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The application of personal glucose meters as universal point-of-care diagnostic tools

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Abstract

Personal glucose meters (PGMs) have been used for the measurement of blood glucose for decades now such that they have become the most used analytical method in the world. They are also well placed to be repurposed for point-of-care testing of other analytes as they are inexpensive, portable and quantitative. Efforts to repurpose PGMs for the detection of any analyte at the point-of-care have been one focus of biosensor research for several years now with a number of successful efforts in the detection of a wide range of analytes. This article reviews the published methods to repurpose a PGM to detect analytes other than glucose, and analyses the potential and the challenges to be overcome in developing a PGM-based biosensor and bring it to market.

Keywords

Personal glucose meter; point of care; biosensor; sandwich immunoassay; competitive immunoassay, functional nucleic acid sensors.

1. Introduction

Since the release of the first commercially successful blood glucose analyser from Yellow Springs Instrument (YSI) in 1975,(Newman and Turner, 2005) and the release of the first personal glucose meter by Medisense in 1987,(Wang, 2001) the success of blood glucose meters has been unparalleled in the medical diagnostics field. More than two decades ago, the ability to regularly self-monitor blood glucose levels and intensively treat diabetes was shown to slow the progression of the disease,(Diabetes Control and Complications Trial Research Group et al., 1993) and years of research and development since have culminated in the Personal Glucose Meters (PGMs) that we recognise today as integral in managing diabetes for millions of patients.(Wang, 2008)

In addition to revolutionising diabetes control in heavily resourced areas, the use of PGMs as point-of-care diagnostic tools in resource constrained areas has also been confirmed, allowing medical professionals to diagnose and treat patients without access to high end analytical laboratories.(Bhavadharini et al., 2016) The ability to transport this critical medical technology to areas with limited infrastructure and limited access to trained personnel is one of the great achievements of PGMs. While the PGM industry moves toward the development of non-invasive, online, and continuous glucose monitoring systems,(Aggidis et al., 2015; Frontino et al., 2010; Vashist, 2012) there is a parallel effort in the research community to repurpose the traditional finger-prick PGMs for the detection of biomarkers other than glucose.(Lan et al., 2016) Such novel application of a hugely successful, cost effective, and portable diagnostic tool could greatly expand the possibilities of point-of-care testing. The need to diagnose, and begin treatment, at a doctor's office, in an ambulance, at home, or in a remote or isolated region (the 'point of care') whilst the patient remains present has been well documented.(Bissonnette and Bergeron, 2015; Bonebreak et al., 2011; Gaydos and Hardick, 2014; Yager et al., 2008; Zumla et al., 2014) It is estimated that effective portable tests could prevent millions of deaths each year through more effective management of transmissible diseases and life-threatening conditions.(Drain et al., 2014; Rasti et al., 2017) In addition to the lives saved, point of care tests have the potential to reduce the transmission and spread of disease (due to rapid diagnosis and immediate intervention), reduce unnecessary use of antibiotics, improve the use of antiviral medications, and reduce the overall economic costs of treating infectious diseases.(Brendish et al., 2015; Turner et al., 2017)

However, the main barriers for the introduction of new point of care tests are the high cost and the time necessary to bring one to the market. Classified as a type of medical devices, these tests need to go through a regulatory process with the relevant authority, such as the Food and Drug Administration (FDA) in USA or the European Medicines Agency (EMA) in Europe. By leveraging an already approved medical technology such as the PGM, the cost and the time of bringing a new product to market could be reduced. Another advantage of using PGMs to develop point of care tests is the ability to quantify the target. Portable tests commercially available for immunosensor applications are mostly based on lateral flow devices, which are qualitative or semi-quantitative.(Vashist et al., 2015; Xuena Zhu et al., 2017) On the other hand, PGMs are inherently quantitative. This means that point of care tests based on repurposed PGMs should be able to provide the clinician with more information regarding the status of a disease, thus helping to identify the most appropriate treatment. This feature further justifies the cost of bringing such a device to the market.

Despite these advantages, the use of PGMs for the detection of biomarkers other than glucose is a recently new area, and a PGM-based point of care test is yet to be commercialised. The first reported use of PGMs to detect other biomarkers was published by Xiang and Lu.(Xiang and Lu, 2011) The Lu group has strongly contributed in establishing this field by developing a variety of strategies for several different analytes.(Xiang et al., 2014; Xiang and Lu, 2012a, 2012b) The first report was based on the target-induced release of invertase, an enzyme that catalyses the conversion of sucrose to glucose (**Figure 1**). The glucose produced by the invertase was then detected using a PGM, thus allowing the quantification of the target. This first successful example have stimulated the interest of other researchers, who have covered analytes as wide-ranging as mycotoxins, DNA, enzymes, metal ions, and pathogenic bacteria,(Fu et al., 2013, 2015, 2014; Tang et al., 2016; Z. Wang et al., 2015; Xue-tao et al., 2014; Y. Zhao et al., 2015) with the majority of the published methods using enzymes or glucose-loaded nanoparticles to generate the glucose that is ultimately measured by the PGM (**Figure 1**).

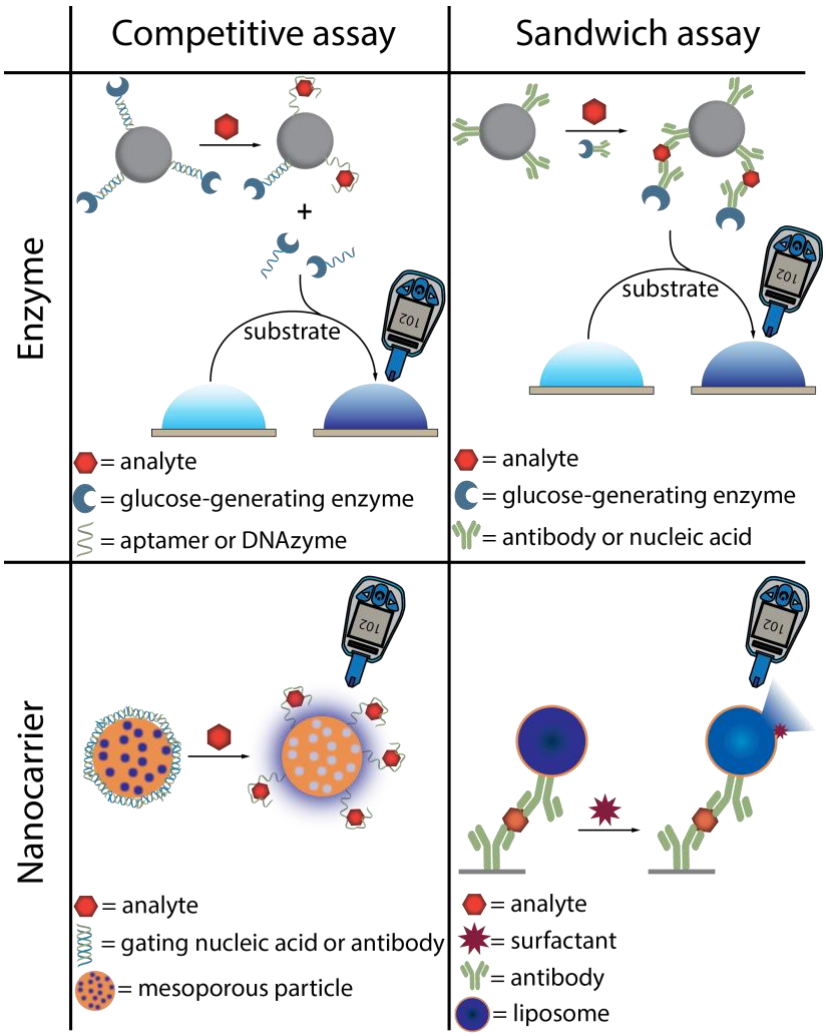


Figure 1. Classification of the strategies to detect analytes other than glucose using a PGMs. Transduction of the analyte into glucose signal is mostly performed either via enzymatic or nanocarrier transduction, in a sandwich or competitive assay format. In enzymatic transduction, the analyte results in capture/activation of an enzyme that converts a substrate to glucose. In nanocarrier transduction, the analyte results in capture of a nanostructure (a liposome or a mesoporous material) carrying glucose solution which can be subsequently released for measurement by the PGM. Several recognition strategies (antibody-antigen interaction, nucleic acid hybridization, DNAzyme, etc.) have been used to detect the analyte.

The recognition species used in the literature include RNA and DNA,(Q. Wang et al., 2014; Xiang and Lu, 2012b) aptamers and DNAzymes,(Gu et al., 2015; Xiang and Lu, 2011) antibodies,(Xiang et al., 2014; Xiang and Lu, 2012a) and molecularly imprinted polymers.(Chen et al., 2015a) These biomolecules have been immobilised on the surface of microtiter plates(Q. Wang et al., 2015a), nanoparticles(Xue-tao et al., 2014) or mesoporous materials(Tang et al., 2014) to capture the analyte from the sample. In several of these strategies the glucose-generating enzyme is directly conjugated with the recognition element, resulting in one per target analyte. However, to improve the detection limit, several authors have linked more than one enzyme per analyte, either by supporting them on a nanoparticle,(Chen et al., 2015a; Fang et al., 2018; Fu et al., 2015, 2014; Hun et al., 2015a, 2015b; Su et al., 2012; Yan et al., 2013; Jin Zhang et al., 2015; L. Zhao et al., 2015) or by loading them inside a liposome(Lin et al., 2016) or a hydrogel.(Yan et al., 2013) Regarding the nanocarriers with encapsulated glucose, two types of nanostructures have been employed; liposomes(Tang et al., 2016; Y. Zhao et al., 2015) and mesoporous particles.(Fu et al., 2013; Gao et al., 2014; Hou et al., 2014; Tang et al., 2014; Y. Wang et al., 2014) PGMs have also been coupled to various DNA amplification methods for nucleic acid detection.(Du et al., 2015; Huang et al., 2017; Wang et al., 2016; Xue-tao et al., 2015a) However, the need for DNA amplification might limit the potential of these approaches to be implemented in a portable device.

An alternative way of expanding the application of PGMs is to use glucose as a marker for conditions other than diabetes. This has been achieved by measuring the glucose concentration in low cerebrospinal fluid as a marker for acute bacterial meningitis cases,(Rousseau et al., 2019) and by monitoring the glucose consumed by the metabolism of bacteria as a way to detect pathogens.(Chavali et al., 2014; Kwon et al., 2018) Recent advances in using PGMs for other sensing has also shown that glucose itself is no longer a requirement as Lu, *et. al* have shown the ability to measure analytes using the internal redox chemistry of the meters directly.(Zhang et al., 2016b) This strategy further expands the possibilities for integrating novel reagents into the detection mechanism.

Several of the approaches published on the repurposing of PGMs were performed in a tube or in a microtiter plate, which requires multiple washing and rinsing steps. For a POC device, the device should be developed to minimize the input needed by the user. Simple fluidic devices have been successfully demonstrated as interfaces between user and PGM. Zhang *et. al* have interfaced the PGM with a lateral flow device to simplify the binding and wash steps.(Zhang et al., 2016a) This approach only required the user to dip one end of an LFD into a sample solution containing analyte, and at the conclusion of the assay the PGM was used to measure glucose from a pad at the other end of the LFD. Zhao *et. al* also reported the use of a lateral flow device, in this case combined with magnetic beads and glucose containing liposomes which were retained within a test zone on the immunochromatographic strips.(Zhao et al., 2017) While the method simplifies the binding and washing steps, it currently still requires that users cut out the test zone and release glucose by addition of 100 µL of Triton X-100 solution. As such automation of this step would be required. Wang and co-workers have reported using a more sophisticated fluidic device to interface with PGMs.(Q. Wang et al., 2014) This approach uses a microfluidic chip with several channels to generate a series of samples containing viral DNA, which can be measured by a PGM one at a time. The method requires several wash steps in which users repeatedly load small volumes of sample or wash buffers (200 µL) followed by blotting with filter paper to 'wash' the channels. While the binding and wash steps of the microtiter format are retained, they are simplified by the use of capillary forces and blotting. The ability to carry out multiplex detection with PGMs is a significant benefit, which may outweigh the burden of washing steps. This is important as there exist many analytes of interest where relative amounts of analyte are the clinically relevant result. HbA1c (glycosylated haemoglobin), for example, is an important analyte in monitoring diabetes, so using PGMs to measure HbA1c would seem to be a straightforward adaptation, and in fact at least one study has shown the measurement of HbA1c using a PGM.(Xiang et al., 2014) However, the clinically relevant measure of HbA1c is the ratio of HbA1c to total haemoglobin, requiring the measurement of both quantities. To achieve this in an unambiguous way, one would need to make two independent measurements with PGMs and compute the ratio. Measuring one analyte at the time, like proposed by Wang and co-workers, is a relatively simple and cost effective approach to performing multiplex analysis with PGMs, but from an implementation standpoint it is perhaps advantageous to focus on analytes whose single concentration measurement is sufficient for diagnosis.

The purpose of this article is to consider how to address the challenges that must be met to realise the use of PGMs as universal point-of-care diagnostic tools. Considerations are limited to off-the-shelf, commercially available PGMs and their associated, unmodified test strips (the modification of the strips have been reported elsewhere(Nie et al., 2010)).

2. Signal transduction and amplification

As with any biosensor, there are three key elements to repurposing PGMs for the detection of any analyte: sample collection, analyte recognition and transduction, and signal quantification. The most challenging is possibly the transduction step, which may require significant amplification depending on the analyte concentration. The detection range for PGMs generally ranges from approximately 20 to 600 mg·dL^{-1} of glucose (1 – 33 mM),(Ekhlaspour et al., 2017) slightly more than one order of magnitude. Biomarkers, however, tend to be present at much lower and wider concentration ranges, meaning that an amplification strategy developed for low concentrations of a given biomarker might not be suitable for high concentrations of the same molecule. In the case of influenza, for example, viral nucleic acid could be present at about 10^{10} copies·mL^{-1} ($2 \cdot 10^{-8}$ mM), although ideally would be detected on the order of 10^{2} copies·mL^{-1} ($2 \cdot 10^{-16}$ mM) to help limit the spread of viral infections through early intervention.(Lau et al., 2010) Cytokines such as Interleukin 6 are interesting biomarkers for many diseases, and they may be present in serum over a wide range of concentrations from roughly 10 to 5,000 pg·mL^{-1} (10^{-10} to 10^{-7} mM).(Hunter and Jones, 2015)

Most PGMs report a change in blood glucose value of either ± 1 mg·dL^{-1} (0.06 mM) or ± 0.1 mmol/L (1.8 mg·dL^{-1}), depending on the geographic region in which they are deployed. This level of resolution is important to consider when designing a transduction strategy since it eventually determines the sensitivity of the diagnostic tool. In fact, it might be impractical to convert a large range of analyte concentrations into the relatively small interval of glucose concentrations measurable by a PGM, since it would result in a biosensor with very low sensitivity. For example, if an analyte which concentration varies over three orders of magnitude was converted within the detection range of a PGM, the result would be that at low concentrations a relatively large increase of analyte would be required to generate (or consume) the 1 mg·dL^{-1} of glucose necessary to achieve a measureable change in the PGM reading (i.e. the slope of the calibration curve would be $1.7 \cdot 10^{-6}$). These concepts are further discussed in the section about meter accuracy.

The two main approaches to signal transduction and amplification are enzymatic generation of glucose, and the use of encapsulated glucose in nanocarriers (**Figure 1**). In the case of enzymatic generation, the analyte results in capture/activation of an enzyme that converts a substrate into glucose. The enzyme turnover number tends to be the limiting factor in achieving a low detection limit and in the time required to achieve detection. In the case of nanocarrier transduction, the analyte results in capture of a nanostructure carrying glucose solution, which can be subsequently

released for measurement by the PGM by the addition of some trigger (chemical, enzymatic, pH, etc.). The amount of glucose encapsulated in these nanocarriers is the limiting factor in achieving a low detection limit.

As amplification may be critical for many analytes, it is worth considering how much glucose needs to be generated/released to result in an identifiable change measured by the PGM. For the purposes of this article it was assumed that a change of 1 $mg \cdot dL^{-1}$ (0.06 mM) can be detected. Also critical is the volume into which the glucose will be generate/released. Many PGMs on the market today require less than 1 µL of fluid to perform the measurement, but realistically one would need to produce at least 10 µL of solution to sample with the test strip. For the purposes of this article it was considered the volume measured by the PGM to be 10 µL.

2.1. Enzymatic transduction

There are several classes of enzymes that have been shown to produce glucose from a variety of substrates.(Mohapatra and Phillips, 2013) However, the performance of the enzymes vary widely in terms of turnover number (a measure of how many substrates one enzyme can convert into a product per second, usually indicated with k_{cat}),(Bar-Even et al., 2011) as well as temperature dependence and specificity. To achieve the lowest detection limits in a point-of-care setting, one would ideally use an enzyme with high turnover number at room temperature. This would minimize the time required to generate an analytical result without the need for temperature control.

The enzymes used in the literature to generate glucose in presence of an analyte are glycosidases, mostly α-glucosidase (also called glucoamylase, EC number: 3.2.1.20), amyloglucosidase (EC number: 3.2.1.33) and fructan β-fructosidase (also called invertase, EC number: 3.2.1.80). The turnover numbers of these enzymes depend on several factors, such as the type of organism from which the enzyme was extracted, the purity and the experimental conditions of the enzymatic reaction (temperature and pH).(Gutíerrez-Alonso et al., 2009; Lan et al., 2016) Considering only the ranges of temperature (21 – 60 °C) and pH (4.5 – 8.8) used in PGM-based assays, the turnover number vary from 49 to 709 s^{-1} for glucoamylase,(Lan et al., 2016; Okuyama et al., 2005) from 1,282 to 2,570 s^{-1} for amyloglucosidase,(del Moral et al., 2018; Nguyen et al., 2014) and from 108 to 3,430 s^{-1} for invertase.(Gutíerrez-Alonso et al., 2009; Lafraya et al., 2011) These ranges are shown in **Figure 2**, together with dashed lines for turnover numbers of 10, 100, 1,000 and 10,000 s^{-1} (as reference for the eyes), and how these values impacts the ability to generate glucose in a timely manner. From the values found in the literature, amyloglucosidase seems to have a narrow range of high turnover number, thus it could ensure a reproducible and fast detection of the analyte for a PGM-based

biosensor. On the other hand, glucoamylase seems to have a wide range of turnover numbers, some of them considerably low. Invertase has also a wide range of turnover numbers, but it also has the highest value reported among these enzymes: 3,430 s^{-1}. This high turnover number is possibly the reason why invertase is the most commonly enzyme used in conjunction with PGMs.(Gu et al., 2015; Q. Wang et al., 2015a; Xiang and Lu, 2013; Zhu et al., 2014b) As shown in **Figure 2**, if the turnover number of the enzyme is 10,000 s^{-1}, only 10 pM of enzyme is required to produce 1 mg·dL^{-1} of glucose in 10 minutes. However, if the turnover number is 1 s^{-1}, 0.1 µM of enzyme is necessary to achieve the same result.

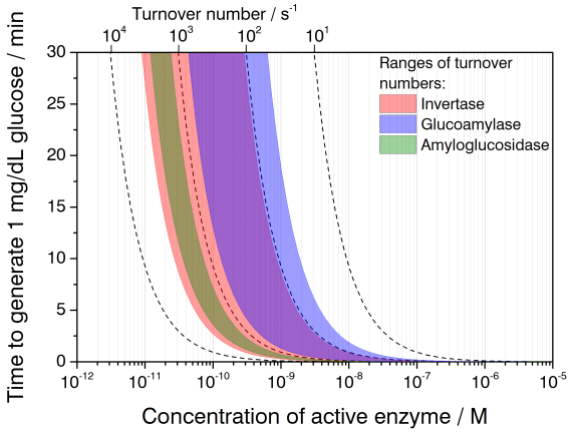
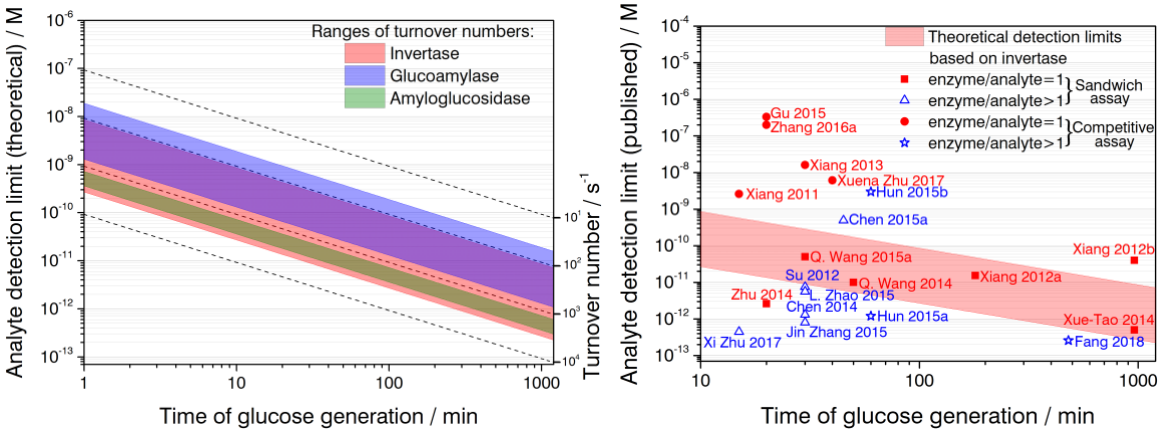


Figure 2. Time required for enzymes with a given turnover number to produce 1 mg·dL^{-1} (0.06 mM) of glucose (the smallest change in glucose concentration that can be measured with PGMs) in a 10 µL volume. The coloured areas are the ranges of turnover number of the three enzymes (invertase, glucoamylase and amyloglucosidase) used in conjunction with PGMs to detect analytes other than glucose. Such ranges were determined from the minimum and maximum turnover numbers published for the three enzymes at a temperature between 21 and 60 °C and a pH between 4.5 and 8.8 (the conditions used in PGM-based assays).

The enzyme turnover number has a significant impact on how much analyte can be detected in a timely manner, but this can be mitigated by other factors such as initial sample volume and the volume in which the glucose signal is finally measured. If it is assumed that each molecule of analyte captured results in one active enzyme for signal transduction, then the limit of detection in a 10 µL PGM measurement volume can be estimated. For example, as shown in **Figure 3 (A)**, an enzyme with a turnover number of 1,000 s^{-1} is theoretically limited to detecting analytes at ~10^{-10} M within 10 minutes if the initial sample is 10 µL and the glucose is also generated in a 10 µL volume. However, the same enzyme could be used to detect analytes as low as ~10^{-11} M if the initial sample volume was increased 10-fold (assuming a corresponding 10-fold increase in the number of captured analytes and active enzymes).

The theoretical range of detection limits associated with the turnover numbers for invertase is shown in **Figure 3 (B)**, and is compared with the experimental detection limits reported in the literature. Published strategies include sandwich and competitive assays, where the analyte molecule is associated with either one or multiple enzymes. The theoretical range of detection limits was obtained in the assumption of one enzyme per analyte, as previously mentioned. Experimental results obtained with one enzyme per analyte molecule in a sandwich assay format (red full squares) correlate well with the theoretical range, while the competitive assays (red full circles) tend to cluster in the top-left part of the graph, being significantly faster but with lower limit of detections. However, an important aspect of competitive assays is that they tend to have fewer steps compared to sandwich assays, and therefore the potential to be more easily implemented in a portable device. On the other hand, the proposed calculations do not describe well sandwich (empty blue triangles) and competitive (empty blue stars) assays obtained with more than one enzyme per analyte molecule, which tend to be more scattered, with some strategies "underperforming" the one-enzyme-to-one-analyte approach, while other strategies managed to achieve femtomolar or lower limit of detections.(Z. Wang et al., 2015; Xue-tao et al., 2015a) **Table 1** summarise the characteristics and the performances of assays based on enzymatic transduction and PGM.



(**A**) (**B**)

Figure 3. (**A**) Theoretical detection limit of analyte versus glucose generation time for enzymes with varying turnover numbers (assuming sample and glucose generation volumes of 10 µL, one molecule of analyte results in one active enzyme, and a minimum detectable change in glucose concentration of 1 mg·dL^{-1}). (**B**) Comparison of the theoretical detection limit over the range of turnover numbers reported for invertase (red shaded area) with the published detection limits reported for selected systems using invertase (symbols). Publications that can be described by the proposed calculations (one single enzyme per analyte) are shown in red (full squares for sandwich assay and full circles for competitive assay). In blue are the strategies not directly described by the calculations, where more than one enzyme was associated with one analyte (empty triangle = sandwich assay, empty star = competitive assay). The numbers near the symbols correspond to the reference.

Reference	Assay type	Analyte recognition	Enzyme	Enzyme:analyte 1:1?	Limit of detection
(Xiang et al., 2014)	Sandwich	Antibody	Invertase	Yes	Insulin, $1 \cdot 10^{-9}$ M
(Joo et al., 2013)	Sandwich	Antibody	Invertase	No	Salmonella , 10 CFU·mL^{-1}
(Ma et al., 2014)	Sandwich	Aptamer	Invertase	Yes	Platelet-derived growth factor-BB, $2.9 \cdot 10^{-15}$ M
(Chen et al., 2015b)	Sandwich	Antibody	Invertase	No	Ractopamine, 0.34 µg·kg^{-1} meat
(Wan et al., 2016)	Sandwich	Aptamer	Invertase	No	E. Coli, 10^{6} CMF·mL^{-1}
(Yang et al., 2015)	Sandwich	Peptide	Invertase	Yes	Protein kinase A, 0.1 mU·µL^{-1}
(Xue-tao et al., 2015b)	Sandwich	DNA	Invertase	Yes	Hg^{2+}, $8 \cdot 10^{-9}$ M
(Xu et al., 2012)	Sandwich	DNA	Invertase	No	DNA, $0.5 \cdot 10^{-12}$ M
(Q. Wang et al., 2014)	Sandwich	DNA	Invertase	Yes	Hepatitis B DNA, $1 \cdot 10^{-11}$ M
(Xiang and Lu, 2012b)	Sandwich	DNA	Invertase	Yes	DNA, $40 \cdot 10^{-12}$ M
(Xiang and Lu, 2012a)	Sandwich	Antibody	Invertase	Yes	Prostate-specific antigen, 0.4 ng·mL^{-1}
					Ochratoxin A, 6.8 ng·mL^{-1}
(Xue-tao et al., 2014)	Sandwich	DNA	Invertase	Yes	DNA, $0.5 \cdot 10^{-12}$ M
(Q. Wang et al., 2015a)	Sandwich	Aptamer	Invertase	Yes	Myoglobin, $50 \cdot 10^{-12}$ M
(Zhu et al., 2014b)	Sandwich	Antibody	Invertase	Yes	Alpha-fetoprotein, 0.18 ng·mL^{-1}
(Su et al., 2012)	Sandwich	Antibody	Invertase	No	Carcinoembryonic antigen, 1 ng·mL^{-1}
(L. Zhao et al., 2015)	Sandwich	Antibody	Invertase	No	Albumin, 0.5 ng·mL^{-1}
(Chen et al., 2015a)	Sandwich	Molecularly imprinted polymer	Invertase	No	Chloramphenicol, 0.16 ng·mL^{-1}
(Zhu et al., 2014a)	Sandwich	DNA	Invertase	No	Telomerase, 67 HeLa cells·mL^{-1}
(Chen et al., 2014)	Sandwich	Antibody	Invertase	No	Transcriptor factor Oct4, 0.05 ng·mL^{-1}

(Su et al., 2013)	Sandwich	Click chemistry reaction	Invertase	No	Cu^{2+}, $10 \cdot 10^{-9}$ M
(Deng et al., 2016)	Sandwich	DNA	Invertase	No	M.SssI CpG methyltransferase, 0.37 $U \cdot mL^{-1}$
(Xi Zhu et al., 2017)	Sandwich	Antibody + aptamer	Invertase	No	Vascular endothelial growth factor, 1.2 $pg \cdot mL^{-1}$
(Fu et al., 2014)	Sandwich	Antibody	Glucoamylase	No	Neuron-specific enolase, 50 $pg \cdot mL^{-1}$
(Fu et al., 2015)	Sandwich	Antibody	Glucoamylase	No	Neuron-specific enolase, 8 $pg \cdot mL^{-1}$
(Taebi et al., 2018)	Sandwich	Antibody	Glucoamylase	No	Hepatitis B surface antigen, 0.3 $ng \cdot mL^{-1}$
(Wu et al., 2015)	Sandwich	Antibody	Glucoamylase	No	Alpha-fetoprotein, 0.03 $ng \cdot mL^{-1}$
(Lin et al., 2016)	Sandwich	Antibody	Amyloglucosidase	No	Thrombin, $1.8 \cdot 10^{-9}$ M C-reactive protein, $0.3 \cdot 10^{-9}$ M
(Hong et al., 2017)	Competitive	DNAzyme	Invertase	No	Platelet-derived growth factor, $0.11 \cdot 10^{-15}$ M
(Xiang and Lu, 2011)	Competitive	Aptamer	Invertase	Yes	Cocaine, $3.4 \cdot 10^{-6}$ M Adenosine, $18 \cdot 10^{-6}$ M Interferon-gamma of tuberculosis, $2.6 \cdot 10^{-9}$ M
(Gu et al., 2015)	Competitive	Aptamer	Invertase	Yes	Melamine, $0.33 \cdot 10^{-6}$ M
(Zhang et al., 2016a)	Competitive	Aptamer	Invertase	Yes	Cocaine, $7.7 \cdot 10^{-6}$ M Streptavidin, $0.2 \cdot 10^{-6}$ M
(Xiang and Lu, 2013)	Competitive	DNAzyme	Invertase	Yes	Pb^{2+}, $16 \cdot 10^{-9}$ M
(Hun et al., 2015a)	Competitive	Peptide	Invertase	No	Prostate-specific antigen, $1.2 \cdot 10^{-12}$ M
(Hun et al., 2015b)	Competitive	Aptamer	Invertase	No	Dopamine, $3 \cdot 10^{-9}$ M
(Jin Zhang et al., 2015)	Competitive	DNAzyme	Invertase	No	Pb^{2+}, $0.8 \cdot 10^{-12}$ M
(Fang et al., 2018)	Competitive	DNA	Invertase	No	DNA, $0.26 \cdot 10^{-12}$ M
(Xuena Zhu et al., 2017)	Competitive	Antibody	Invertase	Yes	8-hydroxy-2'-deoxyguanosine, 1.73 $ng \cdot mL^{-1}$
(Yan et al., 2013)	Competitive	Aptamer	Glucoamylase	No	Cocaine, $3.8 \cdot 10^{-6}$ M

(Liao and Li, 2014)	Competitive	DNAzyme	Glucoamylase	No	Pb^{2+}, $2 \cdot 10^{-12}$ M
(Z. Wang et al., 2015)	Competitive	Electrostatic interactions	Glucoamylase	No	E. Coli, 20 bacteria·mL^{-1}

Table 1 Performances of assays based on enzymatic transduction and PGM.

2.2. Nanocarrier transduction

Encapsulation of glucose solutions offers a different set of benefits and challenges. The kinetics of glucose release may be highly tuneable by choosing the right encapsulation method, with the option of having an almost immediate release. However, it is also critical that the nanocarriers do not leach or release a significant amount of glucose into solution during any capture or wash steps. Assuming that a saturated glucose solution at 25 °C (~5 M) is encapsulated, the ability to release glucose will be dependent on the number of nanocarriers captured and the encapsulation volume. The two common nanocarriers that have been used to encapsulate glucose are liposomes(Tang et al., 2016; Y. Zhao et al., 2015) and mesoporous particles.(Fu et al., 2013; Gao et al., 2014; Hou et al., 2014; Tang et al., 2014; Y. Wang et al., 2014) For liposomes, the size of the encapsulated volume corresponds approximately to the size of the particle (at least for unilamellar vesicles). For mesoporous particles, the encapsulated volume corresponds to the total pores volume. **Figure 4** shows the potential to release glucose from nanocarriers with encapsulation diameter of 10, 100 and 1,000 nm.

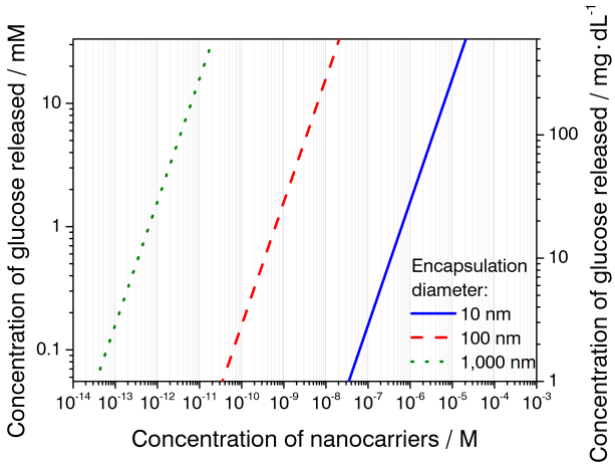


Figure 4. Signal transduction by release of concentrated glucose solution from nanocarriers of varying encapsulated diameter. The encapsulation volume is comprised of 5 M saturated glucose solution at 25 °C. The glucose concentration ranges from the minimum detectable change in concentration of 1 mg·dL^{-1} (0.06 mM) to the maximum measureable value of 600 mg·dL^{-1} (33 mM) reported for many PGMs.

Because the glucose released increases linearly with analyte concentration, the measurement range is the same as the glucose meter. Under the assumptions that one molecule of target analyte will result in the capture of one glucose nanocarrier, and the initial sample volume and glucose release volume are the same, the estimated detection limit for the analyte will be dependent on the encapsulation diameter and on the concentration of the encapsulated glucose solution, as shown in **Figure 5**. According to the calculations, lower detection limits can be achieved by increasing the concentration of the encapsulated solution or the size of the nanocarrier.

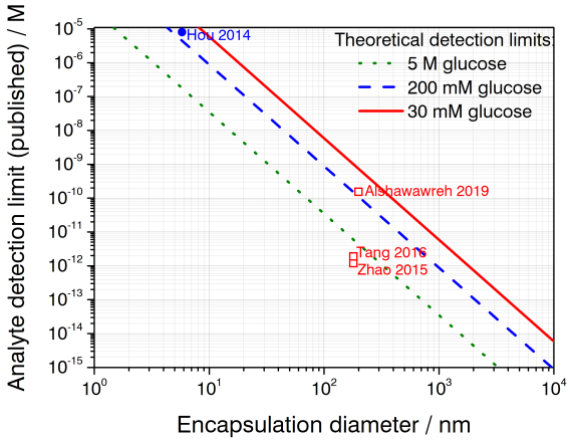


Figure 5. Theoretical detection limit of analyte based on diameter of the encapsulated glucose solution, assuming equal sample and glucose generation volumes, one molecule of analyte per nanocarrier, and a minimum detectable change in glucose concentration of 1 mg·dL^{-1}. Three concentrations of glucose inside the nanocarrier are considered: 30 mM, 200 mM and 5 M. Publications using about 30 mM glucose are shown in red (empty squares), while 200 mM glucose are shown in blue (full circles). The numbers near the symbols correspond to the reference.

This calculation can be compared with the experimental values reported in the literature. Several papers use mesoporous particles to encapsulate glucose, but only the publication by Hou *et al.*(Hou et al., 2014) reported enough details to allow the calculation of the volume of the encapsulated solution (plotted as the diameter of a sphere of equivalent volume). That experimental result – obtained with a 200 mM encapsulated glucose solution– closely matches the theoretical detection limit predicted by the calculations (blue dashed line in **Figure 5**). The other type of nanocarrier used in the literature is liposomes filled with a glucose solution about 30 mM. This strategy has been described in three publications, and the detection limits reported are lower than those achieved by the mesoporous nanoparticles. However, only one experimental detection limit is close to the 30 mM calculated line,(Alshawawreh et al., 2019) while the values for the other two publications diverge significantly, being closer to the 5 M line (**Figure 5**).(Tang et al., 2016; Y. Zhao et al., 2015) It has been reported that the encapsulation efficiency of glucose in liposomes does not increase

monotonically with the glucose concentration, but has a maximum for about 30 mM.(Kirby and Gregoriadis, 1984; Tang et al., 2016) This phenomenon has not been fully explained, but it might be due to the osmotic stress for glucose concentrations above 30 mM, which causes the formation transient pores and the release of the solute.(Alshawawreh et al., 2019)

According to **Figure 5**, the largest influence on the theoretical limit of detection is given by the diameter of the nanocarrier. However, the existence of an optimal size of 200 nm has been reported for liposomes.(Tang et al., 2016; Y. Zhao et al., 2015) This optimal value has been attributed to the formation of multilamellar structures for liposomes above 200 nm, which decreases the volume effectively available for the glucose solution.(Alshawawreh et al., 2019) Therefore, a possible strategy to increase the sensitivity of nanocarrier-based assays would be to prepare micrometer-sized unilamellar vesicles (e.g. giant unilamellar liposomes) filled with glucose solution. This again assumes sufficient space for the binding of vesicles to the surface to scale linearly with their size. **Table 2** summarise the characteristics and the performances of assays based on nanocarrier transduction and PGM.

Reference	Assay type	Analyte recognition	Nanocarrier	Nanocarrier: analyte 1:1?	Limit of detection
(Tang et al., 2016)	Sandwich	Antibody	Liposome	Yes	Aflatoxin B1, 0.6 $pg \cdot mL^{-1}$
(Y. Zhao et al., 2015)	Sandwich	Antibody	Liposome	Yes	Protein 53 phosphorylated, 50 $pg \cdot mL^{-1}$
(Zhao et al., 2017)	Sandwich	Antibody	Liposome	Yes	Protein 53 phosphorylated, 50 $pg \cdot mL^{-1}$
(Alshawawreh et al., 2019)	Sandwich	Antibody	Liposome	Yes	Procalcitonin, $1.53 \cdot 10^{-10}$ M
(Tang et al., 2014)	Competitive	Antibody	Mesoporous silica	No	Aflatoxin B1, 5 $ng \cdot kg^{-1}$
(Gao et al., 2014)	Competitive	Antibody	Mesoporous $NiCo_2O_4$	No	Brevetoxin B, 0.01 $ng \cdot mL^{-1}$
(Y. Wang et al., 2014)	Competitive	DNA	Mesoporous $NiCo_2O_4$	No	Telomerase activity, 80 HeLa $cells \cdot mL^{-1}$
(Fu et al., 2013)	Competitive	DNAzyme	Mesoporous $NiCo_2O_4$	No	Pb^{2+}, $1 \cdot 10^{-12}$ M
(Hou et al., 2014)	Competitive	Aptamer	Mesoporous silica	No	Adenosine triphosphate, $8 \cdot 10^{-6}$ M

(Li and Tang, 2017)	Competitive	Aptamer	Mesoporous titania	No	Cocaine, $5.2 \cdot 10^{-9}$ M

Table 2. Performances of assays based on nanocarrier transduction and PGM.

2.3. Emerging strategies

The assays presented so far are based on the release of a glucose-generating enzyme or a glucose-loaded nanocarrier upon target binding. These affinity-based systems are the majority of the strategies to repurpose a PGM, but other sensing mechanisms have also been published. One of these strategies is particularly suited for the determination of the activity of an enzyme (the target), as shown in **Figure 6**. Such a strategy relies on specifically synthesised substrates that are consumed by the target enzyme. These substrates can either directly release glucose,(Gurale et al., 2016; Mohapatra and Phillips, 2013; X. Zhang et al., 2015) or generate an intermediate species that is converted into glucose by a second enzyme.(Q. Wang et al., 2015b; Jingjing Zhang et al., 2015) Such substrates have been used for the detection of the enzymes b-D-galactosidase,(Mohapatra and Phillips, 2013) alkaline phosphatase,(Mohapatra and Phillips, 2013) α-mannosidase,(Gurale et al., 2016) galactose-1-phosphate uridyltransferase,(Jingjing Zhang et al., 2015) and α-amylase.(Q. Wang et al., 2015b) Moreover, if the target enzyme is present on the surface of a pathogen, its activity can be correlated to the pathogen concentration, as done for E. Coli(Das et al., 2018) and influenza virus H1N1.(Cui et al., 2017; X. Zhang et al., 2015) Some authors have further expanded the choice of these substrates, exploiting the response of some PGMs to species other than glucose, such as galactose, (Cui et al., 2017) catechol and acetaminophen (paracetamol)(Das et al., 2018) and nicotinamide adenine dinucleotide (NADH, see **Figure 6**).(Zhang et al., 2016b)

Another interesting method for the detection of pathogens via PGMs exploits the consumption of glucose by the metabolism of bacteria. By monitoring the decrease of glucose over time, the concentration of bacteria in water samples, or their response to pollutants or antibacterial agents, could be quantified.(Chavali et al., 2014; Fang et al., 2016; Kwon et al., 2018)

As previously mentioned, some authors have coupled nucleic acid amplification methods and PGMs, for the detection of DNA,(Du et al., 2015; Xue-tao et al., 2015a) mercury ions(Huang et al., 2017) and the enzyme telomerase.(Wang et al., 2016) These systems are usually comprised of a first step where the nucleic acid is amplified (**Figure 6**), and a second step where such nucleic acid is detected via enzymatic transduction, either with a sandwich or a competitive assay. Such a combination allows for extremely low detection limits (femtomolar or lower), but usually requires longer reaction

times (16 hour or more). **Table 3** summarise the characteristics and the performances of these emerging PGM-based assays.

Reference	Transduction	Method	Note	Limit of detection
(Mohapatra and Phillips, 2013)	Enzymatic reaction	The target enzyme generates glucose, and its enzymatic activity is directly measured by the PGM	The enzyme substrates is covalently linked to glucose	b-D-galactosidase, 4 $U \cdot mL^{-1}$ (38 nM) Alkaline phosphatase, 2 $U \cdot L^{-1}$ (2 nM)
(Gurale et al., 2016)	Enzymatic reaction	The target enzyme generates glucose, and its enzymatic activity is directly measured by the PGM	The enzyme substrates is covalently linked to glucose	α-mannosidase, 0.01 $U \cdot mL^{-1}$
(X. Zhang et al., 2015)	Enzymatic reaction	The target enzyme generates glucose, and its enzymatic activity is directly measured by the PGM.	The enzyme substrates is covalently linked to glucose	Influenza H1N1, 100 plaque forming units per sample, detected via the activity of its surface enzyme neuraminidase
(Das et al., 2018)	Enzymatic reaction	The target enzyme generates catechol or paracetamol, and its enzymatic activity is directly measured by the PGM	The enzyme substrates is covalently linked to catechol or paracetamol	β-galactosidase and α-mannosidase, 0.01 U per sample E. Coli, 100 $CFU \cdot mL^{-1}$ Influenza H1N1, 1 PFU per sample
(Cui et al., 2017)	Enzymatic reaction	The target enzyme generates galactose, and its enzymatic activity is directly measured by the PGM	The enzyme substrates is covalently linked to galactose	Influenza H1N1, 100 PFU per sample S. pneumoniae, 100 CFU per sample
(Jingjing Zhang et al., 2015)	Enzymatic reaction	The target enzyme generates the intermediate glucose-1-phosphate, which is then converted into glucose and measured by the PGM to quantify the enzymatic activity of the initial enzyme	Alkaline phosphatase converts glucose-1-phosphate into glucose	galactose-1-phosphate uridyltransferase, 7.6 $mU \cdot mL^{-1}$
(Q. Wang et al., 2015b)	Enzymatic reaction	The target enzyme generates the intermediates maltotriose and maltose, which are then converted into glucose and measured	α-glucosidase converts maltotriose and maltose into glucose	α-amylase, 20 $U \cdot L^{-1}$

		by the PGM to quantify the enzymatic activity of the initial enzyme		
(Zhang et al., 2016b)	Enzymatic reaction	Use the dose-dependent response of a PGM to quantify nicotinamide adenine dinucleotide (NADH)	Target-induced consumption or production of NADH through cascade enzymatic reactions	L-lactate, $34 \cdot 10^{-6}$ M
(Chavali et al., 2014)	Bacteria metabolism	Supply E. Coli with known quantity of glucose and monitor the consumption of glucose by the bacteria with a PGM	/	E. Coli, 2 CFU in 100 µL
(Kwon et al., 2018)	Bacteria metabolism	Supply E. Coli with known quantity of glucose and monitor the consumption of glucose by the bacteria with a PGM	The presence of enrofloxacin suppressed the bacterial growth and consequently reduced glucose consumption	Enrofloxacin, 5 $ng \cdot mL^{-1}$
(Fang et al., 2016)	Bacteria metabolism	Supply E. Coli with known quantity of glucose and monitor the consumption of glucose by the bacteria with a PGM	The presence of pollutants (As^{3+}, Ni^{2+}, 4-chlorophenol, and 2,4-dichlorophenol) decreased the microbial glucose metabolism	As^{3+}, 5 $mg \cdot L^{-1}$ IC_{50} Ni^{2+}, 40 $mg \cdot L^{-1}$ IC_{50} 4-chlorophenol, 35 $mg \cdot L^{-1}$ IC_{50} 2,4-dichlorophenol, 14 $mg \cdot L^{-1}$ IC_{50}
(Xue-tao et al., 2015a)	Enzymatic reaction (invertase)	Initial amplification via circular strand displacement polymerase reaction	/	DNA, $5 \cdot 10^{-15}$ M
(Du et al., 2015)	Enzymatic reaction (invertase)	Initial amplification via loop mediated isothermal amplification	/	DNA, 20-100 $copies \cdot \mu L^{-1}$
(Wang et al., 2016)	Enzymatic reaction (invertase)	Initial amplification via DNA-based machine	/	Telomerase, $0.1 \cdot 10^{-15}$ M
(Huang et al., 2017)	Enzymatic reaction (invertase)	Initial amplification via nicking enzyme reaction	/	Hg^{2+}, 10^{-17} M

Table 3. Performances of emerging PGM-based assays published in the literature. Some of these methods measure the enzymatic activity of a target enzyme, rather than the concentration of an analyte, while other methods use a nucleic acid amplification step before the PGM-based detection. CFU = colony forming unit. PFU = plaque forming unit.

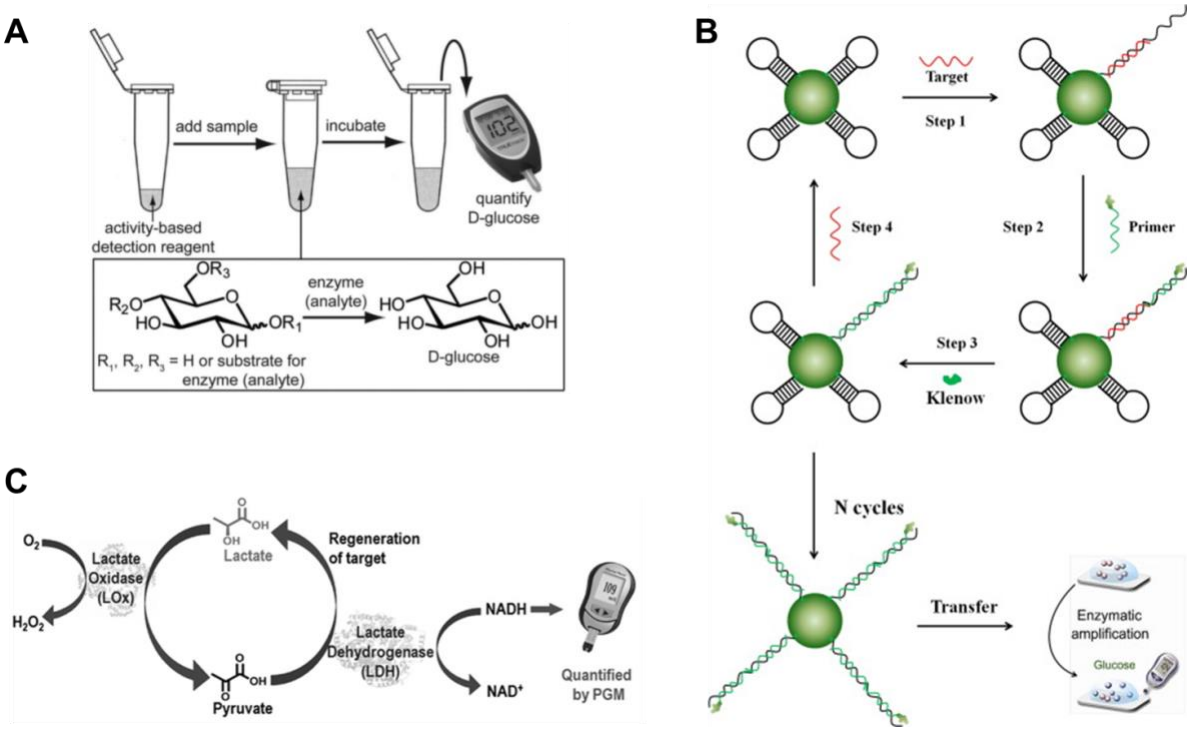


Figure 6. Emerging strategies to detect analytes other than glucose using a PGMs. **(A)** Quantification of enzyme analytes *via* their enzymatic activity towards substrates with covalently attached glucose. The glucose generated is then detected by a PGM. An intermediate enzymatic reaction may also present in some configurations. Reprinted with permission from (Mohapatra and Phillips, 2013). **(B)** Initial amplification of the target DNA based on isothermal circular strand-displacement polymerisation reaction. This amplification caused the polymerisation of an oligonucleotide primer conjugated with invertase, which in turn generated a signal readable by the PGM. Reprinted with permission from (Xue-tao et al., 2015a). **(C)** Quantification of enzyme analytes *via* the generation of the redox pair NADH/NAD^+ that can be detected by a PGM. Adapted with permission from (Zhang et al., 2016b).

3. Quantification

3.1. Meter variability and accuracy

One of the complicating factors in deploying PGM based point-of-care measurements is the fact that one brand of meter may differ significantly from another meter in the amount of glucose being read, even when all the variables that can be controlled by the user have been controlled. This variability in their accuracy has been reported for measurements in whole blood samples, for which PGMs

have been optimised.(Ekhlaspour et al., 2017; Hellman, 2012; Klonoff and Prahalad, 2015; Pfützner et al., 2014) One explanation for this is that although many meters have a similar basic construction, there are some important differences in their internal components – for example the enzyme used to oxidise glucose, the redox mediator, prefiltration membranes, and the redox parameters used for the electrochemical measurement.(Hönes et al., 2008) The enzymes used in PGMs, typically glucose oxidase and glucose dehydrogenase, have slightly different selectivities for glucose and different performance when varying pH, ionic strength, temperature, and oxygen content. When interfacing reagent solutions with PGMs, all of these factors will have an effect on the performance of the meter.

This variability in the accuracy and linearity of different meters may significantly impact the ability to convert glucose readings into a confident measure of analyte concentrations, and needs to be investigated. The international standard ISO 15197:2013 require that 95% of the values reported by PGMs in whole blood must fall within 15 mg·dL^{-1} (0.8 mM) of the reference for values below 100 mg·dL^{-1} (5.6 mM), or within 15% of reference for values greater than 100 mg·dL^{-1}.(Ekhlaspour et al., 2017) Even the best meters can give readings that vary from a reference by 10% when measuring glucose levels below 70 mg·dL^{-1}. This implies that the uncertainty in any given measurement may be significant at the lower end of the PGM limits, thus decreasing the accuracy of a PGM-based diagnostic test. A common method used to demonstrate PGM accuracy is to plot the meter readings against reference values on the 'Parkes error grid' (or 'consensus error grid') as shown in **Figure 7**.(Parkes et al., 2000; Pfützner et al., 2013) The figure includes the error grid (grey dotted lines, with zones A – E which specify risk level), the accuracy requirements stipulated by the standard ISO 15197:2013 (black dashed lines), as well as experimental points collected by measuring solutions of glucose in PBS with the four commercial PGMs. For glucose concentrations below 100 mg·dL^{-1} (5.55 mM) the four meters give similar results, with accuracy within the ISO standard. The readings start to spread more for 100 and 200 mg·dL^{-1} glucose, but still with acceptable accuracy. However, for glucose concentrations above 200 mg·dL^{-1} (11.1 mM), which of course is approaching the limits of the relevant physiologically range, the four meters give considerably different results, with accuracy mostly outside the ISO standard. It is noteworthy that only the Accu-Check Performa appears to be linear over the tested range, while the other meters deviate from linearity above 200 mg·dL^{-1} of glucose. If one considers the discussion above in sections 2.1 and 2.2 on achieving enough glucose, one realises that the higher glucose concentration range will not often be achieved in the repurposing of glucose meters for other assays.

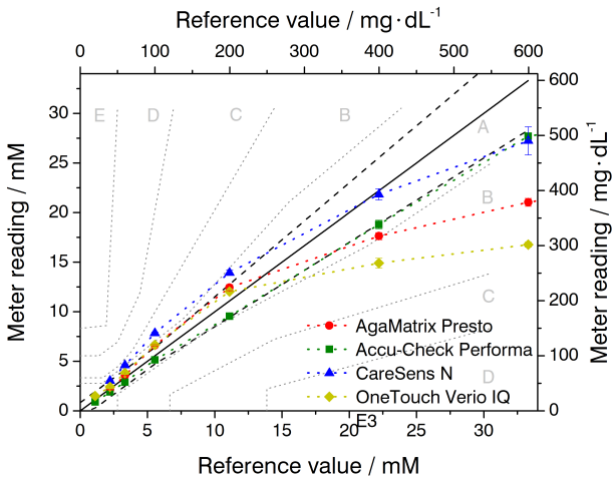
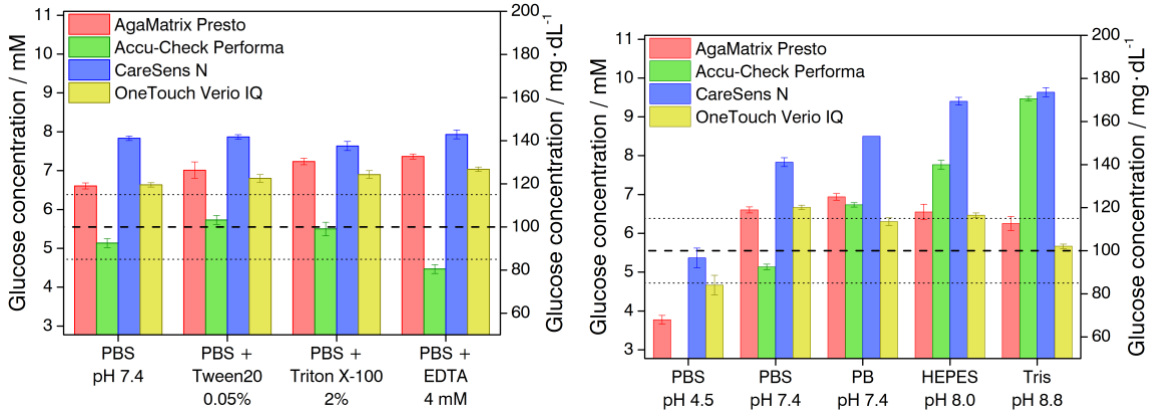


Figure 7. Representation of an ideal meter response (solid black line) and the acceptable deviation specified by international standards (ISO 15197:2013, dashed black lines). The ranges are overlayed on the Parkes Error Grid for type 1 diabetes (also "consensus error grid", dotted grey lines), typically used to visualise the accuracy of PGMs. The graph also shows the experimental data for four commercial PGMS measuring reference solutions of glucose in PBS, pH 7.4. Error bars represent the standard deviation from three measurements. Note that the Parkers Error Grid is specified only from 0 to 550 mg·dL^{-1} of glucose.(Pfützner et al., 2013)

The presence of interfering substances is also well documented to have an effect on glucose quantitation – not just from the enzymatic activity, but also on the electrochemical method employed by a particular meter.(Hellman, 2012; Tang et al., 2000; Vashist et al., 2011) Uric acid, for example, can be oxidized by the PGM electrode.(Ginsberg, 2009) Under normal circumstances this would not be an issue, but if the analyte of interest is being measured in the urine then uric acid interference may be a significant challenge. When using additional reagents to convert an analyte to glucose, or to an electrochemical agent that can be measured by the PGM, care must be taken to avoid interfering substances - both for the enzyme and for the underlying redox chemistry in the meter.(Cho et al., 2015)

In our experience we have seen variable responses to some reagents depending on the meter being used – with some meters there is little to no effect, while others show substantial changes in the measured signals. Five commercially-available PGMs were tested: AgaMatrix Presto, Accu-Check Performa, FreeStyle Lite, CareSens N and OneTouch Verio IQ. Among these meters, the FreeStyle Lite always gave an error message when used with buffer solutions instead of blood, thus it was not used for the following experiments. The remaining four PGMs were tested by measuring the response of a standard glucose solution – phosphate buffered saline (PBS) with 100 mg·dL^{-1} (5.55 mM) of glucose – spiked with additives commonly used in publications employing PGMs as generic diagnostic tools. The results are shown in **Figure 8 (A)**. The accuracy of four meters in PBS with 100

mg·dL^{-1} glucose was quite variable, with the Accu-Check Performa giving the closest reading to the real glucose concentration (-8% error) and the CareSens N the furthest (41% error). EDTA had the largest effect on the meters, since none of them measured a glucose concentration within 15% of the real value. Tween 20 and Triton X-100 caused the reading to slightly increase for all of the meters except CareSense N. Such a meter constantly measured glucose concentration at least 38% higher than the real one, but it was not much affected by the additives tested. In addition to a variety of additives being employed, several different buffer conditions have also been reported for the different repurposing methods. **Figure 8 (B)** shows the reading of four PGMs when measuring 100 mg·dL^{-1} glucose solutions in PBS (reference)(Tang et al., 2016), in PBS adjusted to pH 4.5,(Lin et al., 2016) in phosphate buffer 10 mM pH 7.4 (PB),(Zhao et al., 2017) in 100 mM HEPES(Zhang et al., 2016b) and in 20 mM Tris.(Xue-tao et al., 2015a) Compared to the readings in PBS pH 7.4, an acidic pH caused a significant decrease of the measured glucose concentration for all the meters, and an error message for the Accu-Check Performa, presumably because of denaturation of the enzyme. Accu-Check Performa and CareSens N were also more affected by an increase in pH, measuring a glucose concentration at least 70% higher than the real one in Tris pH 8.8. AgaMatrix Presto and OneTouch Verio IQ were not affected much by basic pH, giving results still within 15% of the real concentration. Overall, OneTouch Verio IQ was the meter least affected by the various conditions tested (additives and pH).



(**A**) (**B**)

Figure 8. **(A)** Variation in the meter reading for a solution of PBS pH 7.4 containing 100 mg·dL^{-1} (5.55 mM) of glucose and various additives that have been used in the literature with PGM-based assays; **(B)** Variation in the meter reading for various buffers containing 100 mg·dL^{-1} (5.55 mM) of glucose that have been used in the literature with PGM-based assays. Error bars represent the standard deviation from three measurements. The dotted lines enclose the region within ±15% of the real glucose concentration.

The redox mediators used also vary from meter to meter. Ferrocene derivatives and ferricyanide may be most widely employed, but quinones, ruthenium and osmium complexes may also be used depending on the meter.(Vashist et al., 2011) These redox mediators cover a wide range of potentials and some may be susceptible to oxidation or reduction by reagents used in the analytical method. If glucose is the ultimate measurement, it would be best to avoid using any oxidising or reducing agents at all to minimise complications with the redox mediators. In our experience, we have seen that some reducing agents can result in a significant increase in the glucose reading, while other reducing agents will suppress the signal from glucose. For example, 10 mM dithiothreitol (DTT) in PBS pH 7.4 containing 100 $mg \cdot dL^{-1}$ of glucose gave an increase of the glucose reading between 7 to 21% for AgaMatrix Presto, CareSens N and OneTouch Verio IQ, while produced an error message in the Accu-Check Performa (data not shown). However, other reducing agents such as tris(2-carboxyethyl)phosphine (TCEP) or glutathione produced a much lower change in the glucose reading in the meters tested. This behaviour may need to be accounted for in cases where glucose is not being measured, but instead the redox probe itself is the target of the measurement, as Zhang and co-workers have demonstrated.(Zhang et al., 2016b) In this case, the glucose oxidase has been bypassed altogether and the redox state of the mediator has been used directly for analyte measurement. However, this approach potentially limits the compatible meters to those with a particular class of redox mediator.

Further, it is worth noticing that the PGMs software allows their use only in a defined range of temperatures, usually from 8 to 30 °C (an error message appears if the temperature is different).

Finally, care must be taken when developing the method to ensure that glucose readings do not fall outside the range of the PGM so as to avoid error messages. If the glucose generated during the assay falls below the lower limit of detection of the PGM (about 10 – 20 $mg \cdot dL^{-1}$ or 0.5 – 1 mM, depending on the meter used), users with no, or very low levels of analyte, will receive error messages and will be unable to determine if the analyte concentration was simply very low, or if there truly was an error in the measurement. As a result, final washes or dilutions may need to be performed with glucose solutions of known concentration, in order to obtain a measurement; the analyte concentration will be proportional to the increase relative to this glucose baseline (a method pioneered by Das et al.(Das et al., 2018)). However, even with this baseline, the requirement for the transduction mechanism is still to generate at least a change of 1 $mg \cdot dL^{-1}$ of glucose in order to resolve a sample.

The net effect of these meter-dependent parameters is that even for methods that are portable between many different brands of PGMs, there will likely need to be a new calibration curve

developed for each meter employed, possibly even for each model of PGM. The pH and composition of wash solutions may need to be tailored to specific meters as well. Precautions also needs to be taken to avoid that a method which operates near the edge of the measureable range for one meter, may fall outside of that range for another meter, resulting in an error message. Or a reagent that does not interfere with the PGM used for method development may interfere significantly with other PGMs.

4. Sample acquisition and preparation

A challenge specific of repurposing a PGM is that sampling and preparation must take into account the level of endogenous glucose, which is present in several biological fluids at different levels. Blood and serum are the most likely matrices to be suitable for targeting biomarkers. However, while 'normal' blood glucose levels in healthy adults occupy a relatively small range of concentrations between 80 – 120 $mg \cdot dL^{-1}$ (4.4 – 6.6 mM)(Wang, 2008), levels for the population as a whole vary over a significantly larger range from below 70 $mg \cdot dL^{-1}$ (3.8 mM) for hypoglycemic patients to over 240 $mg \cdot dL^{-1}$ (13 mM) for hyperglycemic patients. Glucose is also present in saliva, although to a much lesser extent than blood. The level of endogenous glucose in saliva across a range of patients has been reported to vary over nearly an order of magnitude from as little as 0.5 $mg \cdot dL^{-1}$ (0.03 mM) up to 3.6 $mg \cdot dL^{-1}$ (0.2 mM).(Jurysta et al., 2009) Similarly, glucose has been measured in sweat on the order of 0.2 to 5 $mg \cdot dL^{-1}$ (0.01 to 0.3 mM).(Moyer et al., 2012; Watanabe and Miyagi, 1986) Urine and tears typically have low levels of glucose in healthy individuals (below 0.2 mM), although individuals with high plasma glucose concentrations, diabetes and some kidney diseases may exhibit higher glucose levels in those media.(Gerich, 2010; Sen and Sarin, 1980) Endogenous glucose is not detectable in nasal or throat swabs that were taken for the detection of influenza,(X. Zhang et al., 2015) however such biological media needs to be collected by medical professionals.

Three main approaches to deal with endogenous glucose have been explored in the literature – treating it as background signal, diluting it to below the detection threshold, and removing it enzymatically. The simplest of these approaches is to treat endogenous glucose as background signal.(Xiang and Lu, 2011; Yan et al., 2013) In this case, two measurements must be made – one to determine the background level of glucose in the sample, and another to measure the potential increase in glucose as a result of analyte detection. The main challenge for this approach is when it comes to measuring analyte in patients with high levels of endogenous glucose. All glucose meters have an upper limit of detection, and some display non-linear behaviour well below the upper end of

the measuring range (see **Figure 7**). As a result, patients with high blood glucose levels could be at risk of receiving misleading results, or confusing error messages, if the endogenous glucose pushes the result beyond the range of accurate measurements.

A second strategy is to dilute the sample. In the case of whole blood or serum, where endogenous glucose levels are at their highest, this may mean a simple dilution of at least a 100 – 200 fold to ensure that background glucose does not interfere with the analysis. In fact, the majority of methods published to date incorporate several wash steps which effectively dilute the endogenous glucose to negligible levels across the full range of potential patients. Ideally, such washing/dilution steps should happen within the device once the sample has been loaded, otherwise the need for the user to perform these steps may preclude the technology from being used by the general public in the field.

The last method of dealing with endogenous glucose is to use an enzyme to remove it. Yang, *et al.* used glucose oxidase (GOx) to convert β-D-glucose to D -gluconolactone, which does not generate a signal in the PGM.(Yan et al., 2013) The glucose oxidase was then deactivated by heating to ensure that it did not interfere with the subsequent measurements. This method was effective for removing endogenous glucose levels as high as 450 $mg \cdot dL^{-1}$ (25 mM), but it did add 30 minutes to the overall assay time (20 minutes for oxidation of glucose and 10 for deactivation of GOx) and required high temperature for the deactivation step (95 °C). Another enzymatic method that has been employed recently is the use of hexokinase to essentially remove endogenous glucose by converting it to glucose-6-phosphate which does not generate a signal on the PGM.(Zhang et al., 2016b) Using this method, Lu *et. al* showed that they could remove the signal from endogenous glucose in about five minutes. The hexokinase was not deactivated, so this method is only viable where glucose is not the quantity being measured; in this case the internal redox chemistry of the PGM was being exploited to generate a signal, not the response of the meter to glucose in solution.

5. Conclusions and Perspectives

PGMs are incredibly successful point-of-care diagnostic devices, and there has been significant interest recently in repurposing them for analytes other than glucose. There is great promise in developing such approaches as successful implementations could lead to a revolution in cost-effective, portable analytics resulting in point-of-care diagnosis of many diseases.

However, there are significant challenges to using PGMs for other analytes that must be taken into consideration. PGMs have been optimised over the years to be highly specific to glucose with very

limited activity toward similarly structured molecules. This is ideal for blood glucose measurements as it limits the effect of interfering substances, but it also limits the options when designing a method to detect other analytes. The choice of target analyte brings with it potential complications from the sample matrix and endogenous glucose. Also, while multiplex detection has been demonstrated with PGMs, they are better suited to single analytes whose concentration can be directly used for diagnosis.

Signal transduction and amplification are key considerations in how to approach the detection of a particular analyte. PGMs are designed for measuring glucose in biologically relevant ranges (~1 – 10 mM), which presents challenges when trying to measure other analytes, most of which are present well below the 1 mM threshold. The amplification method becomes integral to the potential success as one will likely need to amplify an analyte concentration over several orders of magnitude into a glucose concentration that falls within the relatively narrow range of detection for PGMs. It is also important to consider that PGMs do not measure 0 $mg \cdot dL^{-1}$ of glucose, and the baseline for measuring undetectable levels of analyte must also fall in the range of detection so as to avoid error messages.

In addition, variability between PGMs, and the acceptable variability in measurements for a given meter must be accounted for. Methods developed for one meter will probably, at the very least, require a different calibration curve. At worst, the method may not be transferable to other meters at all if it operates near the detection limits, or if it relies on the underlying electrochemistry of a particular meter. The accuracy of PGMs may also provide challenges in achieving statistically relevant results for small changes in analyte concentration. Whilst PGMs can be used for quantitative detection of analytes other than glucose, the method may need to be developed such that the quantitation takes into account the requirements for meter accuracy stipulated by current international standards.

Finally, to be useful as a point-of-care test for other analytes, the user interface must be easy to use. Ideally, the interface would require the user to simply mix the sample (drop of blood, swab of saliva, etc.) with reagents in one single step and then measure the resulting glucose signal. This would require all the complexity to be built into the reagents and the device, a solution which to our knowledge has not yet been reported in the literature. However, with increasing complexity comes increasing costs, the potential need for additional equipment to manage the flow handling, additional training, etc. A key feature of PGMs is that they are inexpensive to operate and require little to no training, and that benefit may be lost depending on the complexity of fluidic devices designed to interface with them.

Many of these challenges have been addressed on some level in the field, and coupling the recent research advances above with other related efforts, such as specially designed test strips that can be used by PGMs to read non-glucose analytes like ethanol or cholesterol,(Nie et al., 2010) or inkjet-printed glucose detection strips(Gainey et al., 2015) could expand the interest even further. As the challenges in this area are overcome, we may see that PGMs act as a bridge to bringing real diagnostic analytical capabilities to point-of-care scenarios, opening up exciting possibilities.

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Bibliography

Aggidis, A.G.A., Newman, J.D., Aggidis, G.A., 2015. Biosens. Bioelectron. 74, 243–262.

Alshawawreh, F., Lisi, F., Ariotti, N., Bakthavathsalam, P., Benedetti, T., Tilley, R., Gooding, J.J., 2019. Analyst 144, 6225–6230.

Bar-Even, A., Noor, E., Savir, Y., Liebermeister, W., Davidi, D., Tawfik, D.S., Milo, R., 2011. Biochemistry 50, 4402–4410.

Bhavadharini, B., Mahalakshmi, M.M., Maheswari, K., Kalaiyarasi, G., Anjana, R.M., Deepa, M., Ranjani, H., Priya, M., Uma, R., Usha, S., Pastakia, S.D., Malanda, B., Belton, A., Unnikrishnan, R., Kayal, A., Mohan, V., 2016. Acta Diabetol. 53, 91–97.

Bissonnette, L., Bergeron, M.G., 2015. Methods Microbiol. 42, 87–110.

Bonebreak, C.M., Demirci, U., Kuo, W.P., 2011. J. Appl. Oral Sci. 19, 0.

Brendish, N.J., Schiff, H.F., Clark, T.W., 2015. J. Infect. 71, 501–510.

Chavali, R., Kumar Gunda, N.S., Naicker, S., Mitra, S.K., 2014. Anal. Methods 6, 6223.

Chen, J., Wu, W., Zeng, L., 2014. Anal. Methods 6, 4840–4844.

Chen, S., Gan, N., Zhang, H., Hu, F., Li, T., Cui, H., Cao, Y., Jiang, Q., 2015a. Anal. Bioanal. Chem. 407, 2499–2507.

Chen, S., Zhang, J., Gan, N., Hu, F., Li, T., Cao, Y., Pan, D., 2015b. Microchim. Acta 182, 815–822.

Cho, S.J., Cho, C.-H., Kim, K.B., Lee, M.-H., Kim, J.H., Lee, S., Cho, J., Jung, S., Kim, D.-M., Shim, Y.-B., 2015. Anal. Sci. 31, 705–710.

Cui, X., Das, A., Dhawane, A.N., Sweeney, J., Zhang, X., Chivukula, V., Iyer, S.S., 2017. Chem. Sci. 8, 3628–3634.

Das, A., Cui, X., Chivukula, V., Iyer, S.S., 2018. Anal. Chem. 90, 11589–11598.

del Moral, S., Barradas-Dermitz, D.M., Aguilar-Uscanga, M.G., 2018. 3 Biotech 8, 7.

Deng, H., Peng, S.Y., Gao, Z., 2016. Anal. Bioanal. Chem. 408, 5867–5872.

Diabetes Control and Complications Trial Research Group, Nathan, D.M., Genuth, S., Lachin, J., Cleary, P., Crofford, O., Davis, M., Rand, L., Siebert, C., 1993. N. Engl. J. Med. 329, 977–986.

Drain, P.K., Hyle, E.P., Noubary, F., Freedberg, K.A., Wilson, D., Bishai, W.R., Rodriguez, W., Bassett, I. V, 2014. Lancet. Infect. Dis. 14, 239–49.

Du, Y., Hughes, R.A., Bhadra, S., Jiang, Y.S., Ellington, A.D., Li, B., 2015. Sci. Rep. 5, 11039.

Ekhlaspour, L., Mondesir, D., Lautsch, N., Balliro, C., Hillard, M., Magyar, K., Radocchia, L.G., Esmaeili, A., Sinha, M., Russell, S.J., 2017. J. Diabetes Sci. Technol. 11, 558–566.

Fang, D., Gao, G., Yu, Y., Shen, J., Zhi, J., 2016. Analyst 141, 3004–3011.

Fang, J., Guo, Y., Yang, Y., Yu, W., Tao, Y., Dai, T., Yuan, C., Xie, G., 2018. Sens. Actuators B Chem. 272, 118–126.

Frontino, G., Meschi, F., Bonfanti, R., Rigamonti, A., Battaglino, R., Favalli, V., Bonura, C., Ferro, G., Chiumello, G., 2010. Eur. Endocrinol. 9, 21.

Fu, L., Zhuang, J., Lai, W., Que, X., Lu, M., Tang, D., 2013. J. Mater. Chem. B 1, 6123.

Fu, X., Feng, X., Xu, K., Huang, R., 2014. Anal. Methods 6, 2233–2238.

Fu, X., Xu, K., Ye, J., Chen, J., Feng, X., 2015. Anal. Methods 7, 507–512.

Gainey, K.W., Ovington, P., Dean, D., 2015. J. Diabetes Sci. Technol. 9, 1275–1281.

Gao, Z., Tang, D., Xu, M., Chen, Guonan, Yang, H., Chen, G., Ren, H., Liu, Z., Fernandez-Suarez, M., Fortune, S., Toner, M., Lee, H., Weissleder, R., 2014. Chem. Commun. 50, 6256.

Gaydos, C., Hardick, J., 2014. Expert Rev. Anti. Infect. Ther. 12, 657–72.

Gerich, J.E., 2010. Diabet. Med. 27, 136–142.

Ginsberg, B.H., 2009. J. Diabetes Sci. Technol. 3, 903–13.

Gu, C., Lan, T., Shi, H., Lu, Y., 2015. Anal. Chem. 87, 7676–7682.

Gurale, B.P., Dhawane, A.N., Cui, X., Das, A., Zhang, X., Iyer, S.S., 2016. Anal. Chem. 88, 4248–4253.

Gutíerrez-Alonso, P., Fernandez-Arrojo, L., Plou, F.J., Fernandez-Lobato, M., 2009. Fed. Eur. Microbiol. Soc. 9, 768–773.

Hellman, R., 2012. Diabetes. Metab. Res. Rev. 28, 207–209.

Hönes, J., Müller, P., Surridge, N., 2008. Diabetes Technol. Ther. 10, S-10-S-26.

Hong, L., Zhou, F., Shi, D., Zhang, X., Wang, G., 2017. Biosens. Bioelectron. 95, 152–159.

Hou, L., Zhu, C., Wu, X., Chen, G., Tang, D., 2014. Chem. Commun. 50, 1441–1443.

Huang, S., Wang, W., Cheng, F., Yao, H., Zhu, J.-J., 2017. Sens. Actuators B Chem. 242, 347–354.

Hun, X., Xu, Y., Luo, X., 2015a. Microchim. Acta 182, 1669–1675.

Hun, X., Xu, Y., Xie, G., Luo, X., 2015b. Sens. Actuators B Chem. 209, 596–601.

Hunter, C.A., Jones, S.A., 2015. Nat. Immunol. 16, 448–457.

Joo, J., Kwon, D., Shin, H.H., Park, K.-H., Cha, H.J., Jeon, S., 2013. Sens. Actuators B Chem. 188, 1250–1254.

Jurysta, C., Bulur, N., Oguzhan, B., Satman, I., Yilmaz, T.M., Malaisse, W.J., Sener, A., 2009. J. Biomed. Biotechnol. 2009, 430426.

Kirby, C., Gregoriadis, G., 1984. Nat. Biotechnol. 2, 979–984.

Klonoff, D.C., Prahalad, P., 2015. J. Diabetes Sci. Technol. 9, 895–910.

Kwon, D., Lee, H., Yoo, H., Hwang, J., Lee, D., Jeon, S., 2018. Sens. Actuators B Chem. 254, 935–939.

Lafraya, Á., Sanz-Aparicio, J., Polaina, J., Marín-Navarro, J., 2011. Appl. Environ. Microbiol. 77, 6148–57.

Lan, T., Zhang, J., Lu, Y., 2016. Biotechnol. Adv. 34, 331–341.

Lau, L.L.H., Cowling, B.J., Fang, V.J., Chan, K., Lau, E.H.Y., Lipsitch, M., Cheng, C.K.Y., Houck, P.M., Uyeki, T.M., Peiris, J.S.M., Leung, G.M., 2010. J. Infect. Dis. 201, 1509–1516.

Li, G.-Z., Tang, D., 2017. J. Mater. Chem. B 5, 5573–5579.

Liao, J.-Y., Li, H., 2014. Chem. Lett. 43, 1599–1600.

Lin, B., Liu, D., Yan, Jinmao, Qiao, Z., Zhong, Y., Yan, Jiawei, Zhu, Z., Ji, T., Yang, C.J., 2016. ACS Appl. Mater. Interfaces 8, 6890–6897.

Ma, X., Chen, Z., Zhou, J., Weng, W., Zheng, O., Lin, Z., Guo, L., Qiu, B., Chen, G., 2014. Biosens. Bioelectron. 55, 412–416.

Mohapatra, H., Phillips, S.T., 2013. Chem. Commun. 49, 6134.

Moyer, J., Wilson, D., Finkelshtein, I., Wong, B., Potts, R., 2012. Diabetes Technol. Ther. 14, 398–402.

Newman, J.D., Turner, A.P.F., 2005. Biosens. Bioelectron. 20, 2435–2453.

Nguyen, D.H.D., Park, J.-T., Shim, J.-H., Tran, P.L., Oktavina, E.F., Nguyen, T.L.H., Lee, S.-J., Park, C.-S., Li, D., Park, S.-H., Stapleton, D., Lee, J.-S., Park, K.-H., 2014. J. Bacteriol. 196, 1941–9.

Nie, Z., Deiss, F., Liu, X., Akbulut, O., Whitesides, G.M., 2010. Lab Chip 10, 3163.

Okuyama, M., Tanimoto, Y., Ito, T., Anzai, A., Mori, H., Kimura, A., Matsui, H., Chiba, S., 2005. Enzyme Microb. Technol. 37, 472–480.

Parkes, J.L., Slatin, S.L., Pardo, S., Ginsberg, B.H., 2000. Diabetes Care 23, 1143–8.

Pfützner, A., Hengesbach, C., Demircik, F., Schipper, C., Forst, T., Musholt, P.B., 2014. Curr. Med. Res. Opin. 30, 185–190.

Pfützner, A., Klonoff, D.C., Pardo, S., Parkes, J.L., 2013. J. Diabetes Sci. Technol. 7, 1275–81.

Rasti, R., Nanjebe, D., Karlström, J., Muchunguzi, C., Mwanga-Amumpaire, J., Gantelius, J.,

Mårtensson, A., Rivas, L., Galban, F., Reuterswärd, P., Andersson Svahn, H., Alvesson, H.M., Boum, Y., Alfvén, T., 2017. PLoS One 12, e0182005.

Rousseau, G., Asmolov, R., Grammatico-Guillon, L., Auvet, A., Laribi, S., Garot, D., Jouan, Y., Dequin, P.-F., Guillon, A., 2019. Eur. J. Emerg. Med. 26, 41–46.

Sen, D.K., Sarin, G.S., 1980. Br. J. Ophthalmol. 64, 693–5.

Su, J., Xu, J., Chen, Y., Xiang, Y., Yuan, R., Chai, Y., 2013. Biosens. Bioelectron. 45, 219–222.

Su, J., Xu, J., Chen, Y., Xiang, Y., Yuan, R., Chai, Y., 2012. Chem. Commun. 48, 6909.

Taebi, S., Keyhanfar, M., Noorbakhsh, A., 2018. J. Immunol. Methods 458, 26–32.

Tang, D., Lin, Youxiu, Zhou, Q., Lin, Yuping, Li, P., Niessner, R., Knopp, D., 2014. Anal. Chem. 86, 11451–11458.

Tang, J., Huang, Y., Liu, H., Zhang, C., Tang, D., 2016. Biosens. Bioelectron. 79, 508–514.

Tang, Z., Du, X., Louie, R.F., Kost, G.J., 2000. Am. J. Clin. Pathol. 113, 75–86.

Turner, K.M., Christensen, H., Adams, E.J., McAdams, D., Fifer, H., McDonnell, A., Woodford, N., 2017. BMJ Open 7, e015447.

Vashist, S.K., 2012. Anal. Chim. Acta 750, 16–27.

Vashist, S.K., Luppa, P.B., Yeo, L.Y., Ozcan, A., Luong, J.H.T., 2015. Trends Biotechnol. 33, 692–705.

Vashist, S.K., Zheng, D., Al-Rubeaan, K., Luong, J.H.T., Sheu, F.-S., 2011. Anal. Chim. Acta 703, 124–136.

Wan, Y., Qi, P., Zeng, Y., Sun, Y., Zhang, D., 2016. Sens. Actuators B Chem. 233, 454–458.

Wang, J., 2008. Chem. Rev. 108, 814–825.

Wang, J., 2001. Electroanalysis 13, 983–988.

Wang, Q., Liu, F., Yang, X., Wang, K., Wang, H., Deng, X., 2015a. Biosens. Bioelectron. 64, 161–164.

Wang, Q., Wang, H., Yang, X., Wang, K., Liu, F., Zhao, Q., Liu, P., Liu, R., Gould, J., Hood, L., Heath, J.R., 2014. Chem. Commun. 50, 3824.

Wang, Q., Wang, H., Yang, X., Wang, K., Liu, R., Li, Q., Ou, J., Liu, R., Chen, G., Zhou, S., Yang, C., 2015b. Analyst 140, 1161–1165.

Wang, W., Huang, S., Li, J., Rui, K., Zhang, J.-R., Zhu, J.-J., 2016. Sci. Rep. 6, 23504.

Wang, Y., Lu, M., Zhu, J., Tian, S., 2014. J. Mater. Chem. B 2, 5847–5853.

Wang, Z., Chen, Z., Gao, N., Ren, J., Qu, X., 2015. Small 11, 4970–4975.

Watanabe, Y., Miyagi, H., 1986. Japanese J. Clin. Chem. 15, 75–79.

Wu, S., Chen, J., Tian, Y., Tang, X., Li, W., Li, J., 2015. Anal. Methods 7, 1843–1848.

Xiang, Y., Lan, T., Lu, Y., 2014. J. Diabetes Sci. Technol. 8, 855–858.

Xiang, Y., Lu, Y., 2013. Chem. Commun. 49, 585–587.

Xiang, Y., Lu, Y., 2012a. Anal. Chem. 84, 4174–4178.

Xiang, Y., Lu, Y., 2012b. Anal. Chem. 84, 1975–1980.

Xiang, Y., Lu, Y., 2011. Nat. Chem. 3, 697–703.

Xu, J., Jiang, B., Xie, J., Xiang, Y., Yuan, R., Chai, Y., 2012. Chem. Commun. 48, 10733.

Xue-tao, X., Kai-yi, L., Jia-ying, Z., 2015a. Biosens. Bioelectron. 64, 671–675.

Xue-tao, X., Kai-yi, L., Jia-ying, Z., 2015b. Anal. Methods 7, 81–85.

Xue-tao, X., Kai-yi, L., Jia-ying, Z., 2014. Analyst 139, 4982–4986.

Yager, P., Domingo, G.J., Gerdes, J., 2008. Annu. Rev. Biomed. Eng. 10, 107–144.

Yan, L., Zhu, Z., Zou, Y., Huang, Y., Liu, D., Jia, S., Xu, D., Wu, M., Zhou, Y., Zhou, S., Yang, C.J., 2013. J. Am. Chem. Soc. 135, 3748–3751.

Yang, W., Lu, X., Wang, Y., Sun, S., Liu, C., Li, Z., 2015. Sens. Actuators B Chem. 210, 508–512.

Zhang, J., Shen, Z., Xiang, Y., Lu, Y., 2016a. ACS Sensors 1, 1091–1096.

Zhang, Jin, Tang, Y., Teng, L., Lu, M., Tang, D., 2015. Biosens. Bioelectron. 68, 232–238.

Zhang, Jingjing, Xiang, Y., Novak, D.E., Hoganson, G.E., Zhu, J., Lu, Y., 2015. Chem. Asian J. 10, 2221–2227.

Zhang, J., Xiang, Y., Wang, M., Basu, A., Lu, Y., 2016b. Angew. Chemie Int. Ed. 55, 732–736.

Zhang, X., Dhawane, A.N., Sweeney, J., He, Y., Vasireddi, M., Iyer, S.S., 2015. Angew. Chemie Int. Ed. 54, 5929–5932.

Zhao, L., Wei, Q., Wu, H., Li, H., Li, D., Mohapatra, S.S., 2015. RSC Adv. 5, 19439–19444.

Zhao, Y., Chen, X., Lin, S., Du, D., Lin, Y., 2017. Anal. Chim. Acta 964, 1–6.

Zhao, Y., Du, D., Lin, Y., 2015. Biosens. Bioelectron. 72, 348–354.

Zhu, Xi, Kou, F., Xu, H., Lin, L., Yang, G., Lin, Z., 2017. Sens. Actuators B Chem. 253, 660–665.

Zhu, Xuena, Sarwar, M., Yue, Q., Chen, C., Li, C.-Z., 2017. Int. J. Nanomed. 12, 979–987.

Zhu, X., Xu, H., Lin, R., Yang, G., Lin, Z., Chen, G., 2014a. Chem. Commun. 50, 7897.

Zhu, X., Zheng, H., Xu, H., Lin, R., Han, Y., Yang, G., Lin, Z., Guo, L., Qiu, B., Chen, G., 2014b. Anal. Methods 6, 5264–5268.

Zumla, A., Al-Tawfiq, J.A., Enne, V.I., Kidd, M., Drosten, C., Breuer, J., Muller, M.A., Hui, D., Maeurer, M., Bates, M., Mwaba, P., Al-Hakeem, R., Gray, G., Gautret, P., Al-Rabeeah, A.A., Memish, Z.A., Gant, V., 2014. Lancet Infect. Dis. 14, 1123–1135.