A fully integrated microfluidic genetic analysis system with sample-in–answer-out capability

Christopher J. Easley*, James M. Karlinsey*, Joan M. Bienvenue*, Lindsay A. Legendre*, Michael G. Roper*, Sanford H. Feldman†, Molly A. Hughes‡, Erik L. Hewlett‡, Tod J. Merkel§, Jerome P. Ferrance*, and James P. Landers*¶‖

*Department of Chemistry, University of Virginia, Charlottesville, VA 22904; Departments of †Comparative Medicine, ‡Infectious Disease, and ¶Pathology, University of Virginia Health System, Charlottesville, VA 22908; and §Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Bethesda, MD 28092

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We describe a microfluidic genetic analysis system that represents a previously undescribed integrated microfluidic device capable of accepting whole blood as a crude biological sample with the endpoint generation of a genetic profile. Upon loading the sample, the glass microfluidic genetic analysis system device carries out on-chip DNA purification and PCR-based amplification, followed by separation and detection in a manner that allows for microliter samples to be screened for infectious pathogens with sample-in–answer-out results in <30 min. A single syringe pump delivers sample/reagents to the chip for nucleic acid purification from a biological sample. Elastomeric membrane valving isolates each distinct functional region of the device and, together with resistive flow, directs purified DNA and PCR reagents from the extraction domain into a 550-nl chamber for rapid target sequence PCR amplification. Repeated pressure-based injections of nanoliter aliquots of amplicon (along with the DNA sizing standard) allow electrophoretic separation and detection to provide DNA fragment size information. The presence of *Bacillus anthracis* (anthrax) in 750 nl of whole blood from living asymptomatic infected mice and of *Bordetella pertussis* in 1 μl of nasal aspirate from a patient suspected of having whooping cough are confirmed by the resultant genetic profile.

full integration | micro total analysis system | microdevice | pumping | valving

The next revolution in personalized medicine, forensic science, and biowarfare defense will be impelled by analysis systems that provide a quantum leap in terms of functionality, time to result, and cost effectiveness. These systems need to meet several requirements, including a design conducive with low-cost manufacturing, turn-key operation with fast analysis times, and the ability to manipulate small volumes from crude samples. One example is the micrototal analysis system (μ-TAS) described conceptually more than a decade ago by Manz *et al*. (1). Prophetically, they stated that, "... the detector or sensor in a TAS does not need high selectivity, because the sample pretreatment serves to eliminate most of the interfering chemical compounds." There are multiple examples in the literature of steps taken toward the advancement of integrated microfluidic genetic analysis (MGA) systems (refs. 2–4; also see ref. 5 for a comprehensive review); however, after a decade and a half, no *bona fide* microfluidic device has been presented that is capable of nanoliter flow control and integration of an electrophoretic separation with comprehensive sample pretreatment (DNA purification and PCR amplification).

The MGA system described in this report brings together many advances in microfluidics over the last decade, exploiting differential channel flow resistances (6), elastomeric valves (7, 8), laminar flow (9), and electrophoretic mobility within the device, in concert with external fluid flow control from a syringe pump for sample and reagent delivery. Nucleic acid purification through solid-phase extraction (SPE), followed by target sequence amplification by PCR and microchip electrophoretic (ME) amplicon separation and detection is completed in <30 min. This represents a previously undescribed integrated microfluidic system that can accept biological samples as crude as whole blood, extract high-purity nucleic acids, and generate a PCR-targeted amplicon that can be characterized to provide a genotypic readout.

Results

Microdevice Design. The MGA system has a microchannel architecture with three distinct functional domains, two for sample preparation (SPE and PCR) and one for analysis (ME) (Fig. 1). A total of five elastomeric normally closed valves (8) direct flow from a single syringe pump and localize the chemistries and reaction conditions that exist (Fig. 1*b*). The reagents used for DNA extraction in the SPE domain were isolated from the PCR chamber (valve V_1), because these are known PCR inhibitors. The PCR domain, gated from the ME domain by two valves (V_3 and V_4), must be passivated to avoid protein fouling and deactivation of the Taq polymerase. Valves V_3 and V_4 function to gate the two domains and/or pump amplicon from the PCR chamber, whereas the DNA standard from the marker reservoir is injected with valves V_2 and V_5, respectively. The sample is mobilized across the analysis channel for injection, after which the components are separated and detected by laser-induced fluorescence.

Flow Control and Method Development. The major challenge associated with integrating sample treatment steps into a microfluidic format is the incompatibility of SPE reagents (guanidine and isoproponal) with the PCR process. Fluidic isolation of the SPE and PCR domains was accomplished by combining several methods used in microfluidic flow control. Fig. 2 illustrates how differential channel flow resistances, elastomeric valves, and laminar flow are used to isolate SPE solvents from the other domains without compromising DNA extraction. The SPE domain consists of a sample inlet reservoir, a silica extraction bed, a patterned weir, a sidearm for solution loading, and an extraction waste arm (Fig. 2*a Left*). Lysed sample and 80% isopropanol (yellow) are sequentially delivered through the sample inlet and the replaceable silica bed, while distilled deionized water (red)

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Abbreviations: μ-TAS, micrototal analysis system; MGA, microfluidic genetic analysis; SPE, solid-phase extraction; ME, microchip electrophoretic; qPCR, quantitative PCR; PDMS, poly(dimethyl siloxane).

‖To whom correspondence should be addressed. E-mail: landers@virginia.edu.

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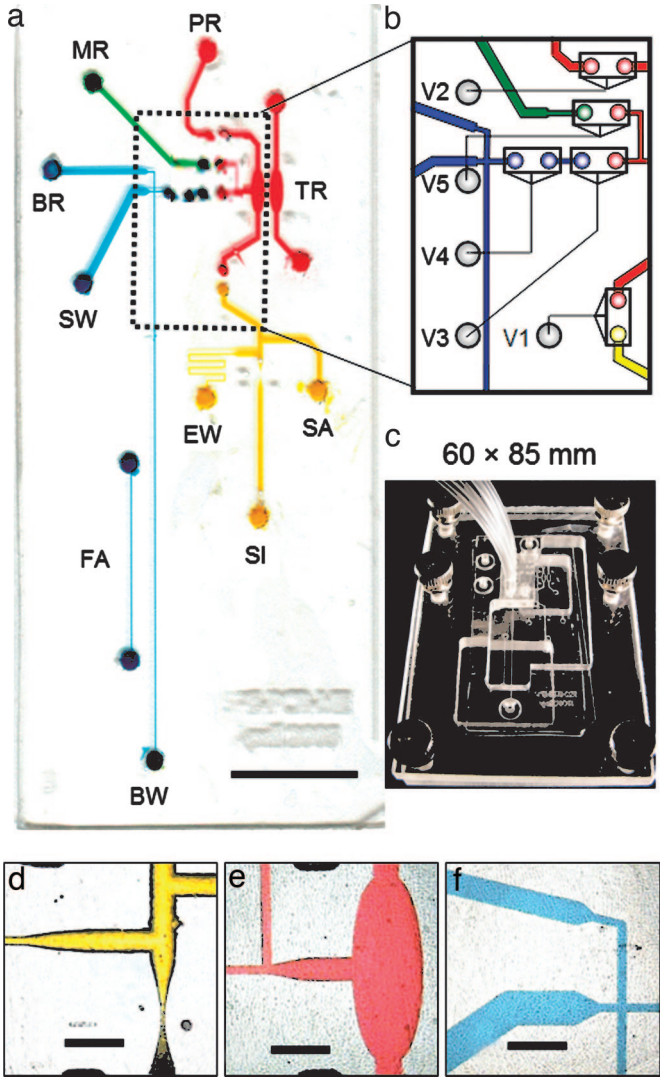

Fig. 1. Images of the MGA device. (*a*) Dyes are placed in the channels for visualization (Scale bar, 10 mm.). Domains for DNA extraction (yellow), PCR amplification (red), injection (green), and separation (blue) are connected through a network of channels and vias. SPE reservoirs are labeled for sample inlet (SI), sidearm (SA), and extraction waste (EW). Injection reservoirs are labeled for PCR reservoir (PR), marker reservoir (MR), and sample waste (SW). Electrophoresis reservoirs are labeled for buffer reservoir (BR) and buffer waste (BW). Additional domains patterned onto the device include the temperature reference (TR) chamber and fluorescence alignment (FA) channel. The flow control region is outlined by a dashed box. Device dimensions are 30.0 × 63.5 mm, with a total solution volume <10 μl. (Scale bar, 10 mm.) (*b*) Schematic of flow control region. Valves are shown as open rectangles. V_1 separates the SPE and PCR domains. V_2 and V_5 are inlet valves for the pumping injection, V_3 is the diaphragm valve, and V_4 is an outlet valve. (*c*) Device loaded into the manifold. (*d*) Intersection between SI and SA inlet channels, with the EW channel tapering to increase flow resistance. (Scale bar, 1 mm.) (*e*) Image of PCR chamber with exit channel tapering before intersecting with the MR inlet channel. (Scale bar, 1 mm.) (*f*) Image of cross-tee intersection. (Scale bar, 1 mm.) The relative sizes of the BR, SW, and BW channels create the difference in volume displacement during the pumping injection and affect how the resistance is dropped under an applied separation voltage.

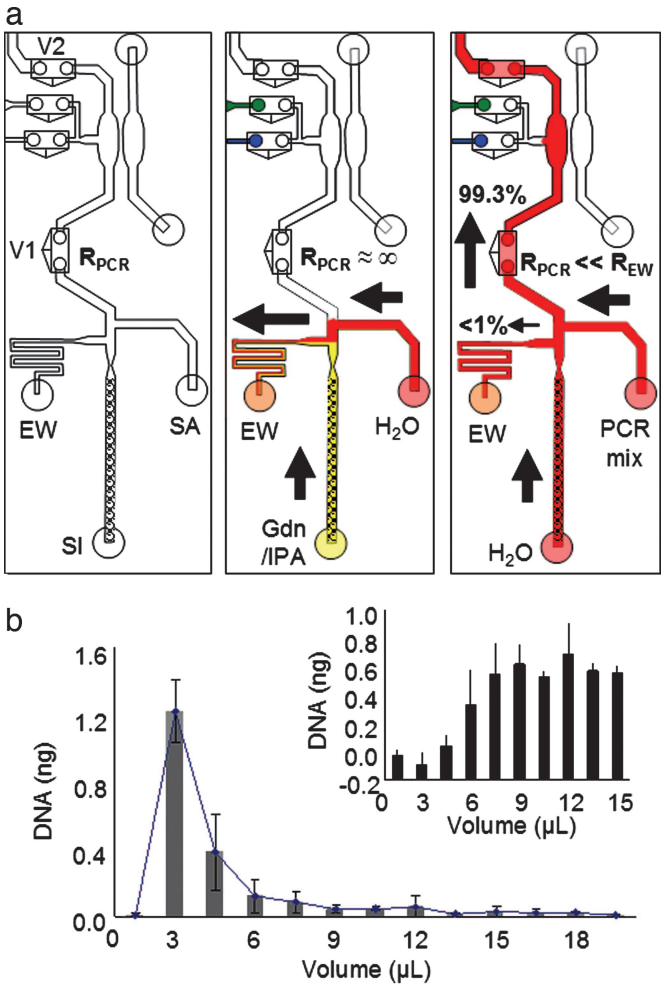

Fig. 2. Data and flow illustrations representing the coupling of SPE and PCR sample preparation steps on the MGA device using elastomeric valves and flow control preset by channel design. (*a*) Flow control between SPE and PCR was accomplished by using differential channel flow resistances, laminar flow, and valving. During the load and wash steps of SPE (center), valve V_1 is closed, making the flow path to PCR highly resistant compared with the extraction waste (EW) path ($R_{PCR} \approx \infty$), and directing all flow to EW. Note that because of laminar flow between the SA and SI channels, the guanidine-HCl and isopropanol solutions (yellow) never contact the valve seats. During the DNA elution step (*Right*), valves V_1 and V_2 are opened, allowing 99.3% of the flow (by calculation) to proceed to the PCR domain ($R_{PCR} \ll R_{EW}$). (*b*) Elution profile of a human genomic DNA extraction from blood using real-time qPCR to determine the amount of DNA eluted from the MGA device. The results demonstrate which volume fractions will be most appropriate for use in downstream PCR amplification in the fully integrated analysis. Replicate breakthrough profiles were also obtained (inset), and the capacity of the solid phase was determined to be 3.3 ng of DNA.

maintains solution flow through the sidearm (Fig. 2*a Center*). With valve V_1 closed during load and wash steps of SPE, the SPE and PCR domains are isolated, and flow is directed toward its only available path, to the elution waste. With this design, problems arising from the incompatibility of the poly(dimethyl siloxane) (PDMS) valves with organic solvents (10) are avoided, because the water (red) effectively serves as a barrier to organic solvents (yellow). During the elution step (Fig. 2*a Right*), valves V_1 and V_2 are opened to allow purified DNA to combine with 2× PCR mixture from the side arm and to be transferred to the PCR chamber. With these valves open, flow is driven to the PCR domain as the more shallow elution waste path functions as a large fluidic resistor. Dominant flow (>99%) through the PCR domain is achieved by a combination of balanced flow resistance ratios (6) and elastomeric valving technology (7, 8).

Having identified a method for fluidic control, the MGA device was tested to ensure contamination-free integration of SPE and PCR. Because the SPE process is not monitored on-line, chromatographic timing was established off-line. Frac-

tions (1.5 μl) were collected from the SPE bed outlet during extraction and evaluated for nucleic acids by fluorescence or for PCR-amplifiable DNA by quantitative PCR (qPCR). The fluorescence assay was used to determine the timing needed for valve V_1 opening to allow eluted nucleic acids to be transferred to the PCR chamber; however, qPCR revealed that the fractions with the largest mass of DNA did not contain the most PCR-amplifiable DNA. This trend is likely the result of PCR inhibition because of residual isopropanol contamination (11). Fig. 2*b* details the qPCR analysis with replicate DNA extractions from human whole blood. The majority of DNA was eluted in 2–5 μl, and fraction 2 consistently provided the most PCR-amplifiable DNA, thereby defining the timing for valve V_1. SPE capacity was determined by flowing human genomic DNA through the bed and measuring the breakthrough volume (Fig. 2*b Inset*), revealing a capacity of 3.3 ng for a whole blood lysate, a mass sufficient for downstream DNA amplification. After completion of SPE, flow control for the remainder of the analysis was maintained by using elastomeric valving/pumping. The valves (8) were used to isolate the purified DNA in the PCR domain during amplification, then to pump from the PCR domain to the ME domain for injection and analysis as described (12, 13).

Fully Integrated Genetic Analysis. In order for a μ-TAS to have value in clinical diagnostics or forensic genetic profiling, it must be capable of accepting whole blood and generating a genetic profile, a difficult task due to the multiple PCR inhibitors associated with this starting sample (14). The utility of the MGA device was evaluated with blood drawn from C57BL/6 mice injected i.p. with *Bacillus anthracis* spores before onset of symptoms. All blood samples were positive for *B. anthracis* colony-forming units, and all mice subsequently succumbed to infection. The blood was mixed with lysis buffer, and a volume equivalent to 750 nl (15–45 ng of murine DNA, exceeding the capacity of the device to ensure saturation) of whole blood was loaded for integrated analysis (Fig. 3*a*). The extraction was completed in <10 min and, upon capture of the purified DNA in the 550-nl PCR microchamber, amplification was invoked by using IR thermal cycling. Subsequently, a 211-bp fragment found on plasmid pX01 of *B. anthracis* was amplified in 11 min.

Postamplification, the product was pressure-injected into the separation domain, with a DNA sizing standard for electrophoretic evaluation (Fig. 3*b*). Injection of PCR-amplified product for ME interrogation has been accomplished, almost exclusively, by electrokinetic mobilization (3, 15–17). However, it has been shown that on-chip pressure injections provide more reproducible and representative sampling (12), and the added control provided by the valves proved essential to integration of multiple processes. Three valves (V_2, V_3, and V_4) provided a diaphragm pumping system (8) with the capability to directly inject amplicon (12, 13), whereas V_5 could be actuated simultaneously with V_2 to perform a coinjection of DNA marker. This method allowed for control of the relative volumes injected into the separation channel from two or more sources (13). In addition, the flow resistances in the separation domain were designed to direct the majority of the flow across the analysis channel and into sample waste (Fig. 1*f*), minimizing the plug width for separation (13).

After injection of amplified material, electrophoretic separation was performed under high fields with laser-induced fluorescence detection. With injection, separation and detection completed in <180 sec, total analytical time for the entire analysis (extraction, amplification, separation, and detection) was <24 min (Fig. 3*a*), an order of magnitude reduction in time relative to analysis using conventional methods. The ability to simultaneously inject DNA standard with amplified material from the PCR chamber provided a simple mechanism for determining both the presence (blank profile in Fig. 3*b*, "positive" in Fig. 3*c*) and size (Fig. 3*c Inset*) of the amplicon. The presence of anthrax in the sample was confirmed by the 211-bp product corresponding to the targeted sequence found on plasmid pXO1 of *B. anthracis*. Moreover, with only a few tens of nanoliters injected from the 550-nl PCR chamber, copious amounts of amplified material remained for replicate analysis. Multiple injection/separation cycles could be carried out after PCR for confirmation of the identity of the product peak, at a cost of only a few hundred seconds (Fig. 3*c*). When eight coinjections of amplicon with DNA standard were carried out, the resultant size was determined to be 211 ± 2 bp by using the local Southern sizing method (18). These results represent a previously undescribed instance in which an integrated microfluidic device was used for all processing and analysis steps in the

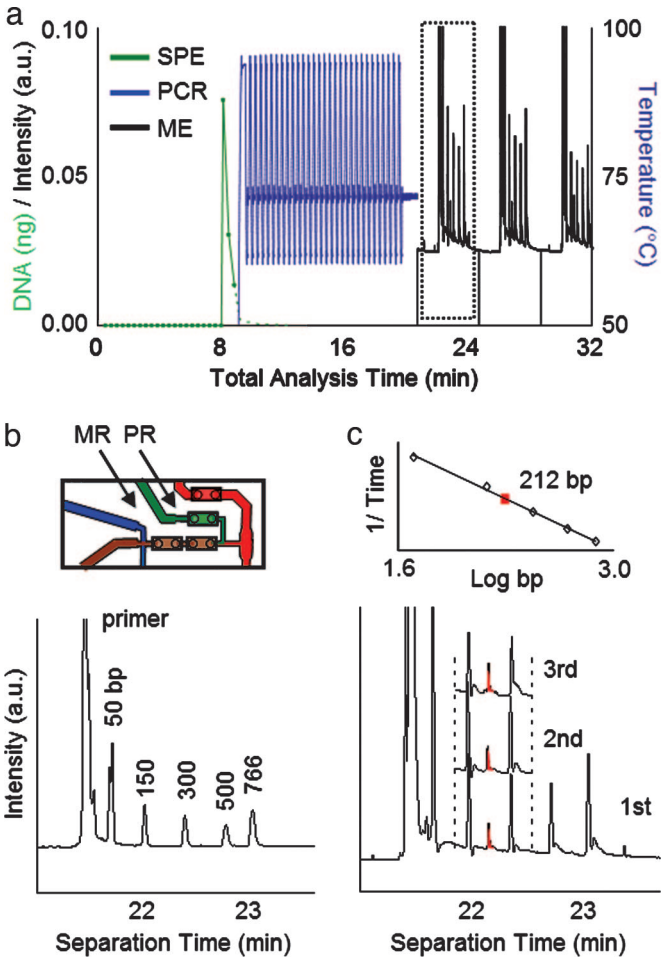

Fig. 3. Integrated detection of *B. anthracis* from murine blood. (*a*) Detector responses during all three stages of sample processing and analysis are portrayed in terms of total analysis time. The SPE trace (green) was taken from an offline DNA extraction of the same murine sample and is representative of the total DNA concentration observed in a typical extraction. The temperature (blue) and fluorescence intensity (black) represent online data, with a total analysis time of <24 min. Three sequential injections and separations were carried out to ensure the presence of amplified product. (*b*) Fluorescence data from an integrated analysis of a blank sample (no DNA loaded) control with marker peaks labeled. The cartoon (*Inset*) represents valve actuation during the coinjection, with the PR and MR pumping inlets indicated by the arrows. (*c*) Zoomed in view of the first separation shown in *a*, with the product peak marked and sized. The second and third runs are overlaid with the time axis cropped. The plot (*Inset*) shows the sizing curve of inverse migration time vs. log (base pairs) with both the sizing standard peaks (open diamonds) and product (red square) plotted for all three runs shown in *a* (error bars included). From these data, the product was sized as 211 ± 2 bp.

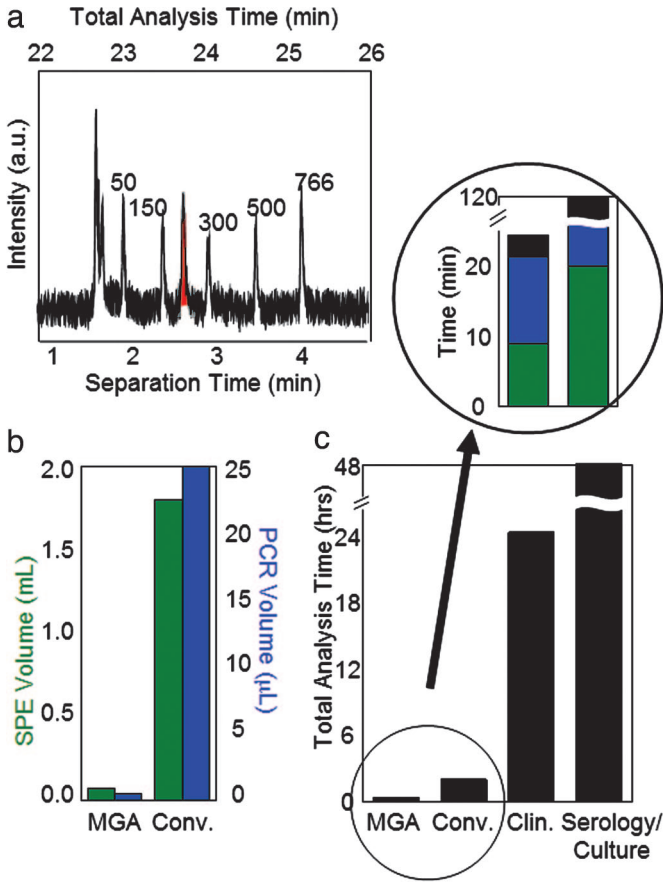

Fig. 4. Fully integrated microchip detection of *B. pertussis* from a human nasal aspirate in only 24 min. One microliter of human nasal aspirate was extracted, PCR was performed on the purified DNA, and products were pressure-injected and electrophoresed. (*a*) The ME trace was plotted alone to show the separation of the coinjected DNA sizing standard (peak sizes labeled in number of base pairs) with the PCR amplicon for product verification. The amplicon (red) migrates between the expected size standards, and sequencing analysis was used to further verify the product (see *SI Supporting Text*). (*b*) Volumes for SPE (green) and PCR (blue) are compared for MGA and Conv., showing a significant reduction for both processes. (*c*) Total analysis times for crude biological samples of the MGA device (from *a*), conventional analysis performed in the research lab (Conv.) and a clinical lab (Clin.), and analysis by serology/cell culture. Analysis times for MGA and Conv. are shown in (*Inset*), with SPE (green), PCR (blue), and ME (black) denoted.

direct analysis of a blood sample to genetically verify the presence of a pathogen in <25 min. Because the early detection of anthrax is critical to the survival of the host by early recognition and administration of antibiotics with postexposure vaccination, the MGA system and its integrated methods provide a microfluidic path to improving biodefense surveillance measures.

To demonstrate the broader utility of the MGA system, a different sample and nucleic acid target was evaluated. A nasal aspirate was obtained from a human patient symptomatic of whooping cough, a respiratory infection caused by the Gram-negative bacterium *Bordetella pertussis*, which can be isolated from the mouth, nose, and throat (19, 20). This infection is characterized by severe spasms of coughing that can last several weeks or months and, although not particularly threatening to those beyond their first year, it can lead to serious complications or fatality in infants (19, 20). Using the same method described above, a volume equivalent to 1 μl of nasal aspirate was prepared in lysis buffer and loaded into the MGA device, with DNA purification carried out as described earlier. The presence of *B. pertussis* can be confirmed by an amplification of a 181-bp fragment of the *IS481* repeated insertion sequence, and after PCR amplification of this target, the amplicon was injected into the separation channel for electrophoretic separation (Fig. 4*a*). Again, coinjection of a DNA sizing standard was used to aid in the sizing of amplified product for comparison with the expected 181-bp fragment, confirmed by off-chip sequencing of the resultant amplicon. With a total analysis time of 24 min, the MGA system could provide physicians with a method to rapidly screen for *B. pertussis* respiratory infection in patients during early infection/exposure or for screening during outbreaks. This technological advance is timely, because >25,000 *B. pertussis* cases were reported in 2004, a 12-fold increase since 1980 (19). The rapid turnaround time not only provides a dramatic improvement over conventional culturing methods for diagnosis [requiring a minimum of 24–48 h (20)] but also presents the possibility of point-of-care testing, a rapidly growing concept applicable to clinical diagnostics, forensics, environmental testing, food safety testing, and biothreat sensing in the field for armed forces.

Discussion

The advantages of the MGA system are obvious: rapid turnaround time, decreased reagent consumption per test, decreased operator variability (human error factor), and improved operator safety. The comparisons in Fig. 4 *b* and *c* showcase the capabilities of a MGA system with respect to reduction of volume analysis time. Fig. 4*c* compares the turnaround time of the MGA system for detecting *B. pertussis* from a sample, relative to conventional molecular-, serologic-, and culture-based methods. The 24-min turnaround time compares favorably with >2 h for analysis using conventional methods, a minimum of 24 h for PCR-based analysis in a clinical microbiological testing lab , and >48 h for serology and/or culturing of the organism (20). Fig. 4*c Inset* highlights the comparison of the MGA system with conventional methods for extraction (green), amplification (blue), and detection (black), assuming standard laboratory instrumentation used by the same operators, with no lost time between processes, and does not take into account "batching-related" delays. Although not insignificant, the 5-fold reduction in analysis time is outweighed by the potential for automation of the integrated analysis, which will further decrease technician labor time and isolate the operator from the analysis. Finally, Fig. 4*b* highlights the value of a microfluidic system with respect to reduced consumption of reagents for DNA extraction and amplification. Microfluidic devices are expected to inherently scale reduction to the analytical system and, consistent with the other elegant microfluidic developments from various (2–4) groups, the MGA system allows for submicroliter PCR. This reduced size not only enhances amplification speed but also provides a 50-fold reduction in PCR volume. Consuming less Taq polymerase, the most costly reagent in this molecular analysis, yields the potential to dramatically decrease the cost per test. Concordantly, the ≈25-fold reduction in volume of reagents used for DNA extraction reduces the hazardous waste that must be disposed of. The microfluidic nature of the MGA system [like microdevices (2–4)] distinguishes it from larger-volume commercial systems (21–23) that do not reap the benefits of submicroliter fluid manipulation.

Although a comprehensive evaluation of device sensitivity for these two diverse sample matrices is ongoing, the proof-of-principle experimentation accomplished with anthrax-infected murine blood suggests the following regarding detection levels with the MGA system. The average day 2 serum level of anthrax in immunoprecipitation-challenged mice was determined to be 2.5 ($\pm$1) $\times$ 10^6 cfu/ml. Of the total blood drawn, only 0.75 μl of blood was loaded onto the silica bed (purposefully overloaded to ensure saturation of the phase for the purification), representing

the equivalent of 1,500–2,000 cfu, which, in this case, is equivalent to the number of starting copies of amplifiable DNA. Having demonstrated the amplification from <10 DNA starting copies with the IR-PCR system used in the MGA device (24), sensitivity on the order of a few hundred starting copies is plausible with the MGA system, but this will only be established with certainty when serial dilution studies are completed.

Although the MGA device shares similarities with other microfluidic devices reported in the literature (3, 17, 25, 26), it is important to define the distinguishing characteristics of this system. First and foremost, in contrast with other systems, the incorporation of a purification step with downstream analytical processing allows for the removal of inhibiting chemical compounds, enabling the input of complex biological samples such as blood, a key requirement of a genetic μ-TAS (1). This MGA system displays a previously undescribed integration of DNA extraction from whole blood with multiple downstream processes (PCR and electrophoretic analysis) on the same microdevice. The second distinction is the simplistic design of this glass MGA device, which avoids costly and time-consuming metallization steps. Circumventing the need to fabricate heaters and/or temperature sensors (2, 3, 17) into the PCR system enhances cost effectiveness so that single-use disposability becomes a realistic possibility.

The addition of DNA purification for the removal of interfering species to already established microfluidic technology for PCR amplification, separation, and detection completes the genetic analysis system and allows relevant genetic profiling for a variety of applications. Through the integration of sample pretreatment with analytical processing for the analysis of biological samples presented here, the goal of the μ-TAS described by Manz *et al*. (1) a decade ago has been realized. In an era witnessing a shift toward point-of-care testing and personalized medicine, the MGA system presented here provides sample-in–answer-out genetic testing. Its virtues are simplicity in function and fabrication, combined with the possibility for turnkey microfluidic detection systems for screening a panel of pathogens. With whole-blood and nasal-aspirate analyses demonstrated, it is clear that a variety of representative candidate samples, including body fluids (urine, blood, semen, etc.), nasal swabs, and fecal matter, could be analyzed in a microfluidic system designed for use in emergency rooms, primary care clinics, and forensic labs. An analytical platform that utilizes disposable, cost-effective microfluidic chips reduces reagent consumption by orders of magnitude, provides turnaround times of 30 min or less, and offers the potential of rapid inexpensive on-site screening. It is reasonable to expect that compact portable instrumentation can be assembled around the small disposable microfluidic device described here to generate a portable and eventually handheld system, applicable in a number of different clinical, biohazardous, and forensic contexts.

Methods

Microchip Fabrication. All glass microchips were fabricated as described (27) by using borofloat glass slides (127 × 127 × 0.7 mm) purchased from Telic (Valencia, CA). Differential etch depths were achieved by using hydrofluonic acid (HF) resistant dicing tape (Semiconductor Equipment Corporation, Moorpark, CA), patterned manually. Dimensions of the device and channel design, as well as device fabrication, are more fully detailed in supporting information (SI) *Supporting Text*.

The four-layer integrated devices (30.0 × 63.5 mm) were assembled as follows. The bottom two glass fluidic layers were etched as described, with access holes drilled into the patterned layer prebonding. After thermal bonding, glass was selectively removed from around the PCR chamber by etching with 49% HF, using HF-resistant tape as a mask. The third (valve) layer consisted of a commercially available poly(dimethyl siloxane) (PDMS) membrane (HT-6240, Bisco Silicones, Rogers, Carol Stream, CT), with a thickness of 254 μm. This unpatterned layer was irreversibly sealed by plasma oxidation (PDC-32G plasma cleaner, Harrick Scientific, Pleasantville, NY) to a fourth glass layer, previously drilled and patterned with valve control channels. These third and fourth layers were aligned, then pressed to seal against the thermally bonded glass microchip, with the third (PDMS) layer in contact with the drilled access holes of the second layer to form pneumatically addressable valve seats in a normally closed configuration (8, 26).

Device Preparation. The glass microchips were cleaned before each experiment (before addition of the valve layer), to regenerate the surface (28). The PCR and ME domains were exposed to a 1:1 methanol:HCl solution for 30 min, rinsed with ddH_2O, and exposed to concentrated H_2SO_4 for 30 min. The SPE domain was cleaned with 2 M HCl for a total of 1 h. The entire device was then rinsed thoroughly with ddH_2O and the PCR and SPE domains dried with nitrogen. The SPE and PCR domains, along with the syringe used to deliver master mix, were silanized by using Sigmacote (Sigma-Aldrich, St. Louis, MO). After silanization, the SPE and PCR domains, as well as the syringe, were rinsed with water and dried under nitrogen.

Macro-to-Micro Interfacing. After conditioning, the device was loaded into a Plexiglas cartridge for interfacing (see Fig. 1*c*). The cartridge consisted of two machined layers between which the device was sandwiched. Buna-N O-rings were used for fluidic (004) and pneumatic (001) seals, with the device held in place by using stainless steel knurled-head screws. The cartridge was machined with access holes and fluidic reservoirs, interconnects for pneumatic control, and openings for IR heating and fluorescence excitation and emission.

SPE. For all extractions using the MGA system, silica beads (5–30 μm) were packed in the SPE domain against the etched weir by using applied vacuum and replaced before each analysis. Flow rates used for all extractions were 4.16 μl·min^{-1} (29). The extraction protocol used for all experiments was adapted from Legendre *et al.* (11); a more detailed description of the protocols used can be found in *SI Supporting Text*.

For generating the real-time qPCR elution profile, a sample consisting of 4 μl of human whole blood, lysed in a solution of 5 μl of proteinase K and 91 μl of 6 M guanidine·HCl, was prepared. The lysed sample was loaded for 6 min and the bed washed with 80% isopropanol (80/20, vol/vol 2-propanol/dd H_2O) for 5 min, with secondary flow of ddH_2O through the sidearm to imitate a fully integrated analysis. Finally, water was passed through the bed, and 13 1.5-μl fractions of eluate were collected for subsequent qPCR amplification ($n = 2$) of the human thyroid peroxidase gene by Taqman chemistry, following the protocol developed by Horsman *et al.* (30).

To generate replicate breakthrough profiles, the same concentration of lysed blood sample as described above was used for consecutive breakthrough plots ($n = 3$), with the silica bed removed and replaced between each run. The sample was flowed through the SPE bed as described, whereas 10 1.5-μl fractions were collected at the SPE outlet. These fractions were fluorescently assayed for DNA concentration (31) using the Picogreen assay (Invitrogen–Molecular Probes, Eugene, OR) according to the manufacturer's instructions.

For the integrated experiments, real clinical samples were used to show the versatility of the device for handling multiple sample types and applications. The first sample evaluated was the detection of anthrax in mouse blood. The C57BL/6 mice were injected with 1×10^9 spores (*B. anthracis* strain 7702) in 100 μl of water. Typically, mice challenged in this manner succumb 5–6 days post-challenge. All of the mice used in this experiment were

positive for cfus in the blood, liver, and spleen by day 2 postchallenge and were asymptomatic when sampled on day 2. The clinical patient sample was a discarded clinical sample from University of Virginia Medical Laboratories, and all patient identification information was removed from the sample before it was obtained. The sample consists of a nasopharyngeal wash that was tested and diagnosed as a strong positive for *B. pertussis*. Samples were prepared by mixing the appropriate volume of sample (8 μl of nasal aspirate or 6 μl of murine whole blood) with 5 μl of Proteinase K, diluted to 100 μl of total volume with 6 M GuHCl and vortexed for 30 sec to mix thoroughly. Human samples were used directly in the analysis, whereas mouse blood samples were first boiled for 10 min. The sample was loaded for 3 min, followed by a 5-min rinse with 80% isopropanol. A preconditioning rinse step was used in which PCR master mix was flowed through R_2 and PCR domain with valves V_1 and V_2 open for 2 min to condition the valve between SPE and PCR. DNA was eluted with water, with the valves closed until the appropriate time as previously determined, followed by subsequent opening and closing of the valves to allow PCR master mixture and eluting DNA to be trapped within the PCR chamber for thermal cycling.

PCR Amplification. For fully integrated analysis, the PCR master mixture was made with the following final concentrations: 20 mM Tris/100 mM KCl (pH 8.3)/6 mM $MgCl_2$/0.8 μM of each primer/0.4 mM dNTP/0.5 units/μl Taq polymerase. The thermal cycling protocols used were 95°C for 30 sec (initial denaturation), then 30 cycles of 95°C for 2 sec, 62°C/55°C for 3 sec (for *B. anthracis*/*B. pertussis*, respectively), and 72°C for 5 sec, followed by a single final extension for 1 min at 72°C after the 30 cycles were completed. The primers for *B. pertussis* amplification were adapted from Loeffelholz *et al.* (32). The primers used in the *B. anthracis* amplification were 5′-CAAATCAGCTCGAAAGTTAGGA (for) and 5′-CAGTAACTGTTCAGAAGGTACATCTGA (rev) for the amplification of a 211-bp fragment of the *virulence B* gene on pXO1 and were designed in-house (33). The noncontact thermal cycling PCR system (see *SI Supporting Text*) was constructed in-house, as described (34). Amplicon from the analysis of *B. pertussis* was removed from the device postanalysis and sequenced at the Biomolecular Research Facility at the University of Virginia.

Microchip Electrophoresis. Glass microchips were cleaned as described above. The separation channels were not dried postcleaning. During PCR, the separation domain was filled with 1.0 M HNO_3. After PCR, the separation channels were rinsed with ddH_2O and filled with the sieving matrix, 3.5% HPC in 80/40 mM Mes/Tris (35) with 1.0 μM YOPRO DNA intercalating dye (Invitrogen–Molecular Probes). The pressure injection and valving instrumentation (see *SI Supporting Text*, for more detailed descriptions of both) were used as described (13). After pressure injection, separation was achieved by applying voltage using a dual polarity high-voltage power supply built in-house using two Spellman high-voltage sources (Hauppauge, NY). For *B. anthracis* analysis, −200 V was applied to the buffer reservoir and 1,050 V to the buffer waste. For the *B. pertussis* analysis, −150 and 790 V were applied. An argon ion laser (Model LS200, Dynamic Laser, Salt Lake City, UT) was used for excitation with a conventional confocal detection setup (×16 objective, 1-mm pinhole). Emission was collected with a PMT (Hamamatsu, Bridgewater, CT) through a 515-nm bandpass filter (Omega Optical, Brattleboro, VT). The instrument and data acquisition were controlled through a LabVIEW application written in-house.

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