A Microfluidic Cartridge System for Multiplexed Clinical Analysis

Vincent Gau, Ph.D.
Genefluidics
Monterey Park, CA

*Walson Lawi,[1] Chris Wiita,[1] Steven T. Snyder,[1] Fang Wei,[2] David Wong,[3] Pak Kin Wong,[4] Joseph C. Liao,[5] David Haake,[6,7] and Vincent Gau[1]**
[1]*GeneFluidics, Inc., Monterey Park, CA*
[2]*UCLA, Los Angeles, CA*
[3]*University of California, Los Angeles, CA*
[4]*University of Arizona, Tucson, AZ*
[5]*Stanford University and Veterans Affairs Palo Alto Health Care System, Palo Alto, CA*
[6]*David Geffen School of Medicine at UCLA, Los Angeles, CA*
[7]*Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA*

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Cartridge-based microfluidics is a promising technology for clinical diagnostics. By miniaturizing the fluid-handling processes required for genomic and proteomic analyses, reagent and specimen volume is minimized along with the size of the system. We demonstrate an automated microfluidic system capable of performing six multiplexed genomic and proteomic analyses simultaneously, by means of an integrated electrochemical sensor and embedded controls.
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Introduction

The advent of miniaturized fluidic processes provides for improvement in the sensitivity, specificity, and the total processing time required for biochemical analysis. By introducing advanced bionanotechnology and innovative transduction principles, a microfluidic-based platform has the potential to greatly expand the scope of point-of-care testing and other resource-limited applications. A key advantage of a microfluidic-based system is its capability in automating molecular analysis. Benefits include reducing errors associated with manual processing and dramatically reducing the quantity of reagents and samples required. With continuous improvement in production cost, scalability, and reliability, microfluidic-based systems can revolutionize current practices in molecular analysis.

We present a physically compact microfluidic-based system with an integrated electrochemical sensor array that is capable of performing multiplexed genomic and proteomic assays simultaneously in a single disposable cartridge. This system produces quantitative results and performs most assays in under an hour.

System overview

Our microfluidic cartridge system has been optimized for conducting complex heterogeneous proteomic and genomic assay experiments. The complete system consists of a disposable microfluidic cartridge and a sensing and control istrument with embedded software. The microfluidic cartridge integrates an electrochemical sensor array, as well as reagent storage and fluid-handling components (FHCs). The sensing and control instrument interfaces with the cartridge by

*Correspondence: Vincent Gau, Ph.D., GeneFluidics, Inc., Molecular Analysis, 2540 Corporate Place, STE B101, Monterey Park, CA 91754; Phone: +1.323.269.0900; Fax: +1.323.269.0988; E-mail: vgau@genefluidics.com

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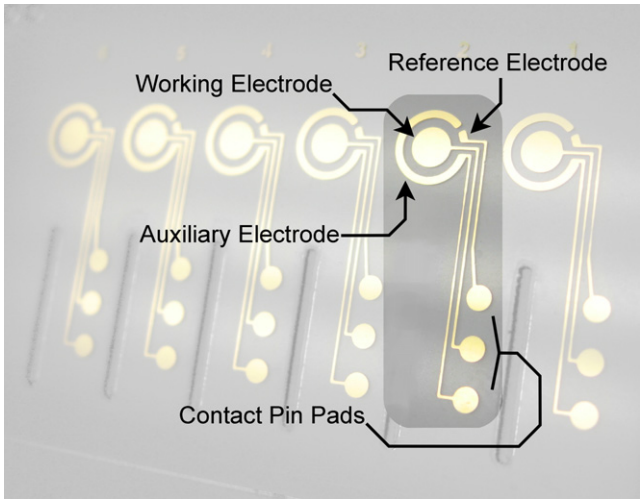

Figure 1. GeneFluidics' electrochemical sensor array.

means of pneumatic and hydraulic manifolds, and data acquisition and potentiostat circuits.

Cartridge System

The microfluidic cartridge integrates reagent storage and fluid handling into a small (90 mm × 48 mm × 7.5 mm) disposable cartridge. All reagents including sample, enzyme, wash, and substrate, as well as a waste chamber, are stored inside the cartridge. Fluid delivery and control valves integrated into the cartridge interface with the instrument's hydraulic and pneumatic manifolds, which control fluidic delivery and distribution inside the cartridge. Each of the cartridge's six independent sensor chambers contains an ultrasensitive electrochemical sensor, allowing for multiplexed and quantitative molecular diagnostic of both protein and genetic targets from raw samples. The hydraulic and pneumatic manifolds, as well as the electrochemical sensor array, are managed by an electronic control system in the instrument.

Electrochemical Sensor and Detection Mechanism

Each sensor chamber of the microfluidic cartridge contains a GeneFluidics electrochemical sensor for amperometric detection of an analyte. The microfluidic system requires a sensor with high sensitivity, small size, and simple assay protocol. The GeneFluidics electrochemical sensor platform has been validated for the detection and species-specific identification of pathogens in previously published successes.[1–9] A clinical study has been performed at Veteran Affairs Hospital Los Angeles that demonstrates accurate detection and identification of bacterial pathogens in clinical urine specimens using the sensor.[3,10]

The electrochemical sensor consists of reference, working, and counter electrodes that are made by depositing a thin layer of gold onto a plastic surface using a shadow mask[1] (Fig. 1). The gold surface is subsequently modified for capturing biotinylated antibodies or nucleic acid capture probes that, during an assay, bind specifically to the analyte molecules on the surface of the working electrode[1] (Fig. 2). The reduction of tetramethylbenzidine (TMB) by horseradish peroxidase conjugated to an antibody or oligonucleotide probe provides a redox current, which is interpreted by the amperometric detection system. Concentration of the analyte is proportional to the steady-state current across the working and auxiliary electrodes of the sensor after the addition of TMB under a bias potential.

Fluid Handling

The microfluidic cartridge's fluid-handling system consists of two components (Fig. 3): the pneumatically operated FHC that controls reagent distribution and houses the sensor chambers, and the hydraulically actuated reagent storage

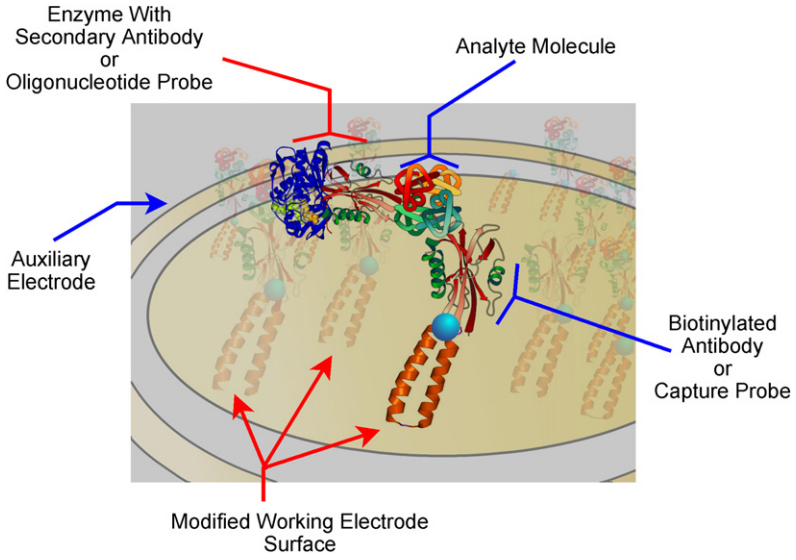

Figure 2. Nanoscale biochemical structures for molecular analysis using electrochemical sensor.

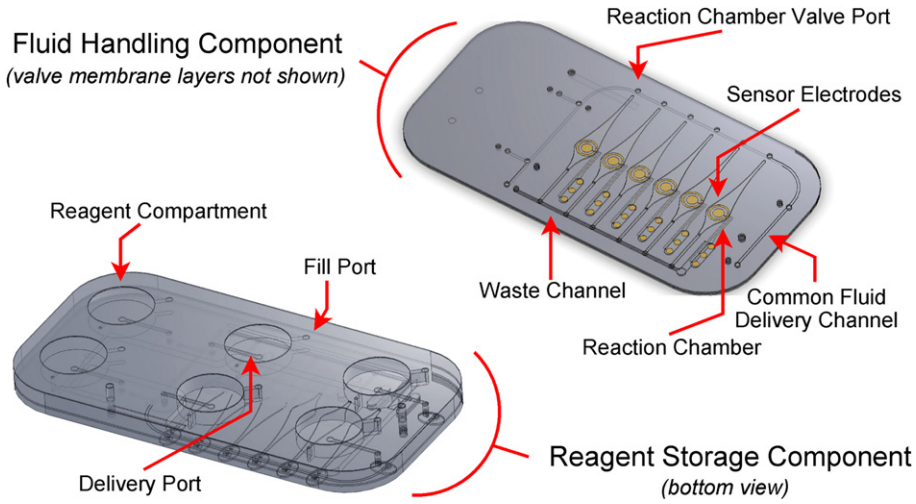

Figure 3. Microfluidic cartridge overview.

component (RSC) that stores and delivers reagents to the FHC (Fig. 4).

Fluid Distribution Component

The cartridge system's FHC consists of the six sensor chambers, a common fluid-distribution channel, isolated microfluidic channels, and unique flexible membrane valves. The proprietary membrane valves are one of the key elements of the FHC. These membrane valves allow for precise control of fluid delivery in the cartridge. The passive valves are made of a plastic laminate containing a bonded flexible membrane. The top of the valve layer seals against a manifold with independently controlled air channels. Pressure applied by the manifold seals the flexible membrane against the valve ports, closing off their respective channels (valve closed, Fig. 5a). If the pressure supply to a valve is eliminated, the flexible membrane will comply with fluid flow, allowing reagent into the desired channel (valve open, Fig. 5b).

A pilot experiment was performed to verify the microfluidic capability of the FHC for multiplexed molecular analysis. Six electrochemical sensors were divided into three groups of precoating conditions, with duplicates of each condition: Antihuman Myoglobin, *E. coli* RNA, and bovine serum albumin (BSA) as a negative control. A cocktail sample solution consisting of buffer spiked with Myoglobin and lyzed *E. coli* 16S rRNA with detector probe solution was loaded manually into the common channel of the cartridge. The delivery sequence to the sensor chambers was controlled by a pneumatic manifold operating on the flexible membrane valves. The signaling enzyme solution, de-ionized (DI) water wash solution, and TMB substrate were sequentially introduced manually through separate inlets. Figure 6 illustrates the signals from Myoglobin, *E. coli* RNA, and the negative control. These results verify the precise control of independent channel delivery provided by the FHC.

Reagent Delivery Component

To fully integrate an assay into the cartridge, a reagent storage and delivery component is necessary. The RSC provides a system of reagent delivery and storage as part of the disposable microfluidic cartridge. It consists of reagent storage compartments, covered by a flexible membrane, into which the required

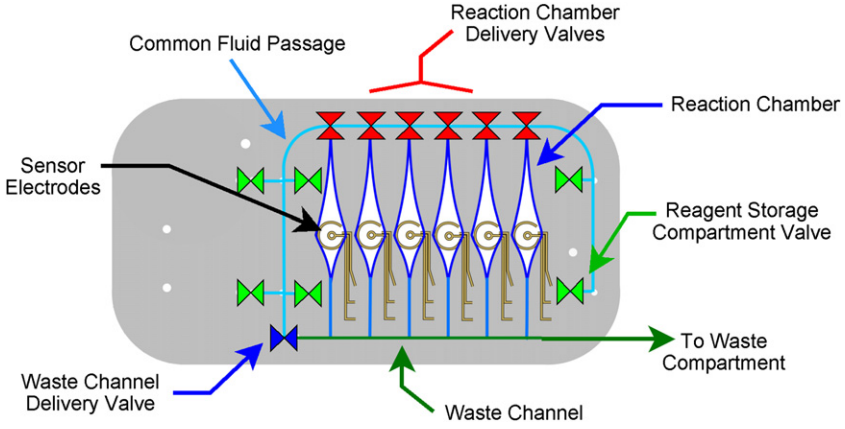

Figure 4. Fluid-handling component schematic.

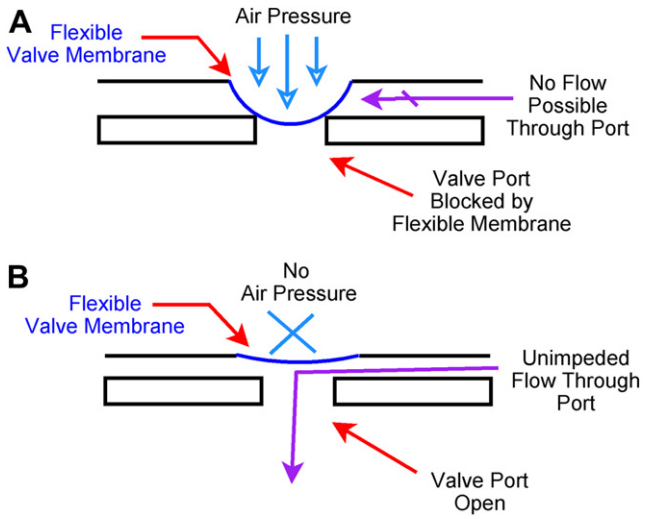

Figure 5. Cross-section view of fluid-handling component's membrane valve: (A) valve closed and (B) valve open.

reagents are preloaded during the final stages of cartridge assembly. When placed into the microfluidic system, the flexible membrane of the cartridge is in direct contact with the flexible membrane of a hydraulic actuator. This actuator is a single hydraulic chamber with a volume precisely controlled by the electronic control system. To deliver a specific reagent to a specific sensor chamber, two valves are opened in the FHC: one reagent control valve and one sensor chamber control valve. An amount of hydraulic fluid equal to the desired amount of reagent is pumped into the hydraulic chamber, deforming both the actuator membrane and the compartment membrane of the desired reagent. The displaced volume of the reagent is delivered into the selected sensor chamber. With delivery complete, the valve pair is switched off, and elastic pressure of both the deformed compartment membrane and a coinciding deformation in the hydraulic actuator membrane creates a vacuum condition in the reagent compartment. This vacuum condition provides a hydraulic lock of that reagent compartment, such that it does not react to changes in hydraulic actuator volume when it is not being actively commanded to deliver.

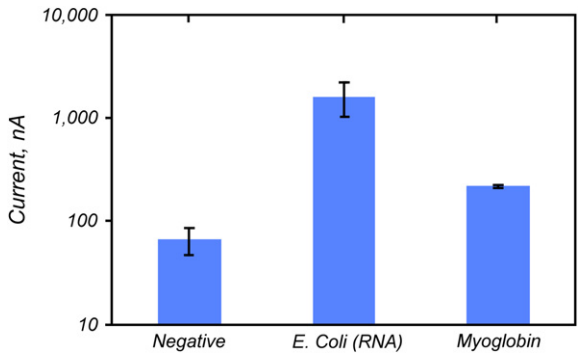

Figure 6. Multiplexed detection of Myoglobin protein and *E. coli* 16s rRNA in fluid-handling component.

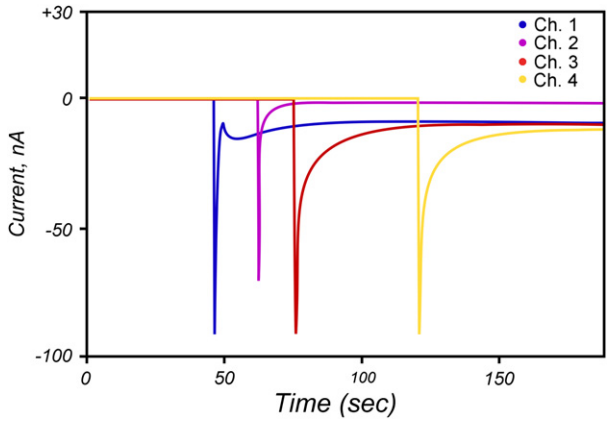

Figure 7. Continuous monitoring of sequential delivery to reaction chambers during a four-assay experiment.

Waste Management

All delivered reagents are routed to the cartridge's waste pouch. This ensures that the instrument is prevented from direct contact with any fluids from the experiment.

Control System

The electronic control system consists of two major components: an embedded component and a host computer system. The embedded component consists of a microcontroller and a multichannel amperometric detection circuit.

Host Computer

The host computer system issues commands to the embedded control system, and receives and stores data from the detection circuit. A set of commands for running a given experiment is called a "recipe." The recipe is designed using software that runs on a Windows XP operating system, and consists of a series of valve control and fluid delivery directives to be carried out by the embedded control system. The recipe can be issued to the embedded component of the control system by either a computer using Windows XP or a smart phone using the Windows Mobile operating system.

Embedded Microcontroller

The embedded microcontroller coordinates valve control, fluid delivery, electrochemical detection, and data acquisition based on the recipe issued by the host computer. The microcontroller's peripherals include a precise digital-to-analog converter (DAC) for control of the detection circuit, an array of analog-to-digital converters (ADCs) for detection, a transistor switch array for valve control, a host computer serial interface, and a fluid delivery motor controller. The microcontroller uses a high-speed serial connection to receive commands from and send acquired data to the host computer system.

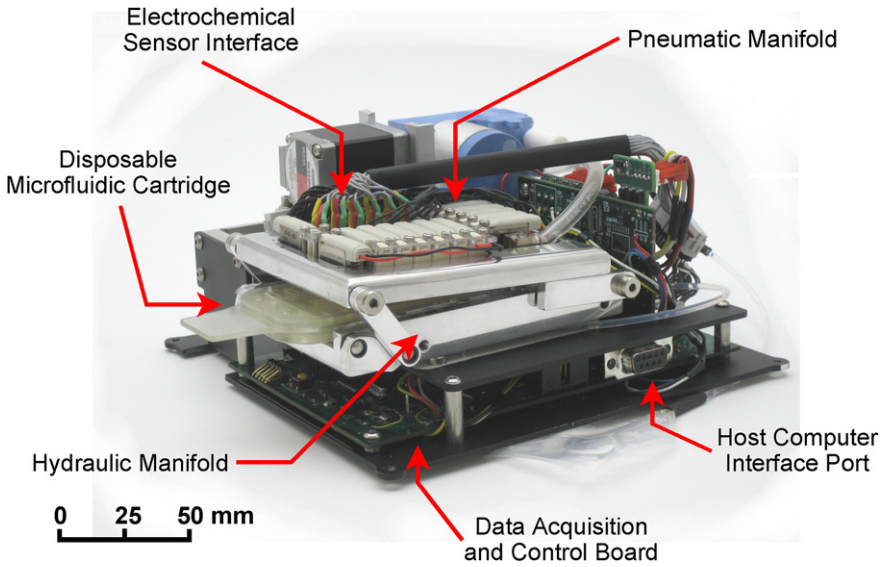

Figure 8. Complete microfluidic cartridge system.

Amperometric Detection Circuit

The amperometric detection circuit consists of a six-channel potentiostat, which interfaces with the cartridge's electrochemical sensors through an array of spring-loaded contact pins. The potentiostat receives its input source voltage from the microcontroller's DAC. The microcontroller's array of ADCs gathers independent amperometric data from each potentiostat channel corresponding to a sensor chamber in the microfluidic cartridge.

Continuous Reagent Delivery Monitoring

Using a small potentiostatic control voltage to measure fluid conductivity, the electronic control system achieves continuous monitoring of sensor chamber conditions during the fluid delivery process (Fig. 7). By providing feedback on each fluid-handling operation, this method of real-time monitoring allows the experimenter to refine a recipe's parameters such as the speed or volume of reagent delivery.

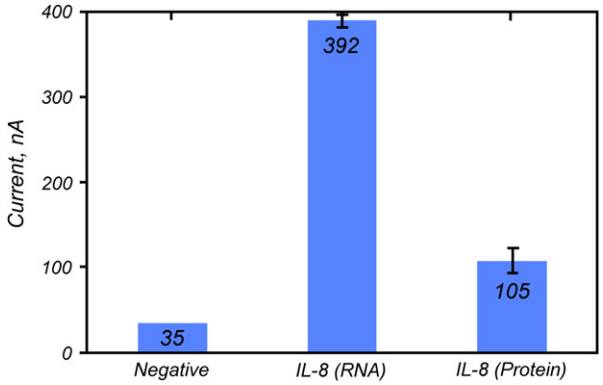

Figure 9. Quantitative IL-8 protein and IL-8 RNA detection using Genefluidics' microfluidic cartridge system.

Results

We demonstrate the coherence of the system integration in a multiplexed proteomic and genomic assay by quantitative measurement of cytokines spiked into a buffer solution. The six electrochemical sensors are divided into two groups of precoating conditions: IL-8 protein and IL-8 RNA. Cocktail sample consisting of buffer spiked with both IL-8 protein and IL-8 RNA was loaded into the cartridge, then BSA buffer (as a prime solution), an enzyme cocktail of IL-8 secondary antibody and detector probe, wash solution, and TMB substrate were loaded into their respective RSC compartments. Figure 8 illustrates the cartridge control system used during the experiment. A smart phone was used as the host computer. The polymer assay protocol described in references 7,8,10 was used for this experiment. The assay process began with delivery of BSA prime solution to all six sensor chambers. Next, the sample was introduced into four of the sensor chambers in the FHC, leaving the two BSA-filled chambers as negative controls. Square wave voltage was applied to the sensors. This pulsing step is necessary in accelerating the binding process by pulling the target closer to the surface as well as preventing nonspecific binding. After 20 pulsing cycles, the sensor chambers were washed with DI water. Secondary IL-8 cocktail enzyme solution was delivered next and binding process was accelerated by 30 pulsing cycles. After a wash step to minimize nonspecific binding, TMB substrate was delivered to each sensor chamber. After a potentiostatic control voltage was applied, the value of the steady state current from all six sensors was recorded.

The measured signal (in nA) from the IL-8 RNA and IL-8 protein was higher than the negative value (Fig. 9). Variance is 1.3% with IL-8 RNA and 13.5% with IL-8 protein. The result demonstrates that our microfluidic system allows for

multiplexed proteomic and genomic assays to be performed concurrently in the same cartridge.

Conclusions and future directions

In designing the compact microfluidic system described here, we have demonstrated how microfluidics and electrochemical sensing technology can be combined to create a powerful and versatile analytical device. Enabling simultaneous genomic and proteomic assays with flexible protocol requirements on a single disposable cartridge holds great promise for a point-of-care application. Further validation of multiplexed assays will be performed by our collaborators through clinical studies using the microfluidic cartridge system described here.

Acknowledgments

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References

1. Gau, J. J.; Lan, E. H.; Dunn, B.; Ho, C. M.; Woo, J. C. A MEMS based amperometric detector for E. coli bacteria using self-assembled monolayers. *Biosens. Bioelectron.* **2001,** *16*, 745−755.
2. Gau, V.; Ma, S. C.; Wang, H.; Tsukuda, J.; Kibler, J.; Haake, D. A. Electrochemical molecular analysis without nucleic acid amplification. *Methods* **2005,** *37*, 73−83.
3. Liao, J. C.; Mastali, M.; Gau, V.; Suchard, M. A.; Moller, K. A.; Bruckner, D. A.; Babbitt, J. T.; Li, Y.; Gornbein, J.; Landaw, E. M.; McCabe, E. R.; Churchill, B. M.; Haake, D. A. Use of electrochemical DNA biosensors for rapid molecular identification of uropathogens in clinical urine specimens. *J. Clin. Microbiol.* **2006,** *44*, 561−570.
4. Liao, J. C.; Mastali, M.; Li, Y.; Gau, V.; Suchard, M.; Babbitt, J. T.; Gornbein, J.; Landaw, E. M.; McCabe, E. R.; Churchill, B. M.; Haake, D. A. Development of an advanced electrochemical DNA biosensor for bacterial pathogen detection. *J. Mol. Diagn.* **2007,** *9*, 158−168.
5. Sin, M. L. Y.; Constantino, V. U.; Gau, V.; Wong, P. K. Scaling analysis of a universal electrode for molecular biosensors. *Nano/Micro. Eng. Mol. Syst.* **2008,** 1151−1155.
6. Sun, C. P.; Liao, J. C.; Zhang, Y. H.; Gau, V.; Mastali, M.; Babbitt, J. T.; Grundfest, W. S.; Churchill, B. M.; McCabe, E. R.; Haake, D. A. Rapid, species-specific detection of uropathogen 16S rDNA and rRNA at ambient temperature by dot-blot hybridization and an electrochemical sensor array. *Mol. Genet. Metab.* **2005,** *84*, 90−99.
7. Huang, T. J.; Liu, M.; Knight, L. D.; Grody, W. W.; Miller, J. F.; Ho, C. M. An electrochemical detection scheme for identification of single nucleotide polymorphisms using hairpin-forming probes. *Nucleic Acids Res.* **2005,** *30(12)*, e55.
8. Wei, F.; Wang, J.; Liao, W.; Zimmermann, B. G.; Wong, D. T.; Ho, C. M. Electrochemical detection of low-copy number salivary RNA based on specific signal amplification with hairpin probe. *Nucleic Acids Res.* **2008,** *36(11)*, e65.
9. Wei, F.; Zimmermann, B.; Li, N.; Ho, C. M.; Wong, D. Electrochemical Detection of Salivary RNA, IEEE Review on Advances in Micro, Nano and Molecular Systems, 7th IEEE International Conference on Nanotechnology, Hong Kong, August 2−5, 2007.
10. Mastali, M.; Babbitt, J. T.; Li, Y.; Landaw, E. M.; Gau, V.; Churchill, B. M. Optimal probe length and target location for electrochemical detection of selected uropathogens at ambient temperature. *J. Clin. Microbiol.* **2008,** *46(8)*, 2707−2716.