test allergic response to mold, Timothy grass, and dust mite allergies with analysis times between 2 and 3 h [19].

Although progress has been made in reducing reaction times for allergen microarray analysis, little has been done to improve the automation of such analysis outside the employment of large robotic workstations. Microfluidic devices provide much promise in the area of automation for microarray analysis and have potential to speed up analysis times due to high surface-to-volume ratios and active mixing. Such devices have performed HIV subtyping using reverse transcription polymerase chain reaction (RT-PCR) followed by microarray analysis [20], performed DNA purification and real-time PCR with the TaqMan probe for infectious disease detection [21], and performed DNA amplification followed by capillary electrophoresis analysis [22,23]. In the area of immunoassays, compact disk-like microfluidic platforms have been developed to carry out automated enzyme-linked immunosorbent assay (ELISA) reactions in the laboratory [24] and in clinical settings [25]. Platforms such as these offer parallel analysis of many samples on the same disk and may be advantageous for a variety of clinical immunoassays. Multiplexed bead-based immunoassays have been in use for years [26], and the use of microfluidics has been proposed to miniaturize these assays as well [27]. The challenge for microfluidic devices in protein-based diagnostics is ensuring that the benefits outweigh the extra cost. Many diagnostic applications already have a rigorous cost structure that makes it difficult to justify the additional cost of microfluidic devices.

In this article, we report the development of a microfluidic cartridge for the automated analysis of specific IgE using allergen extracts. The device was entirely injection molded, and protein was bound to the surface using an inexpensive nitrocellulose coating. The material cost of the device was less than one-tenth that of the activated slide typically employed in microarray experiments. Furthermore, the cartridge was driven by a low-cost analyzer composed of a solenoid actuator array and a low-resolution cooled charge-coupled device (CCD) camera for chemiluminescence detection. The total analysis time for allergen screening on the cartridge was less than 30 min. This system has potential for decentralized allergen screening and near-patient testing where short analysis times, ease of use, and low instrumentation cost are critical.

Materials and methods

Cartridge design

The microfluidic cartridge for allergen screening incorporated five reagent delivery channels consisting of storage tanks and unidirectional pumps, a single reaction zone in which allergen extracts are immobilized, and a waste tank to contain all reaction by-products. Fig. 1 shows a photograph of the cartridge. Each delivery channel used a different-size reagent tank. The tank size was determined by the function of that particular reagent. The cartridge was constructed from three injection molded plastic parts. Transparent general-grade polycarbonate was used for the upper

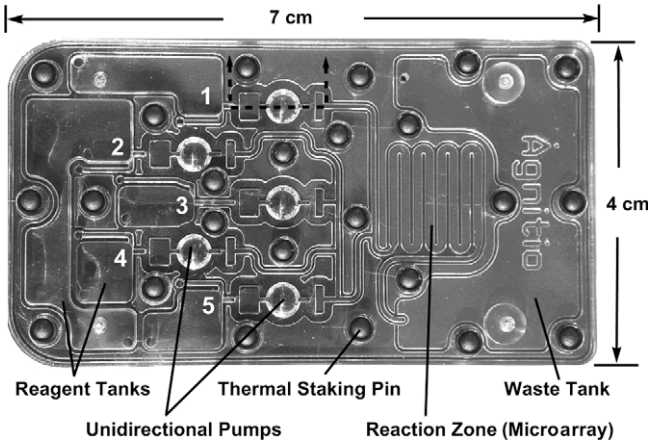

Fig. 1. Injection molded microfluidic cartridge for automated immunoassays. The top view photograph shows five reagent delivery channels and a single reaction zone.

part. Polycarbonate was chosen due to its better stability during thermal assembly processes. The upper part was 3 mm thick. The middle layer was composed of reaction injection molded silicone rubber that was 0.5 mm thick with a hardness of 40 Shore A. Black was the preferred color of this layer due to lower background during chemiluminescence detection. White color additives were found to contain a high percentage of slow-decaying phosphorescent particles that interfered with signal detection. The 3-mm-thick bottom layer was constructed from a copolymer of acrylonitrile, butadiene, and styrene (ABS).

Fig. 2 shows a schematic of the pump structure constructed from these three plastic layers. The unidirectional pump was composed of two passive check valves and a pumping chamber. An external linear actuator was required to displace the silicone diaphragm of the pumping chamber. On displacement, the check valve limits flow in one direction only. The check valves were of different sizes due to the different displacement mechanisms of the pump diaphragm. Because positive displacement forces were provided by the linear actuator, high positive pressures could be achieved and, therefore, the forward check valve was smaller and "tighter." The returning force, however, was due only to the stretching of the silicone diaphragm itself and, therefore, the neg-

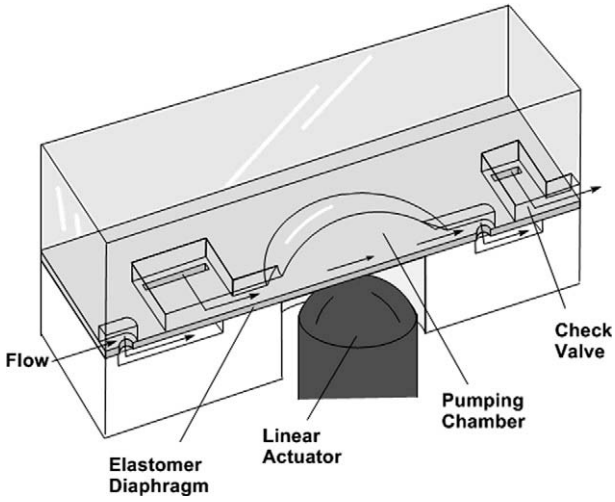

Fig. 2. Cross-sectional schematic of the pump structure corresponding to the dotted line in Fig. 1.