Published in final edited form as:

Methods Mol Biol. 2015 ; 1256: 99–109. doi:10.1007/978-1-4939-2172-0_7.

Detection of Protein Biomarker Using a Blood Glucose Meter

Tian Lan, Yu Xiang, and Yi Lu

Abstract

mHealth technologies are recognized to play important roles in the future of personal care and medicine. However, their full potentials have not been reached, as most of current technologies are restricted to monitoring physical and behavioral parameters, such as body temperature, heart rate, blood pressure, and physical movement, while direct monitoring of biomarkers in body fluids can provide much more accurate and useful information for medical diagnostics. A major barrier to realizing the full potential of mHealth is the high costs and long cycles of developing mHealth devices capable of monitoring biomarkers in body fluids. To lower the costs and shorten the developmental cycle, we have demonstrated the leveraging of the most successful portable medical monitoring device on the market, the blood glucose meter (BGM), with FDA-approved smartphone technologies that allow for wireless transmission and remote monitoring of a wide range of non-glucose targets. In this protocol, an aptamer-based assay for quantification of interferon- γ (IFN- γ) using an off-the-shelf BGM is described. In this assay, an aptamer-based target recognition system is employed. When IFN- γ binds to the aptamer, it triggers the release of a reporter enzyme, invertase, which can catalyze the conversion of sucrose (not detected by BGM) to glucose. The glucose being produced is then detected using a BGM. The system mimics a competitive enzyme-linked immunosorbent assay (ELISA), where the traditional immunoassay is replaced by an aptamer binding assay; the reporter protein is replaced by invertase, and finally the optical or fluorescence detector is replaced with widely available BGMs.

Keywords

Blood glucose meter (BGM); Aptamer; Biosensor; Point-of-care diagnostic; Electrochemical sensor

1 Introduction

Recent advances in mobile health (mHealth) technologies have led to significant changes in ways people can manage their health. A large number of wearable devices have been developed to monitor different parameters of a person's physiological state, such as blood pressure, heart rate, and body temperature, as well as behavior, such as medication adherence and physical movement [1]. While these physical and behavioral results are

³Invertase is an enzyme widely used in industrial applications. It can withstand multiple cycles of heating and cooling, long periods of elevated temperature (65 °C), and low pH. Its optimal activity is observed in acidic pH and elevated temperatures.

⁴To increase assay performance, Grade X invertase (Sigma Aldrich, catalog # I4753) from *Candida utilis* can be used. The same procedures described in the protocol can be directly applied to the Grade X invertase. We have observed an activity enhancement of at least 100 % when using *Candida utilis* invertase versus yeast invertase.

important indicators of human health, they are far from satisfactory in accurate medical diagnostics, which requires direct detection and monitoring of biomarkers in body fluids such as blood, saliva, or urine [1]. Therefore, there is still a huge gap between clinical diagnosis in the hospitals and point-of-care tests using mHealth devices. To bridge such a gap, new technological advances have led to several new products and approaches for remote biomarker monitoring. One example is the mChip system, based on an ELISA assay housed in a microfluidic disposable and an optical reader, which has been developed and field-tested for HIV diagnosis in Rwanda. The diagnostic results are transmitted and stored in the cloud [2]. Despite these advances, very few mHealth devices capable of detecting biomarkers in body fluids are available on the market. One reason for such a situation is the high costs and long cycles associated with developing mHealth technologies and most such devices are dedicated to only one or a few biomarker detections.

To lower the costs and shorten the cycles of developing new mHealth technologies that can be generally applied to a wide range of biomarkers, we proposed and demonstrated an alternative approach of repurposing existing technologies and devices for a much wider range of applications. The blood glucose meter (BGM) is an excellent platform to leverage, because it has gone through decades of research and development, making the current generation of BGMs accurate, well designed for simple operation, low cost, and portable. More importantly, several network-connected smartphone-compatible BGMs [3–5] are available and some of them have already been approved by FDA. In fact, these network-connected BGMs account for the majority of current mHealth devices capable of monitoring biomarkers [1]. Furthermore, BGM technologies are constantly being improved due to the growing number of diabetes [6]. By taking advantage of this highly developed and widely available mHealth device, we and others have developed novel methodologies to transform the binding of non-glucose biomarkers by either aptamers or antibodies into glucose so that network-connected smartphone-compatible BGMs can be used to detect and monitor a wide range of targets, such as metal ions, small organic molecules, protein markers, and nucleic acids [7–12].

In this protocol, a method is described to measure a protein biomarker, IFN- γ , using an unmodified, off-the-shelf BGM [7]. IFN- γ is a cytokine released by immune cells and its level can be a general indicator for many infectious diseases [13]. An aptamer [14–18] is used for the selective binding of IFN- γ . An aptamer possesses similar ligand binding properties as an antibody. Aptamers can be isolated via an in vitro selection process and they are a promising new technology for diagnostic assays in the near future. A more detailed review of current aptamer technology can be found elsewhere [19]. Binding of IFN- γ to the aptamer triggers the release of an enzyme, invertase. As an enzyme used widely in the confectionery industry, invertase is capable of converting sucrose, which cannot be detected by a glucose meter, into glucose. The glucose produced by invertase then can be quantified by an unmodified, off-the-shelf BGM. A scheme of the assay is shown in Fig. 1. Three steps are performed to complete the assay: (1) conjugation of thiol-modified DNA to invertase; (2) immobilization of invertase on magnetic beads; and (3) detection of IFN- γ using a BGM.

In addition to aptamers, antibodies have also been used as target recognition elements in BGM-based assays [9]. The BGM-based immunoassay has been developed using a

sandwich type assay system, where instead of using reporter proteins to generate a fluorescent or colorimetric signal, such as HRP or NADH dehydrogenase, invertase is used to generate glucose, for convenient detection by existing BGMs. This type of sandwich assay has been developed for Prostate Specific Antigen. Simple replacement of the reporter protein in the traditional ELISA provided a more convenient way to convert today's ELISA assay to a BGM based immunoassay.

To apply the BGM-based biomarker detection in mHealth settings, a more simple and elegant solution will need to be developed to perform the different steps of the assay. The solution for mHealth application would encompass two different components: a disposable assay cartridge and a reader. The assay reagents can be stored in the cartridge. The different steps of the assay, e.g., sample collection, pretreatment, binding of biomarkers, release of invertase and glucose production, can be driven by the cartridge. A number of existing technologies can be used to design the cartridge, for example, lateral flow device and various microfluidic designs. Ultimately, the amount of a specific biomarker is correlated to a concentration of glucose, which can be precisely quantified. As the product of decades of engineering and refinement, today's BGM offers an excellent system for convenient and comfortable operation by millions of diabetes. These BGMs are developed with mobile monitoring, cloud storage, Electronic Health Record integration, and remote analysis in mind, such as the iBG STAR[®] and Telcare[®] blood glucose monitoring systems. Future generation BGMs are focused on more accurate and sensitive detection, as well as noninvasive and continuous monitoring. The reader for the BGM-based biomarker assays can be designed or modified based on these existing BGMs to leverage matured technologies. The BGM-based biomarker assays being developed can potentially expand the biomarkers for mHealth application greatly beyond physical body parameters, such as body temperature, heart rate, and exercise tracking.

2 Materials

1. The glucose meter used for the protocol is an ACCU-CHEK Avia glucose meter which can be found in stores. Other glucose meters, such as the iBG STAR[®] and Telcare[®] blood glucose monitoring systems, can also be used for the same application.
2. Streptavidin-coated magnetic beads (1 μ m average diameter) are purchased from Bangs Laboratories (Fishers, IN). The magnetic rack used for separation is purchased from PIERCE Biotechnology (Rockville, IL).
3. Amicon-10K and Amicon-100K (500 μ L capacity) are purchased from Millipore Corporation (Billerica, MA).
4. Grade VII invertase (*S. cerevisiae*) and human recombinant interferon- γ (IFN- γ) are purchased from Sigma-Aldrich (St. Louis, MO).
5. General chemicals and human serum are purchased from Sigma- Aldrich (St. Louis, MO) and are used as received unless otherwise specified.

6. Three different oligonucleotides are used and custom-synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). The list of oligonucleotides is shown in Table 1. Oligos are HPLC purified by the vendor and they are used without further purification.
7. Buffers and solutions used in this protocol:

Buffer A: 100 mM sodium phosphate (NaPi), 100 mM NaCl, 0.05 % Tween-20, pH 7.3.

Buffer B: 100 mM NaPi, 100 mM NaCl, pH 7.3.

Buffer C: 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 100 mM KCl, 1 mM MgCl₂, 0.05 % Tween-20, pH 7.4.

Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) solution: 30 mM freshly prepared in Millipore water.

3 Methods

3.1 Conjugation of Thiol Modified DNA to Invertase (See Fig. 2)

1. A 1 mM solution of thiol modified DNA (Table 1) is prepared in Millipore water. To 30 μ L of thiol-modified DNA, 2 μ L of 1 M NaPi (pH 5.5) and 2 μ L of TCEP solution are added to activate the thiol moiety on the DNA. After 1 h of reaction at room temperature, the unreacted TCEP is removed by washing with Buffer B at least four times using an Amicon-10K. The final volume should be approximately 30 μ L.
2. A 20 mg/mL invertase solution is prepared in Buffer B. To 400 μ L of 20 mg/mL invertase solution, 1 mg of Sulfo-SMCC (Sigma-Aldrich, St. Louis, MO) is added (*see*^{Note 1}). The mixture is vortexed for 5 min and placed on a shaker for at least 1 h at room temperature. After 1 h, any insoluble matter is removed by briefly centrifuging the mixture (15,000 $\times$ g) and retaining the supernatant. The unreacted Sulfo-SMCC in the supernatant is removed by washing with Buffer B six times using Amicon-100K. The final volume should be approximately 30 μ L.
3. The thiol modified DNA and SMCC activated invertase obtained from **steps 1 and 2** are mixed and incubated at room temperature for at least 24 h to form the DNA-modified invertase. After the reaction, the unreacted thiol-modified DNA is removed by washing with Buffer B six times using an Amicon-100K.

¹Sulfo-SMCC may not be completely soluble in buffer during the conjugation of thiol-modified DNA to invertase. This is normal and does not affect the end result of the conjugation. Using DMSO or DMF (for example, 20 % volume of buffer) to increase the solubility of Sulfo-SMCC is not recommended here, because yeast invertase is potentially deactivated in solutions containing high contents of DMSO or DMF and Amicon centrifuges are not able to tolerate solutions with such high DMSO or DMF content.

3.2 Immobilization of Invertase on Magnetic Beads

1. The DNA-modified invertase is washed with Buffer C twice to exchange the buffer. The final concentration of invertase is around 20 mg/mL.
2. A 1 mL suspension of streptavidin-coated magnetic beads (1 mg/mL) in a microcentrifuge tube is placed on the magnetic rack. The magnetic beads are allowed to collect at the bottom of the tube for 2 min. The supernatant is pipetted off and discarded. The magnetic beads are resuspended in 1 mL of Buffer C.
3. A 12 μ L aliquot of 0.5 mM biotin-modified DNA solution is prepared in Millipore water. The biotin-modified DNA is added to the streptavidin-coated magnetic beads from **step 2** and mixed by vortexing for 30 min at room temperature. After incubation, the magnetic beads are washed three times with 1 mL of Buffer C and resuspended in 1 mL of Buffer C.
4. A 12 μ L aliquot of a 0.5 mM IFN- γ aptamer solution is prepared in Millipore water. The aptamer solution is added to the magnetic bead solution from **step 3** and vortexed for 30 min at room temperature. After the incubation, the magnetic beads are washed three times with 1 mL of Buffer C and resuspended in 1 mL of Buffer C.
5. A 1 mL aliquot of DNA-modified invertase (containing around 5 mg/mL invertase) is added to the magnetic beads prepared in the previous step and vortexed at room temperature for 30 min. Excess invertase is removed by washing the magnetic beads with 1 mL of Buffer C five times (*see*^{Notes 2–5}). These DNA-invertase-coated magnetic beads are suspended in 1 mL of Buffer C. For detection of IFN- γ , 60 μ L of DNA- and invertase-coated magnetic beads are used for each sample.

3.3 Detection of IFN- γ Using BGM

1. IFN- γ solutions of different concentrations are prepared in Buffer C. Then 20 μ L of an IFN- γ solution is added to 60 μ L of DNA- and invertase-coated magnetic beads and vortexed for 15 min at room temperature in a 1.5 mL microcentrifuge tube.
2. After incubation with the IFN- γ solution, the tubes are placed on a magnetic rack for 2 min to collect the magnetic beads at the bottom of the tube. Then, 10 μ L of supernatant is withdrawn and added to 3.3 μ L of 2 M sucrose in Buffer A at room temperature.
3. After 30 min of incubation, 5 μ L of the sucrose solution is measured for glucose content using an ACCU-CHEK Avia glucose meter (*see*^{Note 6}). To use the glucose meter, a strip is first inserted into the glucose meter.

²After incubating the DNA-modified invertase with magnetic beads, excess DNA modified invertase can be recycled for further usage by using an Amicon-100K filter and washing with Buffer B.

⁵To scale up production of the aptamer/invertase coated magnetic beads, the amount of materials can be increased, while maintaining the same mass ratio.

After a beep, which indicates the strip is ready for measurement, the sample can be applied directly to the sampling area of the glucose strip. A numeric result will be displayed on the glucose meter after several seconds (*see*^{Notes 7–8}).

4. For a negative control, different concentrations of human serum albumin solutions are tested using the same protocol as described in **steps 1 – 3** above. For detection in 20 % human serum, the same procedures in **steps 1 – 3** may be used without modification.
5. Representative data for IFN- γ detection in buffer C using BGM is shown in Fig. 3a. An IFN- γ dependent glucose response can be observed, and the glucose readout reached a maximum at 400 nM of IFN- γ (*see*^{Note 9}). The limit of detection (LoD) is calculated to be 2.6 nM (based on 3σ), which is similar to the affinity of the aptamer [14]. Existing ELISA assays for IFN- γ typically have a LoD in the sub-picomolar range [20]. The higher LoD obtained from the BGM assay was mainly due to lower affinity of the aptamer, as compared to antibodies. Adoption of antibodies for the BGM assay can improve the assay's sensitivity to a similar level achieved by immunoassays. For example, a BGM-based sandwich assay for Prostate Specific Antigen has been demonstrated with a LoD at 400 pg/mL [9], which is closer to the LoD of existing ELISA assay (typically in the 10–100 pg/mL range) [21–23] (*see*^{Note 10}). Negative controls using human serum albumin result in negligibly low readings from the BGM under otherwise identical conditions.

IFN- γ detection in 20 % human serum has also been tested and a representative result is shown in Fig. 3b. A slight decrease in the maximum glucose signal can be observed due to the presence of serum components; however, the effect on the limit of detection is small (3.4 nM in 20 % human serum). Signal saturation was observed with a lower IFN- γ concentration compared to the assay in buffer. This earlier signal saturation can be a result of a weaker hybridization of the DNA and upon IFN- γ binding to the aptamer, as release of the DNA-invertase conjugate is easier. In addition, serum proteins may block the nonspecific binding of IFN- γ to magnetic beads.

⁶The ACCU-CHEK Avia BGM requires a code chip to be inserted to calibrate different batches of glucose strips—otherwise, the BGM will not perform a measurement. This code chip is obtained from each container of glucose strips. Other brands of BGM may not require a code chip for operation.

⁷The BGM will only report glucose levels between 10 mg/dL and 600 mg/dL. If a measurement is outside this range, either a “LO” (<10 mg/dL) or a “HI” (>600 mg/dL) signal will be displayed, respectively.

⁸Other brands of BGM may also be used; however, due to different chemistry used and calibration, glucose readings will be different for the same sample when different BGM is used from this study. This difference can be minimized if the buffer conditions (pH, ionic strength, viscosity, etc.) are more similar to those of human blood.

⁹The assay has been designed to perform optimally at room temperature (around 23 °C); significant temperature deviation can result in faulty assay results.

¹⁰The principles described in the protocol have been also applied to antibody and nucleic acid hybridization-based assays for the quantification of protein biomarkers and nucleic acid sequences using a BGM [8, 9].

Acknowledgments

This material is based upon work supported by the US National Institutes of Health (RDA035524A and RDK100213A and ES16865) and National Science Foundation (IIP-1330934).

References

1. mHealth G. Device Listing. A global list of commercially available devices in the health-care sector, updated fortnightly 2012. 2012. http://www.gsma.com/connectedliving/wp-content/uploads/2012/04/22-November12_MobileHealth_Device_Listing.pdf
2. Chin CD, Cheung YK, Laksanasopin T, Modena MM, Chin SY, Sridhara AA, et al. Mobile device for disease diagnosis and data tracking in resource-limited settings. *Clin Chem*. 2013; 59(4):629–640. [PubMed: 23327782]
3. iBG STAR. 510(k) Substantial Equivalence Determination Decision Summary (510(k) number: k103544).
4. iBGSTAR. iBGStar[®] blood glucose meter. Sanofi; 2011. (updated 2011; cited 2011 Dec 1) <http://www.bgstar.com/web/ibgstar>
5. Telcare. Telcare. 2013. (updated 2013; cited 2013 09/09) <https://www.telcare.com/>
6. Hones J, Muller P, Surridge N. The technology behind glucose meters: test strips. *Diabetes Technol Ther*. 2008; 10:S10–S26.
7. Xiang Y, Lu Y. Using personal glucose meters and functional DNA sensors to quantify a variety of analytical targets. *Nature Chem*. 2011; 3(9):697–703. [PubMed: 21860458]
8. Xiang Y, Lu Y. Using commercially available personal glucose meters for portable quantification of DNA. *Anal Chem*. 2012; 84(4):1975–1980. [PubMed: 22235863]
9. Xiang Y, Lu Y. Portable and quantitative detection of protein biomarkers and small molecular toxins using antibodies and ubiquitous personal glucose meters. *Anal Chem*. 2012; 84(9):4174–4178. [PubMed: 22455548]
10. Nie Z, Deiss F, Liu X, Akbulut O, Whitesides GM. Integration of paper-based microfluidic devices with commercial electrochemical readers. *Lab Chip*. 2010; 10(22):3163–3169. [PubMed: 20927458]
11. Su J, Xu J, Chen Y, Xiang Y, Yuan R, Chai Y. Personal glucose sensor for point-of-care early cancer diagnosis. *Chem Commun (Camb)*. 2012; 48(55):6909–6911. [PubMed: 22669465]
12. Yan L, Zhu Z, Zou Y, Huang Y, Liu D, Jia S, et al. Target-responsive “sweet” hydrogel with glucometer readout for portable and quantitative detection of non-glucose targets. *J Am Chem Soc*. 2013; 135(10):3748–3751. [PubMed: 23339662]
13. Boehm U, Klamp T, Groot M, Howard JC. Cellular responses to interferon-gamma. *Annu Rev Immunol*. 1997; 15:749–795. [PubMed: 9143706]
14. Liu Y, Kwa T, Revzin A. Simultaneous detection of cell-secreted TNF-alpha and IFN-gamma using micropatterned aptamer-modified electrodes. *Biomaterials*. 2012; 33(30):7347–7355. [PubMed: 22809645]
15. Liu Y, Yan J, Howland MC, Kwa T, Revzin A. Micropatterned aptasensors for continuous monitoring of cytokine release from human leukocytes. *Anal Chem*. 2011; 83(21):8286–8292. [PubMed: 21942846]
16. Lee PP, Ramanathan M, Hunt CA, Garovoy MR. An oligonucleotide blocks interferon- gamma signal transduction. *Transplantation*. 1996; 62(9):1297–1301. [PubMed: 8932275]
17. Balasubramanian V, Nguyen LT, Balasubramanian SV, Ramanathan M. Interferon-gamma-inhibitory oligodeoxynucleotides alter the conformation of interferon-gamma. *Mol Pharmacol*. 1998; 53(5):926–932. [PubMed: 9584220]
18. Tuleuova N, Jones CN, Yan J, Ramanculov E, Yokobayashi Y, Revzin A. Development of an aptamer beacon for detection of interferon-gamma. *Anal Chem*. 2010; 82(5):1851–1857. [PubMed: 20121141]
19. Stoltenburg R, Reinemann C, Strehlitz B. SELEX—a (r)evolutionary method to generate high-affinity nucleic acid ligands. *Biomol Eng*. 2007; 24(4):381–403. [PubMed: 17627883]

20. Wilson AB, McHugh SM, Deighton J, Ewan PW, Lachmann PJ. A competitive inhibition ELISA for the quantification of human interferon-gamma. *J Immunol Methods*. 1993; 162(2):247–255. [PubMed: 8315291]
21. Acevedo B, Perera Y, Ruiz M, Rojas G, Benitez J, Ayala M, et al. Development and validation of a quantitative ELISA for the measurement of PSA concentration. *Clin Chim Acta*. 2002; 317(1–2): 55–63. [PubMed: 11814458]
22. Vessella RL. Trends in immunoassays of prostate-specific antigen: serum complexes and ultrasensitivity. *Clin Chem*. 1993; 39(10):2035–2039. [PubMed: 7691437]
23. Matsumoto K, Konishi N, Hiasa Y, Kimura E, Takahashi Y, Shinohara K, et al. A highly sensitive enzyme-linked immunoassay for serum free prostate specific antigen (f-PSA). *Clin Chim Acta*. 1999; 281(1–2):57–69. [PubMed: 10217627]

Fig. 1.

Design of the aptamer based assay for detection of IFN- γ using a BGM. The assay system is composed of a DNA-modified invertase (*blue* DNA sequence), hybridized with an IFN- γ aptamer (*black* DNA sequence), which also hybridizes with a biotin modified DNA (*green* DNA sequence) that is bound to streptavidin-coated magnetic beads (*brown sphere*). In the absence of IFN- γ , complementary DNA hybridization between the three DNA sequences keeps the enzyme invertase bound on the magnetic beads. Upon addition of IFN- γ , the binding of IFN- γ to aptamer results in the dehybridization of thiol-modified DNA, leading to the release of invertase from the magnetic beads. The released invertase can be separated from the remaining bead-bound invertase by magnetic separation. The released invertase is then used to produce glucose for BGM detection, from sucrose (which is undetectable by BGM)

Fig. 2.

Conjugation chemistry for covalently attachment of a thiol-modified DNA to invertase. The primary group on the surface of invertase is first reacted with a bifunctional linker (Sulfo-SMCC), which contains an amine-reactive *N*-hydroxysuccinimide ester moiety and a thiol-reactive maleimide group. The invertase-SMCC is then reacted with the thiol-modified DNA to complete this step of conjugation

Fig. 3. Representative data of IFN- γ detection in buffer, with human serum albumin as negative control (a); and in 20 % human serum (b)

Table 1

DNA sequences used in the study

IFN- γ aptamer	5'- <u>TGGGGTTGGTTGTGTTGGGTGTTGTG</u> TAAAAAAAAAAAACTA CTCATCTGTGA-3'
Biotin-modified DNA	5'-Biotin-AAAAAAAAAAATCA CAGATGAGTAGT-3'
Thiol-modified DNA	5'-HS-AAAAAAAAAAACAACCAACCCCA-3'

Underlined sequence is the actual functional sequence of the aptamer