A self-contained polymeric cartridge for automated biological sample preparation^{a)}

Guolin Xu, Daniel Yoke San Lee, Hong Xie, Deon Chiew, Tseng-Ming Hsieh, Emril Mohamed Ali, Xing Lun Looi, Mo-Huang Li,^{b)} and Jackie Y. Ying^{b)}
Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, Singapore 138669

(Received 12 April 2011; accepted 22 June 2011; published online 25 July 2011)

Sample preparation is one of the most crucial processes for nucleic acids based disease diagnosis. Several steps are required for nucleic acids extraction, impurity washes, and DNA/RNA elution. Careful sample preparation is vital to the obtaining of reliable diagnosis, especially with low copies of pathogens and cells. This paper describes a low-cost, disposable lab cartridge for automatic sample preparation, which is capable of handling flexible sample volumes of 10 μ l to 1 ml. This plastic cartridge contains all the necessary reagents for pathogen and cell lysis, DNA/RNA extraction, impurity washes, DNA/RNA elution and waste processing in a completely sealed cartridge. The entire sample preparation processes are automatically conducted within the cartridge on a desktop unit using a pneumatic fluid manipulation approach. Reagents transportation is achieved with a combination of push and pull forces (with compressed air and vacuum, respectively), which are connected to the pneumatic inlets at the bottom of the cartridge. These pneumatic forces are regulated by pinch valve manifold and two pneumatic syringe pumps within the desktop unit. The performance of this pneumatic reagent delivery method was examined. We have demonstrated the capability of the on-cartridge RNA extraction and cancer-specific gene amplification from 10 copies of MCF-7 breast cancer cells. The on-cartridge DNA recovery efficiency was 54–63%, which was comparable to or better than the conventional manual approach using silica spin column. The lab cartridge would be suitable for integration with lab-chip real-time polymerase chain reaction devices in providing a portable system for decentralized disease diagnosis. © 2011 American Institute of Physics. [doi:10.1063/1.3614441]

INTRODUCTION

Molecular diagnosis based on nucleic acids detection is a key technique for diseases detection.^{1–3} However, before detection can be performed, purified DNA/RNA samples have to be prepared from raw biological samples. The sample preparation processes of DNA/RNA extraction and purification are time-consuming and labor-intensive. Several steps are required, including pathogen lysis, nucleic acid extraction, impurity washes, and DNA/RNA elution.³ Several lab-chip devices have been reported with an integrated sample preparation feature.^{4–8} These devices typically have limited sample volume handling capacities of ≤ 1 μ l due to the height constraints in the fluid channel or chamber. Such low sample volume poses a challenge on early stage disease diagnosis with a low viral load (<50 copies/ml).^{3,9,10} In addition, the clinical samples commonly have undesired co-existing human or non-specific bacterial cells.^{9,10} The DNA/RNA of these co-lysed impurities may saturate the loading capacity of the miniaturized DNA

^{a)}Paper submitted as part of the 2nd European Conference on Microfluidics (Guest Editors: S. Colin and G.L. Morini). The Conference was held in Toulouse, France – December 8–10, 2010.

^{b)}Author to whom correspondence should be addressed. Electronic addresses: mhli@ibn.a-star.edu.sg and jyying@ibn.a-star.edu.sg. Tel.: (+65) 6824-7000. FAX: (+65) 6478-9085.

FIG. 1. Schematic of the sample preparation system, which consists of a cartridge for DNA/RNA purification, a valve manifold for fluid regulation, and two syringe pumps for fluid pumping. The cartridge contains 8 chambers for raw sample loading (C1), DNA/RNA extraction (X), two impurity washes (C2 and C3), ethanol wash (C4), elution buffer (C5), eluent DNA collection (C7), as well as waste storage (C6). Reagents pumping is achieved using air pressures and vacuum forces, generated by two pumps in a push-pull set-up and regulated by a valve manifold (P1–P5, V1–V2).

capture unit. Thus, in typical clinical laboratory settings, sample preparation is often conducted with a sample volume of 200–500 μl and a high loading of porous silica column or silica-coated magnetic beads.¹¹

Herein we present a novel device for automatic sample preparation in a polymer cartridge that is capable of handling flexible sample volumes of 10 μl to 1 ml (Fig. 1). The low-cost cartridge is made of polymethylmethacrylate (PMMA) and contained no moving components such as pumps and valves. It consists of 8 chambers for the loading and processing of raw sample. Transportation of reagents is conducted with a combination of push and pull forces using compressed air and vacuum, respectively, which are connected to the pneumatic inlets at the bottom of the cartridge. These pneumatic forces are regulated by two pinch valve manifolds and two pneumatic pumps within the desktop system. DNA/RNA extraction is based on a solid-phase method using a silica membrane. The on-cartridge fluidic processes include DNA/RNA extraction, impurity washes (2 $\times$), ethanol flush, elution with elution buffer, and collection of DNA/RNA eluent. The DNA/RNA purification process is automatically performed within this completely sealed cartridge using a desktop unit. The sample preparation could be achieved in 6 min. We demonstrate the successful use of the cartridge for DNA extraction with an efficiency of 54–63%. In addition, we illustrate the capability of on-cartridge RNA extraction from as few as 10 MCF-7 breast cancer cells using our system. Successful extraction is confirmed using a Bio-Rad CFX-96 commercial real-time polymerase chain reaction (PCR) system for CK19 cancer-specific gene amplification.

MATERIALS AND METHODS

Device fabrication

The PMMA sample preparation cartridge (40 mm $\times$ 23 mm $\times$ 30 mm) was designed with SolidWorks, and fabricated by computer numeric control (CNC) machining (Whits Technologies, Singapore) for prototype. After the prototype device verification, the final device was fabricated by cost-effective injection molding. The surface channels were 1 mm in height and 1 mm in width. The through-cartridge pneumatic and fluidic channels were 1 mm in diameter. The chamber volume (Fig. 2) was designed to accommodate the required amount of reagents for the

FIG. 2. (a) SolidWorks drawing of the sample preparation cartridge with chambers for DNA/RNA extraction and purification. The reagents for DNA/RNA extraction could be preloaded into the cartridge, and sealed by adhesive films. The top (b) and bottom (c) views of the cartridge specified with pressure inlets (P1–P5) and vacuum inlets (V1–V2). Pumping of reagents was achieved using air pressures and vacuum forces generated by two pumps in a push-pull set-up. The red and blue arrows indicate the flow directions of air (pressure or vacuum) and reagents, respectively.

commercial DNA/RNA extraction kits, such as QIAamp DNeasy Blood and Tissue Kit and QIAamp Virus RNA Mini Kit.

To remove any oil and contaminant, the cartridge was soaked in 0.2% of detergent (Decon 90, Decon Lab. Ltd.) for 12 h, rinsed thoroughly with de-ionized water, and oven-dried (60 °C, 6 h). Next, the cartridge was dip-coated with 0.5% (w/w) of DuPont AF 1600 fluoropolymer dissolved in 3M FC-40 Fluorinert (filtered with a 10- μ m membrane filter (Vacu-Guard, Whatman)), and oven-dried (60 °C, overnight). The Teflon coating made the surface of chambers and channels hydrophobic to avoid any reagents (like the washing buffers) to stick on to the surface. The Teflon-coated cartridge was soaked in 3% H₂O₂ (MGC Pure Chemicals) for 12 h, rinsed with 0.1% diethyl pyrocarbonate (DEPC) (Sigma-Aldrich) to remove RNases and DNases, and oven-dried (60 °C, 6 h). FujiFilm silica membrane was used for DNA/RNA extraction (FujiFilm Quickgene RNA Cultured Kit S). The membrane was cut from the silica column with its support (to height 4 mm). The membrane was inserted at the bottom of the membrane chamber, as shown in Figure 2(c). The top and bottom of the cartridge were then sealed with MicroAMP optical adhesive film (Cat. 4306311, Applied Biosystems).

Fluidic pumping and regulation

Most planar microfluidic structures require on-chip valves for directing fluids.^{12–16} The necessity of having more components increases the cost per chip. Although surface acoustic wave^{17,18} and magnetic force¹⁹ are able to direct fluids without using valves, the fluids are exposed to air during transportation, which raise the concern for cross contamination. In our system, the fluids were manipulated using a combination of compressed air and vacuum forces. The reagents within our cartridge's chambers were automatically isolated due to gravity. The novelty of our approach was that the cartridge was designed with no moving components for guiding and transferring fluids. The pressure and vacuum forces were generated by two custom-built pumps in the desktop unit (Fig. 3). The pneumatic forces were directed to the appropriate chambers within the cartridge using the pinch valve manifolds. The pumps and pinch valve manifolds constituted the main external pneumatic system, which controlled the fluidic motion within the chambers of the cartridge. This structure has greatly simplified the cartridge assembly and would allow for the mass production of cartridges via injection molding, thereby significantly reducing the cartridge cost. Since the reagents were self-contained within the fluidic

FIG. 3. Photographs of the sample preparation system. (a) The sample preparation cartridge (1) and the desktop system (2). (b) Photograph of the desktop unit with a pinch valve manifold (3), two syringe pumps (4 and 5), a syringe pump controller (6), and a power supply (7). (c) The reagent is automatic isolated in vertical and upside down position due to a combination of gravity and air pressure. (d) The cartridge was connected to the external pressure and vacuum forces via the o-ring sealed interface (8). (e) Details of the o-ring sealed interface with stainless steel tube with 1 mm protrusion high for piercing the sealing film and connecting pressure or vacuum source to the cartridge.

chambers and channels throughout the entire operation, the potential run-to-run and hardware cross contaminations were eliminated.

When the cartridge was loaded into the pneumatic interface slot, the stainless steel tubes (Fig. 3(e)) would puncture the adhesive film covering the inlets of the pneumatic channels. Pressure and vacuum could then be applied to each fluidic chamber through the pressure inlets (P1–P5) and vacuum inlets (V1–V2) of the cartridge (see Figs. 1 and 2). The operation of the sample preparation cartridge is illustrated in Fig. 1 with the status of each pinch valve indicated. The biological sample was first loaded into the sample chamber (C1) by a needle syringe, and the cartridge was then re-sealed with an adhesive film. After the cartridge was loaded onto the desktop system, pinch valves P1 and V1 were opened, while other valves remained closed. The pressure pump and vacuum pumps run at flow rate = 0.5 ml/min. As shown in Figure 1, the pressure source from the pressure pump, passes through the pinch valve P1, through a vertical pneumatic channel (shown in Figure 2(a) also), to the top of the sample chamber C1. In the meantime, the vacuum source from the vacuum pump (Figure 1) passes through the pinch valve V1 and a vertical pneumatic channel, to the top of the waste chamber C6. The pressure source and the vacuum source will not contact with the sample solution. The contamination is avoided.

The biological sample containing the target DNAs was then lysed and filtered through the silica membrane. The DNAs were captured by the membrane, while the filtrate waste was directed to the waste chamber. Like the DNA capturing step, the impurities (trapped within the

silica membrane) were washed out sequentially by wash buffer 1 (C2), wash buffer 2 (C3), and ethanol flush (C4) (flow rate = 1 ml/min). Before elution step, by turn on pinch-valve P4 and V1, the system was extensively flushed with air (flow rate = 10 ml/min; 2 min) from chamber C4 to chamber C6 to remove any remaining wash buffer in the channels and the silica membrane. The purified DNA was then released from the silica membrane with the elution buffer (C5), and directed to the eluent chamber (C7), with only valves P5 and V2 opened. The entire process took only ~6 min. Since all the reagents are preloaded inside the cartridge and the sample preparation processes are conducted within the cartridge automatically, human error and cross-contamination are avoided.

Two in-house fabricated syringe pumps with a volume of 25 ml were used to generate the air pressures and vacuum forces. These syringe pumps were driven by a linear actuator (E43H4N-12, Haydon) and a step motor driver (DCS 4010, Haydon) with a maximum flow rate of 12 ml/min. The air pressures and vacuum forces (connected to inlets P1–P5 and V1–V2, respectively, in Figs. 2(b) and (c)) were regulated by a pinch-valve manifold (P/N 075P2NC12-23S, Bio-Chem Fluidics). These pneumatic forces were connected to the cartridge via o-rings and pneumatic connectors (M-3AU, SMC) with protruded stainless steel tubes (of 1 mm in length), which pierced through the bottom sealing film upon cartridge loading. The entire system was controlled by an industrial controller (PMX-4EX-SA, Arcus Inc.).

The automatic system was powered by a 12-V 35 W power source (S-35-12, MeanWell). The current required for each pinch-valve, linear actuator and controller are 0.24, 0.3, and 0.5 A, respectively. Since only two pumps, two pinch valve and the controller working in the same time, the average power consumption was about 19 W (12 V/1.58 A). Hence, the system can be powered a car battery.

DNA recovery efficiency

The DNA extraction was conducted using the reagents from QIAamp DNeasy Blood and Tissue Kit (Qiagen) based on the manufacturer's instructions. Serial dilutions (50–200 ng/ μ l) of mouse liver DNA (200 μ l) were added with 200 μ l of ethanol (96–100%) and 200 μ l of buffer AL in a 1.5-ml tube. The mixture was then transferred to the sample chamber of the cartridge, which was preloaded with the QIAamp's reagents as follows: 500 μ l of wash buffer AW1 in the wash 1 chamber, 500 μ l of wash buffer AW2 in the wash 2 chamber, 500 μ l of ethanol (96–100%) in the ethanol flush chamber, and 200 μ l of elution buffer in the elution buffer chamber. The top of the cartridge was then sealed with MicroAMP optical adhesive film and loaded into the desktop unit, which performed the DNA extraction automatically. The total DNA eluted was collected in the product chamber and measured using the Nano-drop ND-1000 spectrophotometer (Thermo Scientific).

Control experiments were performed with the same Qiagen kit according to the manufacturer's protocols. Briefly, the sample mixture as in the cartridge experiment was transferred to the spin column, spun at 8000 rpm for 1 min, washed with 500 μ l of wash buffer AW1 (8000 rpm, 1 min), washed with 500 μ l of wash buffer AW2 (14 000 rpm, 3 min), and finally eluted with 200 μ l of AE elution buffer (8000 rpm, 1 min).

RNA extraction from cell and real-time reverse transcription polymerase chain reaction (RRT-PCR)

The RNA extraction of MCF-7 cancer cells was conducted using the reagents from QIAamp Virus RNA Mini Kit (Qiagen) based on the manufacturer's instruction. Serial dilutions (10–10⁴ cells in 70 μ l of phosphate buffered saline (PBS)) of MCF-7 breast cancer cells were added with 280 μ l of cell lysis AVL buffer and 2.8 μ l of carrier RNA (1 μ g/ μ l in AVE buffer) in a 1.5-ml tube. The mixture was incubated at room temperature for 1 min. Next, 280 μ l of ethanol (96–100%) was added to the mixture, which was then transferred to the DEPC-treated cartridge (sample chamber). The cartridge was preloaded with essentially the same amount of QIAamp's reagents as in the DNA extraction experiment, except that the elution buffer volume

pre-loaded was 100 μl . After automatic RNA extraction, the eluted total RNA was collected in the product chamber) for RRT-PCR.

Control experiments were performed with the same Qiagen kit according to the manufacturer's protocol. Briefly, the sample mixture as in the cartridge experiment was transferred to the spin column, spun at 8000 rpm for 1 min, washed with 500 μl of wash buffer AW1 (8000 rpm, 1 min), washed with 500 μl of wash buffer AW2 (14 000 rpm, 3 min), and eluted with 100 μl of AVE elution buffer (8000 rpm, 1 min).

Keratin, type I cytoskeletal 19 (CK19) gene from MCF-7 cells was selected for characterization of RNA extraction efficiency. RRT-PCR of CK19 was performed in a Bio-Rad CFX96 system with 20 μl of reaction mixture, which included 10 μl of $2\times$ RT-PCR Mix (Cat. 4392587, Applied Biosystems), 0.5 μl of $40\times$ TaqMan RT Enzyme Mix (Cat. 4392939, Applied Biosystems), 1 μl of $20\times$ LC Green Plus (Idaho Technology, USA), 8 μl of extracted RNA, and 400 nM of CK19 primers (forward primer: ATGAAAGCTGCCTTGGAAGA; reverse primer: TGATTCTGCCGCTCACTATCAG; Sigma Aldrich). The RRT-PCR process included: 48 $^{\circ}\text{C}$ (10 min) for the reverse transcription process, followed by 95 $^{\circ}\text{C}$ (10 min) and 50 cycles of 95 $^{\circ}\text{C}$ (15 s) and 60 $^{\circ}\text{C}$ (1 min) for gene amplification and detection.

RESULTS AND DISCUSSION

Performance of fluidic transfer

The displacement velocities for the pressure and vacuum pumps were the same. Before the fluidic pumping began, the pressure pump was at its maximum volume position (~ 25 ml), and the vacuum pump was at its minimum vacuum volume position. Once fluidic transfer was initiated, the respective pinch valves would start their operation. Pressure was applied to the fluidic chamber (e.g., C1 to C2), and vacuum was applied to the waste chamber (C6) or eluent chamber (C7).

The relationship of the pump displacement volume and the fluid volume transported from reagent chambers is illustrated in Fig. 4. The fluid volumes transported were very similar at a given pump displacement volume for pump displacement velocities of 0.1 ml/min and 0.2 ml/min. Although there was an initial compression volume of ~ 47.4 μl before the fluid would start to move, the volume of fluid transported has a linear relationship with the pump displacement volume. Hence, the fluidic transportation could be easily controlled.

FIG. 4. The fluid volumes transported as a function of the displacement volumes of vacuum and pressure pumps are very similar at pump displacement velocities of ($\diamond$) 0.1 ml/min and ($\square$) 0.2 ml/min. Their averaged linear curve (—) has a slope of 0.907, with $R^2 = 0.998$. Error bar: standard deviations, $n = 4$.

Efficiency of on-cartridge DNA extraction

Nucleic acid assays based on PCR (Refs. 6, 20, 21) or biosensors^{22–24} are relatively robust. However, a variety of contaminants can inhibit amplification and affect gene detection.^{3,11} In order to circumvent this problem, DNA must be extracted and purified from a raw sample through a variety of lysis protocols and purification techniques.^{4,25–30} The most common method is chemical lysis of the raw sample, followed by DNA purification using a solid-phase extraction method.

Rat total DNA was used for the on-cartridge DNA extraction and purification. The sample was first lysed, and the genes were protected in the lysis buffer with high ionic concentration. When transferred to the sample chamber of the cartridge, the lysis buffer containing genes was first passed to the silica membrane chamber. The DNA was adsorbed onto the silica membrane surface, while the unbound impurities passed through the membrane to be stored in the waste chamber. Purification of the bounded DNA was achieved by passing the washing buffer twice and ethanol flush once through the silica membrane. The membrane was then dried by passing air through it. The adsorbed DNA was released into the elution buffer with low ionic concentration using nuclease-free water to dilute the DNA.

To test the efficiency of the on-cartridge DNA extraction, the mouse liver total DNA was selected to mimic the clinical biological sample with co-existing human total DNA and virus DNA. ND-1000 spectrophotometer was used to measure the DNA concentration of the sample loaded in the cartridge and in the elution buffer after the on-cartridge sample preparation. The volume of DNA loaded in the cartridge and the eluent volume were both 200 μl . Since the measurement limits of the ND-1000 spectrophotometer were 2–1000 $\text{ng}/\mu\text{l}$, the input pure sample DNA concentration was selected to be 50–200 $\text{ng}/\mu\text{l}$.

The DNA recovery efficiency via the on-cartridge DNA extraction is shown in Fig. 5. The concentration of recovered DNA has a linear relationship with the concentration of DNA loaded into the cartridge. The recovery efficiency varied from 54% to 63%. Using a manual spin column to process the same DNA with the same Qiagen reagents, an average DNA recovery efficiency of $54 \pm 5\%$ was obtained ($n=3$, input DNA concentration = 200 $\text{ng}/\mu\text{l}$). This demonstrated that our automated cartridge-based process has a comparable or better performance as compared to the conventional spin column process.

RNA extraction with MCF-7 Cells

RNA extraction in the sample preparation for RRT-PCR requires several steps.^{27,31} Similar to the purification of DNA, the RNA adsorbed onto the silica membrane surface was purified

FIG. 5. The recovered DNA concentration (●) and DNA recovery efficiency (■) as a function of the input DNA concentration. The volume of DNA sample loaded into the cartridge and the eluent volume were both 200 μl . The DNA recovery efficiency was ~ 54 –63%. Error bar: standard deviations, $n=3$.

by passing the washing buffer twice and ethanol flush once through the silica membrane. The adsorbed RNA is then released into the elution solution under a low ionic concentration. These manual, labor-intensive processes have been integrated in our on-cartridge RNA extraction. Reagents from Qiagen QIAamp Viral RNA Mini Kit were used to extract 10 – 10^4 MCF-7 breast cancer cells by our cartridge. The Qiagen Kit employed Carrier RNA ($2.8\ \mu\text{g}$) to enhance the RNA extraction,^{32,33} which made it difficult to measure the recovery rate of the target RNA directly by measuring the quantity of eluted RNA using spectrometer, since the excess amount of Carrier RNA would co-elute with the target RNA. We have utilized the RRT-PCR method to tackle this problem.

The RNA from on-cartridge extraction was examined using Taqman One-Step RRT-PCR Kit. The kit combined reverse transcription and cDNA amplification in the same mixture. A commercial thermal cycler (CFX-96, Bio-Rad) was employed to amplify the CK19 gene in the cancer cells. The enzyme reverse transcriptase (RT) process was conducted at 48°C for 15 min. The RNA strand was first reverse transcribed into its DNA complement (cDNA). The CK19 gene was amplified under denaturation of the DNA complement at 95°C for 10 s, followed by a combined annealing and extension step at 60°C for 1 min. The total number of amplification thermal cycles was 50; the RRT-PCR curves are shown as red lines in Fig. 6.

Control experiments were performed with the Qiagen spin column using the same reagents to extract RNA from the same amount of breast cancer cells. The RRT-PCR curves are shown as black lines in Fig. 6. To test for any contamination in the RRT-PCR reagents, negative controls with no template RNA and with mouse liver total RNA sample (7801, Ambion) that did not have CK19 gene were run using the same RRT-PCR reagents, and amplified under the same thermal cycling conditions as for the samples. As shown by the blue and pink lines in Fig. 6, nothing was amplified in these negative controls, indicating that the RRT-PCR amplification was specific.

The cycle threshold (C_T) values for 10 and 10^4 cells were 29.3 and 25.6, respectively, using our cartridge for RNA extraction. The C_T values were 30.3 and 26.6, respectively, when spin column was employed for the RNA extraction. Ideally, for a 10-fold difference in input cell number, the difference in C_T values should be ~ 3.3 . However, we found that the differences in C_T values between 10^4 and 10^3 cells, between 10^3 and 10^2 cells, and between 10^2 and 10 cells were 1.7, 1.7, and 0.3, respectively. When using the spin column, the corresponding

FIG. 6. The real-time fluorescence curves of total RNA extracted from (*) 10 , ($\times$) 10^2 , ($\blacksquare$) 10^3 , and ($\blacklozenge$) 10^4 MCF-7 cells using the cartridge (red lines) and the Qiagen spin column (black lines), reverse transcribed and amplified using Bio-Rad CFX-96. The blue and pink lines were negative controls that have been run using water and rat total RNA with no CK19 gene, respectively.

differences in C_T values were 0.8, 2.6, and 0.3, respectively. This could be due to two reasons: (i) the input cell number might not be accurate at the low cell concentrations, and (ii) the gene expression of individual breast cancer cells might be different.

CONCLUSION

In conclusion, we have successfully developed a novel self-contained cartridge and a desktop unit for automated DNA/RNA purification from raw sample within 6 min. The cartridge used a combination of compressed air and vacuum forces for fluidic transfer. The on-cartridge DNA recovery efficiency was 54–63%, which was comparable to or better than the conventional manual approach using silica spin column. In addition, the on-cartridge RNA extraction was successfully demonstrated using $10\text{--}10^4$ MCF-7 breast cancer cells as samples. RRT-PCR of CK19 cancer-specific gene was achieved with as little as 10 MCF-7 cells. This cartridge system could also be integrated with components such as real-time polymerase chain reaction and DNA microarray to provide a point-of-care device for rapid disease diagnosis.

ACKNOWLEDGMENTS

This work was funded by the Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research, Singapore). The authors would like to thank Dr. Yong Yeow Lee and Ms. Li Shi Lim for providing MCF-7 breast cancer cells and CK19 primers.

- ¹C. D. Chin, V. Linder, and S. K. Sia, *Lab Chip* **7**, 41 (2007).
- ²S. E. Joanna and C. Z. Maria, *Rev. Med. Virol.* **12**, 375 (2002).
- ³Y. Huang, E. Mather, and J. B. M. Madou, *Anal. Bioanal. Chem.* **372**, 49 (2002).
- ⁴N. C. Cady, S. Stelick, and C. A. Battb, *Biosens. Bioelectron.* **19**, 59 (2003).
- ⁵L. A. Legendre, J. M. Bienvenue, M. G. Roper, J. P. Ferrance, and J. P. Landers, *Anal. Chem.* **78**, 1444 (2006).
- ⁶K.-Y. Lien, S.-H. Lee, T.-J. Tsai, T.-Y. Chen, and G.-B. Lee, *Microfluid. Nanofluid.* **7**, 795 (2009).
- ⁷L. Liu, W. Cao, J. Wu, W. Wen, D. C. Chang, and P. Sheng, *Biomicrofluidics* **2**, 034103 (2008).
- ⁸R. H. Liu, M. J. Lodes, T. Nguyen, T. Siuda, M. Slota, H. S. Fuji, and A. McShea, *Anal. Chem.* **78**, 4184 (2006).
- ⁹J. Dockter, A. Schroder, B. Eaton, A. Wang, N. Sikhamsey, L. Morales, and C. Giachetti, *J. Clin. Virol.* **45**, S39 (2009).
- ¹⁰A. M. Caliendo, A. Valsamakis, Y. Zhou, B. Yen-Lieberman, J. Andersen, S. Young, A. Ferreira-Gonzalez, G. J. Tsongalis, R. Pyles, J. W. Bremer, and N. S. Lurain, *J. Clin. Microbiol.* **44**, 1726 (2006).
- ¹¹H. Tian, A. F. R. Huhmer, and J. P. Landers, *Anal. Biochem.* **28**, 175 (2000).
- ¹²R. H. Liu, J. Yang, R. Lenigk, J. Bonanno, and P. Grodzinski, *Anal. Chem.* **76**, 1824 (2004).
- ¹³R. Pal, M. Yang, R. Lin, B. N. Johnson, N. Srivastava, S. Z. Razzacki, K. J. Chomistek, D. C. Heldsinger, R. M. Haque, V. M. Ugaz, P. K. Thwar, Z. Chen, K. Alfano, M. B. Yim, M. Krishnan, A. O. Fuller, R. G. Larson, D. T. Burke, and M. A. Burns, *Lab Chip* **5**, 1024 (2005).
- ¹⁴C.-Y. Lee, G.-B. Lee, J.-L. Lin, F.-C. Huang, and C.-S. Liao, *J. Micromech. Microeng.* **15**, 1215 (2005).
- ¹⁵S. H. Lee, S.-W. Kim, J. Y. Kang, and C. H. Ahn, *Lab Chip* **8**, 2121 (2008).
- ¹⁶L. Xie, *IEEE Trans. Adv. Packaging* **32**, 1521 (2009).
- ¹⁷L. Y. Yeo and J. R. Friend, *Biomicrofluidics* **3**, 012002 (2009).
- ¹⁸A. Qi, L. Yeo, J. Friend, and J. Ho, *Lab Chip* **10**, 470 (2010).
- ¹⁹P. Neuzil, L. Novak, J. Pipper, S. Lee, L. F. Ng, and C. Zhang, *Lab Chip* **10**, 2632 (2010).
- ²⁰R. Rauch, L. Stevens, R. Lenigk, J. Yang, D. B. Rhine, and P. Grodzinski, *Anal. Chem.* **74**, 3063 (2002).
- ²¹N. C. Cady, S. Stelick, M. V. Kunnavakkam, and C. A. Batt, *Sens. Actuators B* **107**, 332 (2005).
- ²²D. Boehm, P. Gottlieb, and S. Hu, *Sens. Actuators B* **126**, 508 (2007).
- ²³N. V. Zaytseva, V. N. Goral, R. A. Montagna, and A. J. Baeumner, *Lab Chip* **5**, 805 (2005).
- ²⁴Q. Luan, Y. Xue, and X. Yao, *Sens. Actuators B* **147**, 561 (2010).
- ²⁵T. Wilson and J. Carson, *Lett. Appl. Microbiol.* **32**, 326 (2001).
- ²⁶K. A. Wolfe, M. C. Breadmore, J. P. Ferrance, M. E. Power, J. F. Conroy, P. M. Norris, and J. P. Landers, *Electrophoresis* **23**, 727 (2002).
- ²⁷J. West, M. Boerlin, A. D. Jadhav, and E. Clancy, *Sens. Actuators B* **126**, 664 (2007).
- ²⁸J. Pipper, M. Inoue, L. F.-P. Ng, P. Neuzil, Y. Zhang, and L. Novak, *Nature Med.* **13**, 1259 (2007).
- ²⁹U. Lehmann, C. Vandevyver, V. K. Parashar, and M. A. M. Gijs, *Angew. Chem. Int. Ed.* **45**, 3062 (2006).
- ³⁰K. J. Shaw, D. A. Joyce, P. T. Docker, C. E. Dyer, J. Greenman, G. M. Greenway, and S. J. Haswell, *Lab Chip* **9**, 3430 (2009).
- ³¹M. C. Breadmore, K. A. Wolfe, I. G. Arcibal, W. K. Leung, D. Dicks, B. C. Giordano, M. E. Powers, J. P. Ferrance, S. Feldman, P. M. Norris, and J. P. Landers, *Anal. Chem.* **75**, 1880 (2003).
- ³²K. J. Shaw, L. Thain, P. T. Docker, C. E. Dyer, J. Greenman, G. M. Greenway, and S. J. Haswell, *Chim. Acta.* **652**, 231 (2009).
- ³³S. J. Read, *Mol. Pathol.* **54**, 86 (2001).