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Impact of Laboratory Standards on the Accuracy of Blood Glucose Meters and Its Clinical Effect on Insulin Dosing.

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Engenharia Biomédica

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Abstract

Diabetes Mellitus is characterized by inexistent or insufficient insulin production, with consequent hypoglycemia. To overcome this deficiency patients need to administer exogenous insulin to cover high blood glucose levels. As a result the amount of drug injected is dependent on blood glucose concentration, measured by patients with handheld blood glucose meters. These devices, however, can have different accuracy depending on various properties and also the laboratory standard used to calibrate them. Therefore it was first made a review of BGMs, its technology, history, regulatory requirements, quality requirements and limitations, such as, user handling and technical errors.

Secondly, and since it is not clear what is the impact on insulin dose when measuring glucose with BGMs performing and calibrated in a different way, the quantitative effect of these differences on correct insulin dosing was investigated.

With that purpose, insulin doses based on results for each BGM and two reference methods were calculated and compared. A separate analysis was made for low, normal and high blood glucose to distinguish meters with better performances in each level.

The basis of this work was an accuracy study where 10 meters were compared with two enzymatic methods following ISO standards. Accuracy compliance to ISO:15197:2013 criteria for the evaluated meters was also summarized.

Resumo

A diabetes mellitus é uma doença caracterizada pela inexistente ou insuficiente produção de insulina, com consequente hiperglicemia. De modo a ultrapassar esta limitação, os doentes necessitam de administrar insulina para cobrir níveis elevados de glicemia. Assim, a quantidade de insulina administrada depende da concentração de glicose no sangue, que é medida com aparelhos portáteis chamados medidores de glicose no sangue. Estes dispositivos, no entanto, possuem valores diferentes de exactidão resultado de diferenças tecnológicas e químicas e, também, do aparelho de calibração.

Primeiro, para perceber o funcionamento dos medidores de glicose, foi feita uma revisão da história, tecnologia regulamentação, requisitos de qualidade e limitações, tais como, erros técnicos e do utilizador.

Seguidamente, foi investigado as diferenças no doseamento de insulina baseado em valores adquiridos com dispositivos com tecnologias e desempenhos diferentes. Para isso, doses de insulina foram calculadas e comparadas utilizando valores de glicose no sangue medidos com 10 dispositivos diferentes e dois métodos laboratoriais de referência. Uma outra análise foi feita de modo a perceber quais os medidores com melhor desempenho em vários níveis de glicemia.

Este trabalho é fundamentado num estudo laboratorial que analisou a exactidão de 10 medidores de glicose utilizando dois métodos laboratoriais de referência. O estudo referido seguiu os procedimentos referidos na norma internacional ISO 15197.

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1 Introduction

1.1 Diabetes mellitus

Diabetes Mellitus is a chronic disease that affects millions of people worldwide. According to the World Health Organization's (WHO) "Global Status Report on Noncommunicable Diseases" of 2014, the prevalence of diabetes was estimated to be 9% in people older than 18. The same report says 1.5 million deaths in 2012 were related to diabetes [**1**]. By 2040, the International Diabetes Federation (IDF) predicts 642 million people will live with this disease [**2**].

Definition and classification

It is a metabolic disorder characterized by chronic hyperglycemia due to insufficient or inexistent insulin production by pancreatic β-cells in the islets of Langerhans or an acquired resistance to the hormone's effect.

Etiologically diabetes can be classified in two major categories, called type 1 and type 2 diabetes [**3**]. Further clinical cases are gestational diabetes and other specific types.

Type 1 diabetes is caused by cellular-mediated autoimmune β-cell destruction in pancreatic islets. As a result insulin is not produced and patients must rely on exogenous insulin administration to survive [**3**]. It is frequently detected in early teens.

On the contrary, type 2 characterized by "adult-onset" (age≥45), though in recent years increasing number of children have been diagnosed [**4**], is strongly associated with obesity (about 70 to 80% of diabetic patients are obese), lifestyle and genetic predisposition [**5**].

Insulin action in its target tissues can be impaired due to obesity. Therefore, today's elevated rate of obesity (15% of women and 11% of men older than 18 are obese), has increased the prevalence of diabetes type 2. Globally, 90% of diabetic

patients are type 2 [**1**]. Although in early stages of disease overall insulin production is normal, there is a delay in its release and it is relatively low to tissues sensitivity. This factor puts stress in pancreatic cells that have to compensate by producing more insulin. Over time, due to insulin resistance, there is progressive β-cell dysfunction and lower insulin availability. Thus in the first stages it can be managed with healthy diet and regular exercise but, in most cases, insulin therapy will be required in later stages [**6**]. However, patients' unwillingness to change lifestyle habits leads to an accelerated decline in patients' health and needing insulin administration, even earlier. Increasingly sedentary lifestyle is indicated as one of the reasons for the growing number of new type 2 diabetes diagnoses[**1**]. Coupled with diabetes onset at a younger age, it is possible that the number of insulin dependant type 2 patients will grow.

Insulin is an anabolic hormone that facilitates glucose transport to muscle cells and adipose tissue and, in the form of glycogen, liver storage. In the pre-prandial state insulin levels are low and there is protein and lipid catabolism, forming ketone bodies, hepatic gluconeogenesis and glycogenolisis in order to keep plasma glucose concentration stable and available for the brain and other tissues (such as red blood cells). When there is a spike in glucose after a meal if insulin is not sufficient or there is a decreased responsiveness to its effect, metabolism is the same as in fasting with increasing hyperglycemia [**5**]. These elevated glucose levels lead to microvascular complications such as retinopathy, neuropathy, and nephropathy. Eventually macrovascular complications appear, through the process of atherosclerosis, namely coronary artery disease, peripheral arterial disease and stroke [**7**].

1.1.1 Insulin therapy

Insulin therapy is essential when there is insulin deficiency [**8**]. In such cases daily insulin doses are administered. Initial sources of insulin for clinical use were animal pancreases (cow; pig) [**9**]. In 1972 pharmaceutical companies started production of synthetic "human" insulin manufactured using recombinant DNA

technique [**9**]. In 1996 FDA approved the first insulin analog [**10**]. Insulin analogs have a modified amino acid sequence that allows shorter/longer action profiles [**11**].

Insulin is injected through subcutaneous injections ensuring that it arrives to the bloodstream. Other formulations, less popular, allow alternative administration routes through inhalers [**9**].

One type of insulin is not sufficient to mimic insulin secretion of a healthy person throughout the day. Various types of insulin, according to their action profile, are available. Bolus insulins (rapid-; short-acting) have a prompt onset of action and short duration. They are administered before meals or snacks. Basal insulins (intermediate-, long-acting) have a longer acting profile lasting to up to 30 hours [**8**].

Rapid-acting insulin starts acting within 15 min of injection with peak after 2h and is cleared after 4 to 6 hours. It is used for high glucose correction or to cover glycemic spikes such as those after a meal. Therefore it is administered before meals or snacks [**12**].

Short-acting insulin reaches blood stream after 30 min, with a peak of action between 2 to 3 hours. It last from 3 to 6 hours [**12**].

Intermediate-acting insulin has a profile of action more extended with an onset between second and fourth hour after injection. Its peak is around hour 4 to 12. Its effectiveness can last up to 18 hours [**12**].

Long-acting insulin or basal insulin has duration up to 24h (newer formulations can last over 30h [**13**]) and does not have a defined peak of action. It is administered in the evening before bed or in the morning before breakfast, usually reaches bloodstream after 90 minutes [**12**] (new formulations' onset develops over a period of 6h [**13**]). It is designed to mimic small amounts of insulin secretion throughout the day in response to glucose release by the liver and to lower morning fasting plasma glucose levels.

WHO Expert Committee of Biological Standardization established an international Standard for human insulin. By definition 1 International Unit (IU) is

the activity of 0.03846 mg of human insulin [**14**]. Since insulin analogs may differ from human insulin they are labeled in units (U) [**15**]. Insulin Units are related to its biological action, which is the blood glucose-lowering activity. Insulin is stored in vials with different concentrations (number of insulin units per ml; U-100; U-500). Vials with smaller amounts are also available (U-40).

Syringes or insulin pens can be used to inject insulin. Insulin pens are commonly used due to easiness of use. A variety of insulin pens are available today for adults and children. Pre-filled pens already come with one vial and are non-reusable after all units were administered. Reusable pens can be used with more than one vial. Pens are graduated to measure insulin in units. Patients have to choose, in the pen, the intended dose in units, insert the lancet subcutaneously and the insulin pen will inject the selected dose. Typically, insulin pens measure insulin in 2, 1 or 0.5 U increments.

Continuous subcutaneous insulin infusion is another way of delivering insulin that closely resembles physiological insulin release. Rapid acting insulin is continuously being injected in small doses and in response to measured glucose [**16**]. Insulin pumps can deliver doses in even smaller increments than insulin pens.

Insulin regimens are usually 40%-50% basal insulin and 50%-60% bolus insulin [**8**][**17**]. Basal insulin doses are calculated based on glucose concentration in the morning (fasting). Insulin titration may follow a titration scheme provided by the HCP (Health Care Practitioners) where number of units will increase until blood glucose target range is reached.

Mealtime insulin doses are intended to cover carbohydrate intake and to correct for high blood sugar. Doses to cover carbohydrate intake are determined by the amount of carbohydrate in the meal and the amount of carbohydrate disposed by 1 U of insulin. One unit usually covers from 6-30 g of carbohydrates. This value may depend on time of day, exercise and vary from person to person [**8**]. Doses to correct for high blood sugar are calculated based on pre-prandial blood glucose, target BG and insulin sensitivity factor (ISF). ISF expresses what glucose concentration decrease is achieved by injecting one unit of insulin. This parameter

is, at least for diabetes patients with pump therapy or intensified therapy, one major parameter in diabetes therapy.

Important factors that affect insulin dose and insulin availability are, carbohydrate count; differences in insulin absorption and measured blood glucose [**18**]. Error effects will be associated to insulin action profile. An error in bolus dose can represent an immediate emergency (30 min) whereas in basal insulin errors will have a more lasting effect. An important cause of concern is hypoglycemia and in particular nocturnal hypoglycemia (related to basal insulin) hence the fear and reluctance of many patients in using insulin particularly in intensified regimens.

1.2 Guidelines and recommendations on diabetes diagnosis and management

Many internationally recognized organizations are committed to diabetes research and patient care. The European Association for the Study of Diabetes (EASD), American Diabetes Association (ADA), American Association of Clinical Endocrinologists (AACE) and International Diabetes Federation (IDF) are some with more exposure.

To help patients and HCPs manage diabetes and inform them of the latest development in treatment they publish in websites and scientific journals information, guidelines and recommendations to improve quality of care.

Recommendations and guidelines are written by groups of people considered specialists in the field, supported by clinical evidence. They reflect a consensus opinion among the group. Diagnosis, monitoring and therapeutic actions in diabetes are some of the topics discussed.

Diagnosis of Diabetes Mellitus

Diabetes is diagnosed by testing the presence of hyperglycemia either by plasma glucose criteria or glycated hemoglobin (HbA_{1c}) criteria. Cut-off values are

established based on thresholds of glycemia associated with microvascular disease. Tests are performed with laboratory methods using venous samples.

Table 1.1 represents parameters and respective cut-off values for diabetes diagnosis.

Table 1.1: Diagnosis of Diabetes. FPG, fasting plasma glucose; 2hPG, 2-hour plasma glucose; OGTT, oral glucose tolerance test; HbA1c, glycated hemoglobin; PG, plasma glucose

Parameter	Cut-off Value
FPG	≥126 mg/dL (7mmol/l)
2-hour PG OGTT	≥200 mg/dL (11.1mmol/l)
HbA1c	≥6.5% (48 mmol/mol)
Random PG	≥ 200 mg/dL (11.1mmol/l)

In the presence of classic hyperglycemia symptoms (polyuria, polydipsia unexplained weight loss), a single test in diabetes range is enough for classification. If not, the test previously used should be repeated on a different day, except if random PG was measured, in which case an alternative method is recommended.

HbA1c test represents the percentage of glycated hemoglobin in erythrocytes and is a measure of glycemic control of the past 2 to 3 months (average time for erythrocyte turnover). When using HbA1c to diagnose diabetes it is important to take into consideration factors that can lead to misleading results. Such factors are age, ethnicity and medical conditions (hemolytic anemias, hemoglobinopathies, iron deficiency). This test is not intended for diagnosis in children, adolescents, pregnant women or when suspected T1DM. It must also be performed using a standardized, validated assay.

In patients with likely T1DM, confirmation by laboratory test should not delay treatment initiation in order for prevent rapid deterioration.

Glycemic Targets

ADA, IDF, CDA (Canadian Diabetes Association) recommend an HbA1c target < 7% or less for adult non-pregnant patients. Patients with no significant risk of hypoglycemia or adverse events can target to < 6,5%. Conversely a less stringent

goal (≤ 8%) can be set for patients with risk of hypoglycemia, limited life expectancy, advanced micro and macrovascular complications and other comorbidities. Premeal capillary blood glucose should be targeted between 80-130mg/dL and the postprandial peak below 180mg/dL [**8**]. AACE proposes fasting and premeal blood glucose below 110 mg/dL and 2h post-prandial < 140 mg/dL.

Even though these recommendations are based on clinical evidence of improved outcomes, they represent a guide for glycemic control. Goals should be tailored to individual needs.

Managing Diabetes - Lifestyle interventions

Diabetes care must comprise lifestyle changes, some of which are diet modification, increase in exercise, sufficient amount of sleep, smoke cessation and moderation in alcohol consumption.

Medical nutrition therapy is beneficial for glycemic control in DM. Glycated hemoglobin can be reduced by 0.3-1% in T1DM and 0.5-2% in T2DM [**8**][**19**]. Patients should be educated about nutrition therapy at the time of diagnosis. Strategies for meal planning, grocery shopping, healthy food choices should be addressed. Regarding relative distribution of calories across macronutrients, there is not one perfect proportion for each one. Energy sources may range from 45-60% carbohydrate, 15-20% protein, and 20-35% fat. Food choices can be individualized considering preference, religion and geographic region with metabolic goals in mind. Emphasis should be given to food with high fiber content and low glycemic load in detriment of high sugar content. Whole grains, vegetables, fruits, legumes and dairy products should be the preferred source of carbohydrates [**8**]. Preference to food with high content in polyunsaturated and monounsaturated fatty acids should be given with limited intake of saturated fatty acids and avoidance of trans fats.

Education on carbohydrate count is essential to patients with flexible insulin regimens since administered dose will depend on carbohydrate amount. Fixed

insulin regimens depend on steady carbohydrate intake. Patients will benefit from education on meal planning and portion control.

In overweight or obese T2DM patients, weight loss has been shown to be beneficial to reach glycemic targets and reduce need for pharmacologic interventions [**8**]. Lower healthier body weight can be achieved following a nutritionally balanced diet, reduced energy intake and regular exercise [**20**].

Regular physical activity is associated with increased cardiorespiratory fitness, improved glycemic control, improved insulin sensitivity, blood pressure reduction, improved lipid profile and maintenance of weight loss [**21**]. It is recommended for people with diabetes to do moderate-intensity aerobic exercise at least 3 days a week (at least 150 min cumulatively) with no more than 2 consecutive days between exercise days [**8**][**21**].

Resistance training is also related with improved glycemic control and decreased insulin resistance. Patients should perform resistance exercise twice a week [**21**].

Exercise may prompt hypoglycemia in patients using insulin. Dose reduction or carbohydrate intake adjustment can prevent dangerously low blood glucose. Blood glucose should be checked before exercise and carbohydrates should be ingested if measurement is low (<100 mg/dL)

Another important part of care in diabetes is education on self-management. It helps people with diabetes to make informed choices regarding treatment and facilitates effective self-management throughout their life [**22**].

Pharmacological therapy

T1DM requires insulin therapy immediately at diagnosis. Patients should be treated with multiple dose insulin injections (3 or more injections per day of basal and prandial insulin) or continuous subcutaneous insulin infusion (pump therapy) [**8**]. Diabetes Control and Complication Trial established the advantages of

intensified insulin therapy in reducing microvascular complications. Although risk of severe hypoglycemia was approximately three times higher, new developed insulin analogs are associated with lower rate of hypoglycemia [**23**].

Bolus insulin comprises 50-60% of total daily insulin (TDI) distributed by the necessary premeal doses. Doses must account for carbohydrate intake, glycemic index of each food and measured BG.

When lifestyle interventions alone cannot maintain glycemic goals additional oral pharmacologic therapy is one choice for T2DM management. There are several agents with different physiological effects. They act to reduce glycemic level mainly by decreasing hepatic glucose production, elevating insulin sensitivity in target tissues or improving insulin secretion [**5**].

Biguanides (Metformin) are generally the first recommended oral anti-hyperglycemic agent [**8**] [**24**] [**25**]. They act to suppress hepatic glucose production and raise periphery tissues sensitivity to insulin. If after approximately 3 months, at highest possible dose, HbA1c target is not achieved, combination therapy should be considered with a second oral agent. Subsequently a third agent can be added. Choice of oral agent needs to be individualized considering patient characteristics (degree of hypoglycemia, height, comorbidities), agent effects (blood glucose lowering efficacy, effect on height, side effect) and costs to provide best possible care while minimizing side effects [**8**].

Insulin therapy in T2DM is advantageous and recommended when patients present hyperglycemic symptoms and elevated glycemia or high HbA1c or when other methods fail to help patients achieve glycemic goals. If insulin is necessary therapy should start with basal insulin once daily.

ADA/EASD guidelines propose initial basal insulin dose of 10 U/day or 0.1-0.2U/Kg/day and adding 10-15% or 2-4 U to previous dose once or twice a week for dose titration while BG is above target. If glycemic control is not reached guidelines recommend adding preferably one oral agent (Glucagon-like peptide-1, GLP-1) that stimulates glucose release or prandial insulin.

Several schemes are available for prandial insulin initiation and titration. Initially only one dose before largest meal and if targets are not met adding injections before 2 or 3 meals [**8**] [**17**]. Adjustments can be done by adding 10-15% previous dose or 1-2 U, two or three times a week while postprandial glucose is not at target [**8**] [**24**].

Monitoring Diabetes

Monitoring diabetes is needed to evaluate disease progression and effectiveness of treatment. Glycemic control may be assessed with two principal tools: SMBG or laboratory tests for glycated hemoglobin.

Glycated hemoglobin is a measure of average glycemic control and should be tested every 3 months or 6 if values are consistently in target range.

SMBG is used as an aid to guide and assess interventions and detect hypoglycemia. Understanding how to perform SMBG, what results mean and what are the appropriate actions is essential for optimal use of SMBG. Regular measurements give patients immediate feedback about intervention effects (exercise, food, medication) on glycemic control. If performed and recorded regularly helps establish glycemic patterns that can be correlated to therapeutic actions.

Frequent self-testing of blood glucose in insulin dependant patients on intensified insulin therapy is of the upmost importance and has been related to HbA1c reduction. Patients are advised to test BG before meals and snacks and at times after meals, before exercise, at bedtime, when they think BG is low, after treating low BG until values normalize and before dangerous/serious tasks like driving. T2DM patients treated with once daily basal insulin plus oral agents should test at least once every day at different times [**26**]. If patients are on non-insulin therapy or medical nutrition therapy, SMBG is recommended to control treatment effectiveness [**8**].

1.3 Self monitoring of blood glucose (SMBG)

The Diabetes Control and Complications Trial showed that tight glycemic control, achieved with intensive insulin therapy, can slow the progression of microvascular complications for type 1 patients [**23**]. Similarly, another study, conducted by UK Prospective Diabetes Study (UKPDS) Group, reached identical conclusions for type 2 patients [**27**]. Both studies are a testimony to the importance of intensified glycemic control and insulin therapy for which daily self-monitoring of blood glucose (SMBG) is key.

1.3.1 History

Self-monitoring of blood glucose started in 1963 with the development of dry chemistry test strips Dextrostix® (Miles Laboratories, Elkhart, IN, USA; now part of Bayer), by Ernest Adams and his research team, which displayed a blue colour with intensity proportional to glucose concentration. It required a drop of blood of 50-100μl that had to be wiped after 1 minute. Glucose concentration was then estimated comparing the paper strip's colour to a colour-concentration chart [**28**]. In 1971, Anton Clemens at Miles Laboratories, patented the first device for self-monitoring of blood glucose [**29**]. The device, called Ames Reflectance Meter, using reflectance photometry was able to detect and quantify reflected light from Dextrostix® test strips. This meter, for today's standards, was bulky, heavy (1.2 kg) and expensive; however, it was an improvement from visual evaluation of test strips. Further improvements were made releasing meters easier to handle, less expensive and capable of storing data.

Although Clarke and Lyons proposed the first glucose biosensor in 1962 using an amperometric enzyme method [**30**], biosensors were only available to end users around 1987 when MediSence (Waltham, MA, USA; now Abbott Diabetes Care, Alameda, CA) launched ExacTech® [**31**]. It was very innovative in terms of portability and appearance with two types offered. The customer could choose

between a pen and a credit card sized meter. Detection and quantification of glucose was done through an enzyme coupled with an electron transfer molecule.

From then on the market of glucose sensing gradually moved from photometric to electrochemical technology.

Operator-dependant steps that were potentially error sources were also minimized or removed. For example, wiping of test strips and timing was no longer necessary; sample size was reduced with the introduction of capillarity filling [**28**].

It is worth mentioning another generation of glucose meters used for continuous *in vivo* blood glucose monitoring. The electrochemical sensor is placed subcutaneously though a flexible catheter in the form of a needle of less than 1 mm in diameter [**32**]. Continuous glucose monitoring (CGM) permits a better understanding of glucose levels' progression with real-time values every 1 to 5 minutes.

Since that first blood glucose monitor (BGM), technology for blood glucose measurement evolved substantially. Today meters for self-measurement fit in the palm of a hand, require samples of about 1μL or even smaller, are fast (5s) and are easy to use requiring very little input from the user.

1.3.2 Technology and chemistry

BGMs need to be able to detect glucose from a complex blood sample and convert its concentration into a measurable signal. The majority of commercially available SMBG systems use electrochemistry and have two fundamental components: a biorecognition agent and a transducer. Bioreconignition is done through an enzyme specific for glucose and a redox mediator acts as transductor.

Glucose measurement in BGMs is based on oxidation-reduction reactions. There is electron transference from glucose molecules first to the enzyme, then the mediator and lastly picked up by an electrode. So when glucose is present in the sample a flux of electrons is generated proportional to glucose concentration. Figure 1.1 is a schematic representation of reactions happening in a GOD test strip. The

enzymes and mediators are capable of participating in several reactions because, as seen in Figure 1.1, after each reduction there is an oxidation to return the molecules to their initial state.

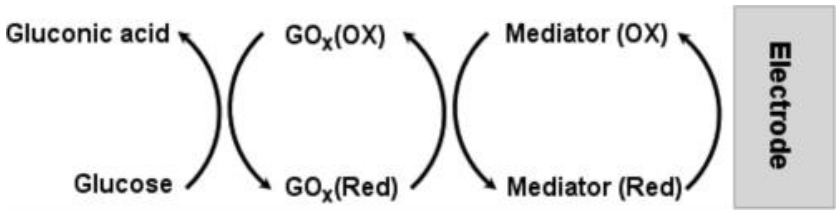

Figure 1.1: Oxidation and reduction reactions that occur in a test strip GOD based. OX and Red represent the oxidised and reduced state respectively. Reprinted from [33] Copyright 2008, American Chemical Society

Enzymes

They are oxidoreductases that act in the reducing end of glucose, the hydroxyl group, to form gluconolactone. In handheld BGMs those enzymes are glucose oxidase (GOD) or glucose dehydrogenase (GDH) [**34**]. In laboratory analysers Hexokinase (HK) is also used but not GDH.

The reaction catalyzed by GOD is described in eq. (1.1)

$$glucose + O_2 \xrightarrow{GOD} gluconolactone + H_2O_2 \qquad (1.1)$$

GOD oxidises glucose transferring two electrons to the enzyme cofactor, flavin adenine dinucleotide (FAD) reducing it. Oxygen is the natural acceptor of electrons from GOD, forming hydrogen peroxide (eq. (1.1)). H_2O_2 is an active oxidant that can nonspecifically oxidize metabolites from the sample, such as uric acid and bilirubin, causing interferences in the measurement. For that reason, other molecules are used as mediators (eq. **(1.3)**), but oxygen can still compete with them for electrons. When using a GOD based meter, sample type, i.e. venous, arterial or capillary, will render different results in terms of glucose concentration exactly due to differences in oxygen partial pressure.

Since GDH is unable to use O_2 as the electron acceptor oxygen content in the sample no longer interferes with measurement. While GOD only uses FAD as coenzyme, GDH also uses nicotine adenine dinucleotide (NAD) or nicotine adenine dinucleotide phosphate (NADP) and pyrroloquinoline quinone (PQQ). The cofactor plays an important role in enzyme specificity. GDH(PQQ) can react with maltose, xylose and galactose [**35**]. GDH(NAD) reacts with xylose and GDH(FAD) reacts with maltose, mannose, galactose and lactose but in very low percentages [**36**]. When using a meter, health care practitioners (HCP) or patients should be aware of the possible interferences and choose devices accordingly.

Mediators

Mediators are organic or inorganic molecules capable of existing in the oxidized or reduced form. In other words, they react rapidly to accept and donate electrons [**36**].

Subsequent to glucose oxidation by the enzyme (eq. (1.2)), electrons are transferred from the cofactor to the mediator reducing it (eq. **(1.3**)). The enzyme, now in the reduced form, can again take part in more reactions with glucose. The mediator can also be regenerated when oxidized by the electrode (eq. **(1.4**)). It is this reoxidation that gives the signal current needed to measure glucose concentration.

$$glucose + GOD_{(ox)} \rightarrow gluconolactone + GOD_{(red)} \quad (1.2)$$

$$GOD_{(red)} + 2M_{(ox)} \rightarrow GOD_{(ox)} + 2M_{(red)} \quad (1.3)$$

$$2M_{(red)} \rightarrow 2M_{(ox)} + 2e^{-} \quad (1.4)$$

As seen in eq. 1 the pair O_2/H_2O_2 can act as a mediator. However, in BGM other molecules are used as, for example, hexacyanoferrate III/hexacyanoferrate II; Hexaammineruthenium(III) chloride/Hexaammineruthenium(II) chloride [**37**]. The lower the redox potential, less interference from other bioactive molecules will

occur [**36**]. Mediators are chosen according to their redox potential along with solubility and rate of dissolution, stability in mixtures with proteins, redox potential, availability and cost [**32**].

Test strip design

Test strips are small thin multilayered plastic strips where the chemical components for detecting glucose are housed. Each manufacturer has its own specific design, but overall test strips are made roughly in the same way.

Two layers of plastic in the bottom and top give support. Each test strip has at least 2 or 3 electrodes. Usually there is a working electrode and a reference and auxiliary electrode. Fill detection electrodes can also be present to detect when sufficient amount of sample is introduced. One common material for electrodes is carbon ink that is screen printed on test strips. Reference and auxiliary electrode can be made of other materials e.g. screen printed ink of Ag/AgCl.

To start the reaction in the test strip the meter has to apply an electrical potential to the electrodes. After analyzing the current time response of the strip, the meter then converts the signal into a glucose concentration shown in the display.

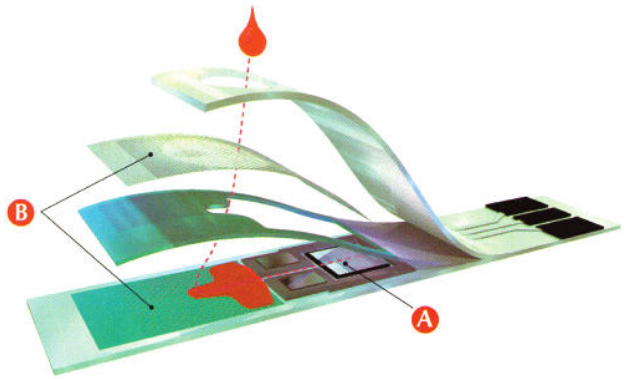

Figure 1.2: Squematic view of test strip layers; (A) electrode system; (B) hydrophobic layer. Reprinted from **[**33**]**

SMBG devices take whole blood samples to measure glucose concentration but provide a glucose result that is plasma equivalent. Due to differences in glucose

concentration between samples a conversion factor needs to be applied. In system accuracy testing the most common conversion factor is given by eq (1.5):

$$G_{plasma} = 1.11 \times G_{whole\_blood} \quad (1.5)$$

G_{plasma} refers to glucose concentration in plasma and G_{whole_blood} glucose concentration in whole blood. The value 1.11 is the International Federation of Clinical Chemistry (IFCC) recommended conversion factor although there are other conversion factors, some of them hematocrit dependent.

Interferences

The complex enzymatic reaction in BGMs can be altered by a series of interfering agents. They can be brought in by environment factors, manufacturing process or blood sample composition.

Altitude affects meters performance due to differences in O_2 partial pressure. As described before, O_2 is the natural acceptor of electrons in GOD based test strips. Since the electrode can only pick up electrons from the mediator, glucose measurement is compromised by changes in O_2 partial pressure. For instance, at high altitude, where pO_2 is lower, glucose values will be increased [**38**].

Temperature and humidity change reaction kinetics with different consequences for different types of test strip [**38**]. Furthermore, mediator can be reduced at higher temperatures. A mediator reduced by temperature will, therefore, lead to an elevated signal not related with glucose concentration [**39**]. Some meters have internal thermometers to correct temperature differences. It is necessary, however, to avoid measurements when temperature changes rapidly.

Test strip manufacturing process can prompt changes in enzyme coverage and test strip lot differences that bring variability. Enzyme coverage is proportional to the produced current. Loss of enzyme area will lead to underestimation of glucose [**38**].

Blood composition of the sample can also bring interferences. Most meters have a percentage range of hematocrit 20-60% where they can measure glucose. Outside those boundaries, values are no longer reliable. Hematocrit interferes with diffusion of glucose in the sample to the site of reaction. BGMs measure glucose in whole blood and are calibrated to provide a result that is plasma equivalent. Since erythrocytes contain intracellular glucose at a different concentration than plasma, variations in hematocrit can cause errors [**38**]. Some meters can compensate for this effect. Anemia, certain types of cancer, chronic and end-stage renal disease, malnutrition or specific diet deficiencies, rheumatoid arthritis, and other conditions lower HCT [**40**]. Patients with underlying conditions or medication that can alter hematocrit should not use handheld meters sensitive to hematocrit.

Partial pressure of O_2 in the sample, as in atmosphere, also interferes with measurements [**41**]. Since pO_2 is different in arterial, venous or capillary blood, only capillary blood should be used to measure blood glucose concentration with SMBG systems intended for capillary samples. Alternate site testing should only be performed when explicitly approved in BGMs' labelling.

Certain chemical substances present in the blood sample, either endogenous or due to medication or pathologies, can have a competitive reaction in three steps of the reaction on a test strip. Competing with the enzyme subtract (1), competing with the mediator (2) or competing with the electrode (3).

Table 1.2: Effect of different substances on glucose readings [1] – GOD; [2] – GDH. Adapted from [38]

Substance	Effect on reading	Step
O_2	Variable	2
Uric acid	Increase[1]	3
Galactose	Increase[2]	1
Xylose	Increase[2]	1
Acetaminophen	Decrease[1]	3
L-dopa	Variable[1]	3
Tolazamine	Variable[1]	3
Ascorbic acid	Variable[1]	3
Icodextrin	Increase[2]	3

Due to rapid changes in hematocrit, pO_2, medication SMBG is not approved for patients in critical care or neonatal care. Also when choosing a device HCP should take into account the medication of the patient and check manufacturer label for possible interferences.

Although technical limitations can lower accuracy, user handling is also an important part in obtaining good results. Some basic steps such as hand washing and control testing are very important when measuring blood glucose with BGMS. These will be discussed later in "Importance of accuracy" section.

1.3.3 Requirements

In the European Union (EU) BGMs for self-testing are regulated by directive 98/79/EC on *in vitro* diagnostic (IVD) medical devices. IVDs are a specific category of medical devices "intended by the manufacturer to be used in vitro for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information: concerning a physiological or pathological state; (…) or to monitor therapeutic measures." [**42**]. BGMs are classified as moderate risk devices (list B, Annex II).

Demonstration of compliance with essential requirements described in directive 98/79/EC will allow application of CE (*Conformité Européene*) Mark and free marketing in EU. Notified bodies are a part of the approval process for BGMS. These independent third parties evaluate the quality of documentation provided by the manufacturer and can ask for additional information.

The directive does not describe specific technical factors. Instead, it defines broad essential requirements for any IVD regarding safety for all users. More detailed technical evaluations are described in European harmonized standards (e. g. ISO 13485- Quality Management Systems).

The International Organization for Standardization (ISO) is an international body that specifies standards to ensure safety and quality of products and services.

Performance requirements regarding system accuracy of BGMs for self-testing are specified in ISO 15197. This standard was revised in 2013.

Analytical System accuracy

Previous minimum system accuracy was defined as: 95% of values should be within either ±15 mg/dL at glucose concentration <75 mg/dL or within ±20% at glucose concentrations ≥75 mg/dL. There was no restraint regarding outliers (5% of results could fall anywhere).

Minimum system accuracy criteria are now defined as:

"95 % of measured glucose values shall fall within either:

- ±0,83 mmol/l (±15 mg/dL) of the average measured values of the reference measurement procedure at glucose concentrations < 5,55 mmol/l (<100 mg/dL) or
- ±15 % at glucose concentrations ≥ 5,55 mmol/l (≥ 100 mg/dL)." [**43**]

Glucose concentration cutoff from absolute value to percentage was raised from 75 mg/dL to 100 mg/dL. This, however, does not mean a relaxing in criteria. Figure 1.3 evidences that new criteria are more stringent for values larger than 75 mg/dL. For values below 75 mg/dL, existing accuracy requirements are maintained.

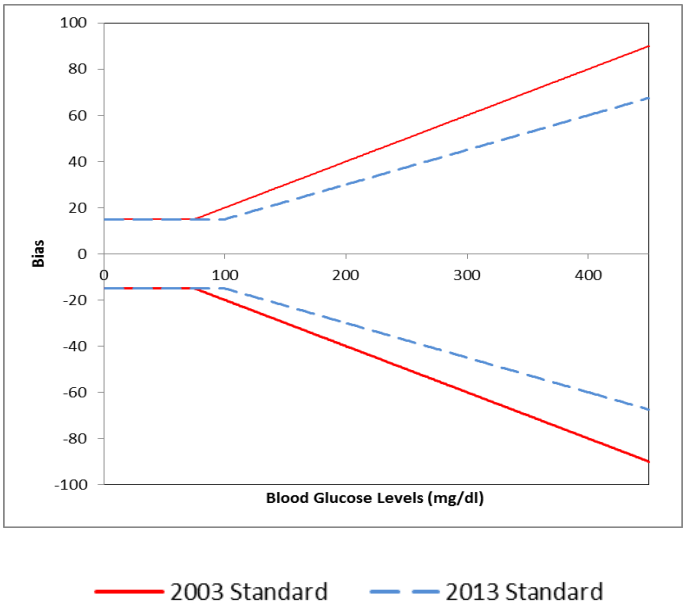

Figure 1.3: Difference plot with system accuracy limits. According to ISO 15197:2003 (full line) at least 95% of results shall be within ±15 mg/dL at BG concentrations <100 mg/dL and within 20% at BG concentrations ≥100 mg/dL. The 2013 revision (doted line) stipulates that at least 95% of results shall be within ±15 mg/dL at BG concentrations <75 mg/dL and within ±20% at BG concentrations ≥75 mg/dL).

The revised standard adopts a risk-based approach with the Consensus Error Grid (CEG) for T1DM. Whereas before there were no requirements for 5% of results, now 99% of measured values are required to fall within zones A and B of CEG (explained in next section).

1.3.4 Importance of accuracy

Measurement variability in BGM can be related to numerous factors introduced since the manufacturing process to the time of user handling. In general, sources of interferences can be related to the monitoring system, as described earlier, calibration process or user errors [**44**]. All three can be present and contribute to the final system accuracy.

BGM System accuracy criteria and procedures for its assessment are defined by the International Organization for Standardization in ISO 15197. The standardization of these procedures is important to define minimum requirements

and to have comparability between laboratory studies. These studies determine system accuracy and demonstrate meter's compliance to the international standards, which is mandatory for CE marking. But regular and independent studies are also important to ensure constant adherence of BGMS to international standards. However, such studies are not compulsory and there is no EU-wide independent institution that evaluates BGMS quality. Manufacturers themselves generally do these studies. But variability between test strip batches may affect measurement quality [**45**]. Individual test strip lots from EU marketed BGMS have been reported to not fulfil minimum system accuracy criteria [**46**][**47**].

It is important to point out that an analytically accurate meter does not assure optimal performance in daily use. User's handling proficiency is a big part of the measurement. In a standardized laboratory study, performed by trained personnel, where there is a controlled environment set for optimal performance and where interferences can be reduced to a minimum, analytical accuracy can be evaluated exclusively. However, these conditions do not reflect patients' daily measurements where sampling conditions and device handling may influence measurement results. Figure 1.4 summarizes conditions in daily life that may influence system accuracy.

User errors

Among the most frequent user errors are failing to clean site before drawing the sample and incorrect test strip and meter handling [**48**].

An unclean hand containing traces of glucose-containing products can substantially increase glucose concentration values [**49**] [**50**]. More so with microsample meters since small amounts of contaminating substances have greater influence when sample volume is smaller [**38**]. In a study where subjects' fingers had been exposed to fruit previous to measurement showed that for 88% of subjects' values were more than 10% higher compared to control measurement using the fist drop of blood. Using the second drop of blood improved results. However, 11% of patients still obtained values 10% higher than control [**51**]. Only washing hands with

soap and water and drying them provided satisfactory results. Traces of other substances, for example hydrogen peroxide, found in hand sanitizers and ascorbic acid, found in fruit, may act as a reducing agents and affect measurements as well.

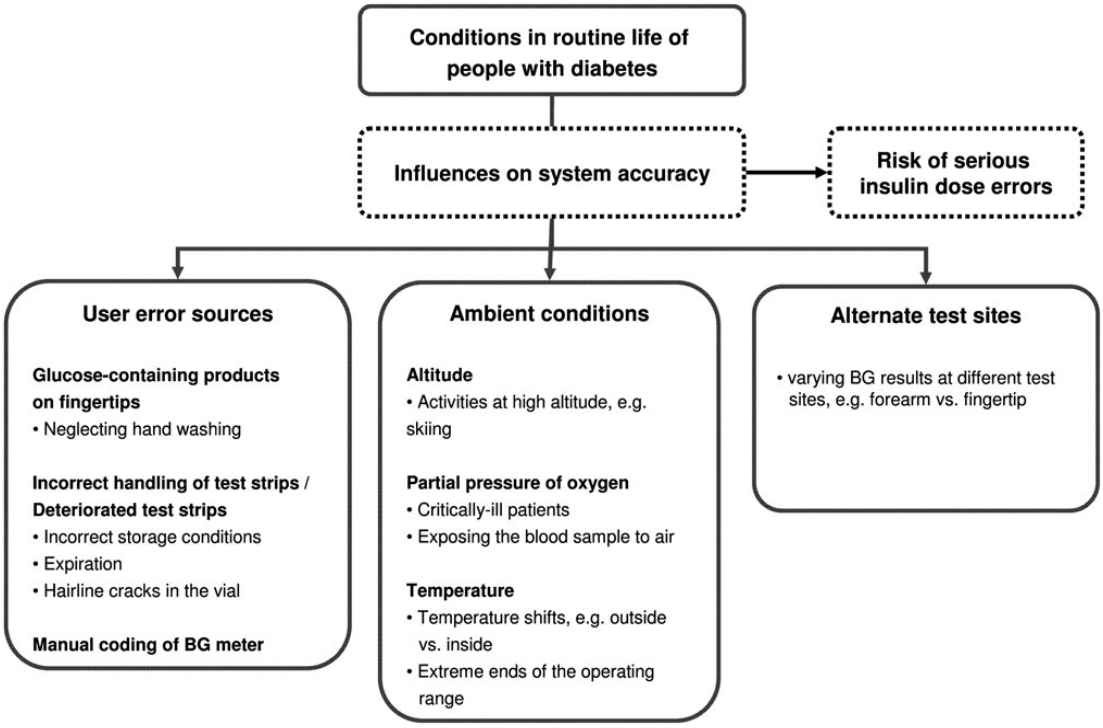

Figure 1.4: Sources of error in routine blood glucose testing. Reprinted from **[53]**.

Incorrect test strip handling

Test strips due to their complex enzymatic reaction are a very sensitive part of BGM. Its lifetime is about 2 years when stored in appropriate humidity and temperature conditions, as per manufacturer recommendations, for optimal performance [**38**]. A study comparing performance between test strips of open *versus* closed vials in different conditions of humidity, temperature and light exposure concluded that test strips from open vials deteriorated faster than tests trips in closed vials. Even test strips from closed vials stored incorrectly under direct light or high humidity did not remain "analytically stable" lasting 28 of the 50 study days [**39**]. Patients should be aware of and respect manufacturer's storage recommendations.

Mechanical stress applied to the test strip and test strip reuse have also been reported as common mistakes patients make [**52**][**53**]. The later is, in most cases, owed to test strip cost.

Incorrect meter handling

When performing the measurement some aspects need to be taken in consideration. Sample evaporation, with consequent elevation of glucose, and contact with oxygen can alter sample composition. Correct sample application on the test strip and swift measurement reduce those errors. These simple measures can be improved with training and make obtaining reliable results easier.

Alternate site testing from forearm, palm, thigh and earlobe is available with some meters. These options are described to be less painful and so more appealing for frequent testing. However due to differences in skin blood flow, blood glucose results can have a lag time especially during rapid glucose excursions delaying hyper- or hypoglycemia detection [**54**].

Adding up meter's technical limitations and user errors can make a difference in the final result. It is both important to have an analytically accurate system and correct measurement technique.

To minimize user error SMBG manufacturers must provide clear labeling with instructions for use, understandable to patients, so that good measurements can be obtained without further training. Evidently patients should take the time to read them carefully and adhere to them. At the time of first SMBG contact training should also be provided by the HCP who should also be familiar with instructions for use. Another strategy, adopted by manufacturers, is to reduce the number of operator-dependant in each measurement and, consequently, diminishing mistakes likelihood. For example when test strip coding was absent, user related error was lower compared with meters that still employed that technology [**55**].

When using blood glucose meters patients should be aware of its performance under different environmental conditions, know meters' limits, technical limitations and possible interferences. Knowing these users can take measures to mitigate errors and make a conscious interpretation of the results given by the meter. Frequent testing with control solution can help establish if device is performing correctly.

At home a patient has to rely on values given by BGM systems to make appropriate therapeutic decisions. Blood glucose values, measured with handheld BGMs, are a source of information to optimize glycemic control and prevent acute chronic complications of diabetes. In managing diabetes the measured value is taken as the "true" glucose concentration and treatment decisions are made based on that supposition. Preprandial glycemia for example is a key value to calculate prandial insulin dose. This dose will determine postprandial glycemic excursions and over time global metabolic control [**56**].

Results positively biased can prevent patients from detecting hypoglycemia or lead to the administration of an excessive amount of anti-hyperglycemic drugs. A patient whose glucose concentration is already low can have a severe hypoglycemic episode if low glycemic levels are undetected. On the other hand, if a meter is negatively biased hyperglycemia can remain concealed allowing chronically elevated BG levels associated with risk of developing diabetes-related complications. Even within accuracy limits results may vary substantially. According to revised ISO of 2013 standards the cut-off is ±15 mg/dL for glucose levels of <100mg/dL and ±15 % for glucose levels of ≥100 mg/dL. This means that a result of 60 mg/dL, for example, could be between 45 mg/dL and 75 mg/dL. ADA sets an alert value for hypoglycemia at plasma glucose concentration of ≤70 mg/dL, this, of course, varies between patients and may shift depending on patients' glucose concentration history. Following ADA hypoglycemic cutoff, the former result would be identified as hypoglycemic whereas the latter wouldn't. Fear of hypoglycemia is one of the most threatening factors in patients' perspective and a barrier for insulin therapy adherence [**56**]. A hypoglycemic event may prompt confusion, loss of

consciousness and, at the extreme, death. Therefore, it is a concern to accurately measure BG in low glycemic ranges.

On the other side of the scale a value of 400 mg/dL could be reported between 340 mg/dL and 460 mg/dL prompting different therapeutic decisions [**48**].

One way of measuring the clinical effect of basing treatment decisions on BG measurements is by error grid analysis. In this qualitative approach BGM measure values are compared with a designated comparison method and plotted on a grid divided by 5 zones according to clinical outcomes (Figure 1.5). Zones range from A (no effect on clinical action) to E (Altered clinical action, could have dangerous consequences). Table 1.3 describes potential clinical actions of errors falling in zones A, B, C, D and E. This assessment reflects expert opinion based on evidence available previous to 1994 and may be obsolete with new analytical accuracy criteria, insulin types and clinical practice. It is however a good tool to classify outlier data points from BGM according to the seriousness of altered clinical action.

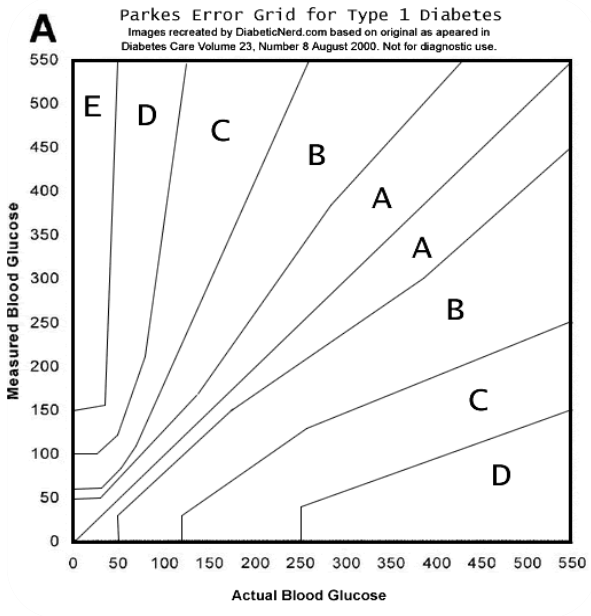

Figure 1.5: Parks error grid developed for T1DM.

Table 1.3: Definitions of the error grid zones

Risk level (CEG zone)	Risk to diabetic patient
A	No effect on clinical action
B	Altered clinical action – little or no effect on clinical outcome
C	Altered clinical action – likely to affect clinical outcome
D	Altered clinical action – could have significant medical risk
E	Altered clinical action – could have dangerous consequences

When looking at accuracy one should have in mind the purpose SMBG. SMBG is recommended to determine insulin doses, to achieve glycemic goals and detection of hypoglycemia [**57**]. How relevant errors are and which level of accuracy is needed will depend on what way values are used for in therapeutic decisions.

A diverse population of patients with varied therapeutic regimens and different diabetes types and acuteness uses BGMs. Evidently they have different needs in terms of SMBG system accuracy. For patients in insulin therapy early detection of hypoglycemia is essential. These patients need especially accuracy in the hypoglycemic range. Patients in intensified insulin and insulin pump therapy are advised to check blood glucose before meals and exercise, at bedtime, sporadically posprandially, when hypoglycemia is suspected among others [**8**]. Accuracy across all glucose ranges is expected.

Not only the BG value is important in insulin dosing, also carbohydrate intake estimation, accuracy of insulin pens, differences in insulin's metabolic effect, amount of insulin still active from previous doses and subsequent physical exercise. All these factors affect postprandial glycemic excursions. Nevertheless, an error in the first step, i.e. BG value, will be amplified by possible subsequent errors or variations [**56**][**58**].

Calibrating method also plays an important role in meters accuracy. There is not a standardized method to calibrate meters. Manufacturers may choose the most convenient one. It is typically based on either hexokinase or glucose oxidase. Studies have showed methodological differences between laboratory methods using HK and

GOD of about 8% [**59**]. This discrepancy yields different results with meters calibrated with either method. Nevertheless, many BGMS exhibit larger measurement error than the bias between HK and GOD based methods. Thus laboratory method plays a vital role only in high quality BGMS.

Hematocrit was included in the interfering substances to be tested.

New analytical accuracy criteria were motivated by an understanding of SMBG importance in supporting diabetes management. Lifestyle and therapeutic decisions are based on BGM values. Many diabetes patients have hypoglycemia unawareness and SMBG is the only practical means for detecting asymptomatic hypoglycemia. Additionally, BGM technology improvements, since publication of first edition, ensured that manufacturers could comply with stricter criteria.

BGMS are compared to laboratory devices but there is not one harmonized method for accuracy evaluation. Typically, laboratory devices are based either on the hexokinase or glucose oxidase method.

Laboratory devices for comparative accuracy studies (following ISO 15197) need to comply with traceability requirements established in ISO 17511. Figure 1.6 shows one example of a traceability chain for glucose measurement in body fluids. A traceability chain usually starts with the definition of a measurement unit, followed by a primary measurement method and a primary calibrator material. For glucose, the accepted primary method is ID-GC/MS (Isotope Dilution Gas Chromatography-Mass Spectrometry), but this does not exclude other methods. The primary calibrator material can be, for example, Standard Reference Material 917c (glucose powder) from the US-based National Institute for Standards and Technology (NIST SRM 917c) or NIST SRM 965b (glucose in human serum). The traceability chain is then composed of secondary (and successive) measurement methods and secondary (and successive) calibrator materials.

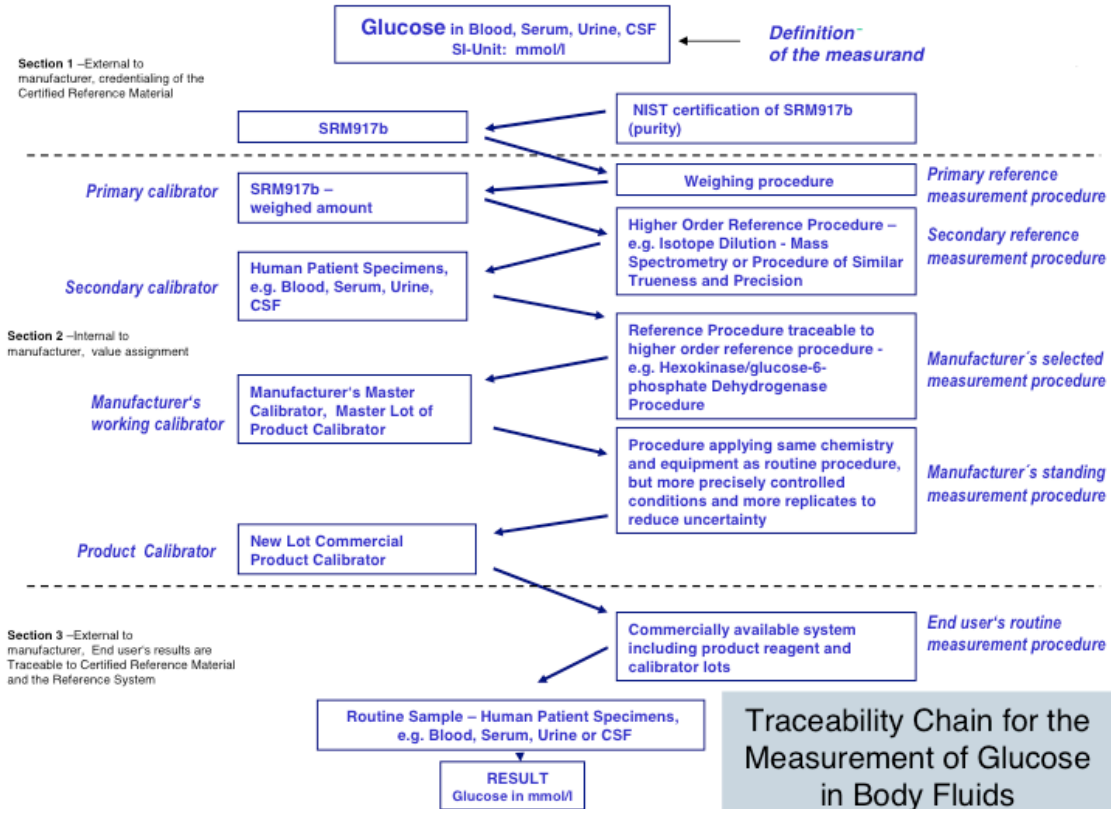

Figure 1.6: Traceability chain for the measurement of glucose in body fluids. Reprinted from [60]

ID-GC/MS is considered the most accurate reference method for glucose. However only a few laboratories perform this method and manufacturers calibrate BGMS to alternative methods that are easier to handle. These methods should, nonetheless, have ID-GC/MS in their traceability chain.

An internationally recognized reference method would improve comparability of results and help interpret performance of BGMS.

2 Introduction to the Problem

Several studies have demonstrated considerable variations in measurement quality between BGM systems. Even among marketed systems some studies have reported BGMs not compliant with ISO system accuracy criteria [**61**] [**46**]. Since the revision of ISO 15197 in 2013, more stringent requirements were proposed. After a transitioning period of 3 years, in 2016, in order to be marketed in EU, all systems will have to prove compliance to the new international standard.

System accuracy of 10 market available BGMS was evaluated in a laboratory study conducted by Dr. Guido Freckmann at the Institut für Diabetes-Technologie Forschungs- und Entwicklungsgesellschaft mbH an der Universität Ulm (IDT), Germany. Following ISO 15197:2013, which allows any method conforming to established traceability requirements to be used for reference measurement, system accuracy was assessed against two laboratory methods based on Hexokinase or Glucose Oxidase.

For one system considerable number of values were measured with test strips from two vials. Elevated results from control solution measurements with test strips from these two vials were detected during the study. Manufacturer's investigation attributed this to possible vial exposure to moisture. For this reason, even though ten systems were evaluated, only results with respect to 9 systems were described.

In the study, 7 systems showed compliance with system accuracy specifications. More than 95% of results were within accuracy limits, with all 3 lots tested, independent of the comparison method used. Two systems did not comply with minimum accuracy requirements irrespective of comparison method used. Looking at individual lots of these two systems, 90% to 94% of results were within the established system accuracy limits when evaluated against the manufacturer comparison method (GOD) and 84% to 99% if compared with the alternative laboratory method (HK).

Regarding clinical accuracy all systems showed 100% of results within consensus error grid zones A and B.

Relative bias of individual lots to each laboratory method was also calculated. A difference in relative bias between lots of each system bigger than 5% (5.4%; 7.6%) was found in 2 systems.

Previous studies have reported differences between laboratory methods [**59**]. Yet, few studies have weighted analytical accuracy results against two different laboratory comparison methods. Now that any traceable laboratory reference method can be used in comparison accuracy studies, independent of manufacturer calibration method, the question whether calibration method can have impact on BGM accuracy is relevant. Variations were observed, in the referred laboratory study, between results obtained with both laboratory methods. Differences were reflected in a systematic mean difference of approximately 3%, indicating that these differences may have "considerable impact" on results from system accuracy studies.

The variability found in marketed BGMs in Europe brings the issue of clinical implications of using poor quality BGM.

Boyd and Bruns' simulation model of the use of BGM to adjust insulin doses weighted insulin dose errors against device imprecision and bias. Variability of 10% led to 16% to 45% of incorrect insulin doses. To achieve 95% correct insulin doses, precision and bias would have to be less than 2%. Another simulation evaluated the percentage of insulin errors with various BGMs, for different degrees of bias (-1.35% – 4%) and imprecision (CV: 4.84% – 7%). Insulin doses where on target in 64% to 82% of cases.

Correct glycemic measurement and insulin dosing help achieve glycemic targets with smaller amount of glycemic excursions. This can be translated in better metabolic control and less diabetes related complications. SMBG is the primary tool to achieve these aims. Yet, it is agreed that different patient groups require different levels of BGM accuracy within the clinically relevant BG ranges, hypoglycemia, euglycemia, and hyperglycemia [**62**]. Among those patient groups are type 2

diabetes patients, type 1 diabetes on intensified insulin therapy and patients in intensive care.

Patients following an intensive insulin regimen are recommended to test BG levels at least 6 times a day [**8**]. BGM accuracy for these patients is important across all glucose concentration ranges but specifically in the hypoglycemic range. Type 2 diabetes mellitus patients do not necessarily need accuracy in low ranges. A distinction between BGM performances is essential to help patients and HCP choose systems that will offer better performance, according to clinical need.

There is concern whether BGM performance can be a source of insulin errors even with meters compliant with accuracy criteria. In addition, a categorization of BGM according to accuracy in several glucose ranges (low, normal and high) is desirable. With that intent insulin, dosing errors were calculated based on data from BGMs measurements.

In order to evaluate BGMS performance in different glycemic levels, errors separated by glucose ranges were considered.

3 Methods

A laboratory study was performed at IDT under principal investigator Dr. Guido Freckmann with trial registry number (ClinicalTrials.gov): NCT01909687.

Study duration was between 03.07.2013 to 22.10.2013. 164 different subjects entered the study. Subjects were type 1 or 2 diabetes mellitus or non-diabetic. Previous to the study, each patient was checked for interfering substances as well as inclusion and exclusion criteria p.e. pregnancy, lactation period, acute disease, chronic disease.

Investigated systems were: Accu-Chek® Aviva, Contour® XT, FreeStyle InsuLinx, Contour® next USB, BGStar®, OneTouch® Verio® IQ, Accu-Chek® Performa, mylife™ Pura™, Glucocard™ G+ and MyStar™ Extra. It was used for each system three test strip lots. All test strips lots and 9 of the investigated systems were purchased at pharmacies. MyStar Extra® was not freely available in Germany and so it was obtained from the distributor (Sanofi-Aventis Deutschland GmbH, Germany). Control measurements were done each day to ensure systems proper functioning.

Two laboratory methods were used to perform comparison measurement: a glucose oxidase based method (YSI 2300 STAT Plus™ glucose analyzer, YSI Incorporated, Yellow Springs, OH, USA) and a Hexokinase based method (Cobas Integra® 400 plus, Roche Instrument Center, Rotkreuz, Switzerland) referred to as YSI and Cobas respectively. Traceability requirements according to ISO 17511 were confirmed by the manufacturers.

Study was performed in three parts: in the first and second part 3 different systems in each part were evaluated changing the order of the investigated systems every 1/3 of subjects. In the third, 4 systems were assessed. Order was changed every ¼ of subjects.

Temperature at which measurements were made was 23°C ±5°C. Humidity was maintained between 37,5 % – 57,8 %. Sample hematocrit had to be between 20% - 60% and glucose concentration measured with laboratory method had to be

within systems' measurement rage 10 mg/dL – 600mg/dL (or according to manufacturer specifications).

Procedures specified in international standard ISO 15197:2013 were followed for system accuracy evaluation.

Procedure

Each patient was asked to wash hands with soap and water and dry them before sample collection. Samples were collected from fingertip capillaries by skin puncture. Individual samples were tested in duplicate for each system lot using test strips from the same vial and two devices. To ensure that test strips from 10 different vials were used, vials were changed every 10 subjects, approximately.

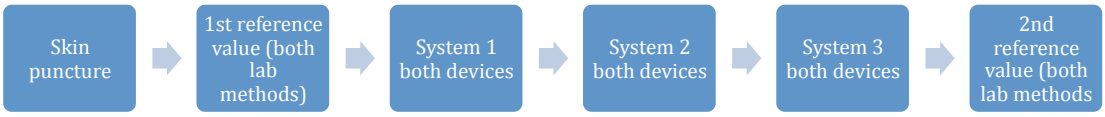

Figure 3.1: Testing sequence

Aliquots were collected from each sample immediately before the first and immediately after the last measurement with up to 4 systems for duplicate measurement with the comparison methods.

Sample stability was confirmed by checking that the difference between the first aliquot and second was ≤4mg/dL at BG concentrations ≤100 mg/dL and ≤4% at BG concentrations >100 mg/dL.

Each sample was allocated to a bin according to glucose concentration mean value measured with the respective reference method as specified in Table 3 section 6.3.5 of ISO 15197 and described in Table 3.1.

Table 3.1: Blood glucose concentration of samples according to ISO 15197:2013 [1]

Bin #	Percentage of samples [%]	Glucose concentration [mg/dL]
1	5	≤ 50
2	15	> 50 – 80
3	20	> 80 – 120
4	30	> 120 – 200
5	20	> 200 – 300
6	15	> 300 – 400
7	5	> 400

To attain the defined distribution of glucose concentrations only in the range <50 mg/dL and >400 mg/dL samples can be adjusted. Sample adjustment was performed by incubation to allow glycolysis to take place or by glucose supplementation with a stock solution of 40% glucose in 0,9% NaCl.

At least 100 fresh capillary blood samples were collected and prepared according to device manufacturer' instructions. This ensured 200 data points for each system lot witch is 600 data points per BGMS.

Insulin dose error

Insulin doses were calculated based on BGMS measurements. A simplified model of pre-meal insulin dose was used.

Before meals insulin doses need to cover carbohydrate intake and correct for high blood sugar. Insulin dose to cover carbohydrates is calculated taking meal carbohydrate content divided by insulin-to-carbohydrate ratio. To correct glycemic levels difference between measured BG and glycemic gloal divided by their Insulin Sensitivity Factor (ISF) is used. Carbohydrate intake was excluded from the proposed model in order to relate BGM's values to insulin doses. Carbohydrate intake and carbohydrate estimation error was consequently dismissed.

True value of glucose concentration is not accessible due to inherent error when using a laboratory method. Results from the manufacturer designated comparison method were assumed true and insulin doses calculated based on those results were considered correct.

Intended insulin doses were then calculated using the following equation:

$$ID_{reference} = \frac{BG_{reference} - BG_{goal}}{ISF} \quad (3.1)$$

$BG_{reference}$ is the blood glucose concentration measured with a laboratory/reference method; BG_{goal} is the target value for blood glucose after insulin administration; *ISF* is the insulin sensitivity factor. If we substitute $BG_{reference}$ by BG_{BGM}, blood glucose measured with a BGM (eq. 2.2), we then have insulin dose related to BGM values, ID_{BGM}.

$$ID_{BGM} = \frac{BG_{BGM} - BG_{goal}}{ISF} \quad (3.2)$$

This is considered the dose a patient would inject.

Error in insulin dose is then the difference between $ID_{reference}$ and ID_{BGM} .

$$Error = ID_{reference} - ID_{BGM} \quad (3.3)$$

Where the signal of the equation, plus (+) or minus (–), translates to over or underdosing, respectively.

$ID_{reference}$ and ID_{BGM} were rounded to the nearest 0,5 IU given that typical for insulin pens measure in 0,5 IU increments.

For each system and each test trip lot insulin dose errors were calculated, taking both laboratory methods as reference, with the objective of determining the percentage of insulin doses within 0,5 IU of the intended dose. Percentage of insulin above 1IU and 2 IU was also determined. Percentage of underdosing was calculated for doses 2 IU or more below intended dose.

It was checked, as well, percentages of target insulin doses, overdose and underdose based only on unadulterated samples.

Furthermore, dose errors were calculated per glycemic level following glucose concentration distribution of Table 3.1 to assess witch meters performed better for each glycemic range. In this assessment only the manufacturer comparison method was used to calculate intended insulin dose for each system.

4 Results

Please see Appendix A through E.

5 Discussion and Conclusion

The aim of this thesis work was to assess errors in insulin dosing that market available BGM systems might cause and also to investigate which systems might be more appropriate to type 1 or type 2 diabetes.

5.1 Significance of this study

Patients suffering from diabetes and on insulin therapy depend on blood glucose monitoring systems daily. Glucose concentration is the main parameter to calculate insulin doses. Patient's metabolic control is linked with correct insulin level in the blood stream and therefore with the correct dosage injected. The major risk of incorrect under dosage is hypoglycemia. This can be a consequence of incorrectly high BG value. Symptoms of hypoglycemia are confusion, anxiety, palpitations and others. Severe hypoglycemia may cause coma and death. Insulin overdosing, on the other hand, prevents the achievement of glycemic goals and is an impediment to the delay of micro and macrovascular complications. This limits the years of healthy life. Also the cost in healthcare increases substantially due to diabetes related complications [**63**]. It is, therefore, in the best interest of HCP and patients to achieve balanced metabolic control. For these reasons, it is important that BGMs measure BG concentration accurately for correct insulin dose and that patients use them appropriately.

5.2 Methodology

This work establishes a relationship between BGM performance and insulin dosing errors with two laboratory methods (HK and GOD) as reference.

In this study, it was obtained data regarding glucose concentration from 164 patients. All patients were tested with 10 systems twice (two devices per system) and using 3 different test strip lots. At least 100 values per device per lot were obtained, in conformity with ISO specifications. In the end, 600 different data points per BGM system were generated. In addition, samples were measured with two laboratory methods to be used as reference.

In order to determine the accuracy of devices under study, BGM measurements were compared with reference values. In the study led by Dr. Freckman, two systems did not fulfil with ISO 15197: 2013 system accuracy (OneTouch Verio IQ and GlucoCard G+). Those systems showed less than 95% of results within stipulated criteria. In fact, GlucoCard G+ did not fulfill system accuracy criteria with none of the 3 tested lots, irrespective of the laboratory reference method used [**64**]. Contour Next showed 100% of results within system accuracy criteria irrespective of comparison method.

Insulin doses were calculated from the original 600 BG data points. It were also calculated insulin doses from two laboratory reference methods. Results from BGM insulin doses were then compared with results from both laboratory methods to determine insulin dosing errors

5.3 Summary of results

In the present study, it was system 3 that showed on target insulin doses closest to the respective manufacturer reference method. Both systems that did not fulfil accuracy criteria were among the ones with lowest percentage of on target insulin along with system 2.

Overdose equal or above 2 U was relatively low, not surpassing 5%. Overall the study showed that the 10 BGMs under study give BG concentration values with limited impact in insulin doses.

This study revealed differences in insulin dose error within BG concentration ranges allowing to distinguish between BGMs suitable for diabetes type 1 or type 2.

It can be argued that type 1 patients need small error across all ranges and especially in the hypoglycemic range. On the contrary, type 2 patients do not necessarily need the same in the low glycemic range.

System 5, system 3 and system 7 showed insulin dose errors not superior to 1.5 U across all ranges and 100% of on target insulin doses in the hypoglycemic range.

In particular cases, such as patients treated with intensive insulin therapy or insulin pump, more accurate measuring devices are needed. In these groups, insulin dose errors of 1 U can have a more pronounced impact than in other patients. In this study, overdose by 1 U or more was as high as 12.5% (YSI reference).

Also the threshold of 1 U was studied because of the significant physical and psychological consequences of hypoglycemia. A benchmark for diabetes therapy assessment is HbA1c, established by the publication of the Diabetes Control and Complications (DCCT). The study showed a direct relationship between glycemic control and the development of micro and macrovascular complications. It showed as well that intensively treated patients reached HbA1c levels faster and vascular complications were delayed. This, however, came with higher rate of hypoglycemic events. As it was referred previously, new insulin formulations have lower risk of hypoglycemia.

When looking at results from unadulterated samples, errors are lower than compared with all samples. This is the consequence of excluding samples with BG concentration above 400 mg/dL where in general BGMs are less accurate.

5.4 Limitations of the study

There were some limitations in the development of this study, which should be pointed out. These limitations refer to the study methods and to the simplification of the dose error model. A laboratory study does not mimic everyday conditions in which patients carry out the measurements. As discussed, user error plays a significant part in the quality of results, as do ambient conditions. It is

nonetheless agreed that errors derived by poor analytical performance will be propagated with subsequent interfering factors.

Another limitation is knowing the exact value of glucose concentration. Inherent to the measurement of a biological substance is the impossibility of knowing its true value. In this study, BGM results were compared to two laboratory methods and the respective results were assumed as the true value.

Finally, the last limitation in this study was the simplification of error model. Insulin dose calculation was based on the equation used by patients to calculate mealtime insulin doses. This equation includes a carbohydrate component to cover carbohydrate intake. This component was disregarded to link BGM performance to insulin dose.

In conclusion, accuracy of BGM systems must be guaranteed before it is available to customers as well as in post-marketing stages. HCP and patients should be informed about technical aspects of BGMs and its performance in order to make informed choices regarding its use. Special consideration should be given in cases where BGM is used to calculate insulin doses and if it will be used by type 1 or type 2 diabetes patients.

5.5 Future outlook

Diabetes is a chronic disease, which requires constant management and is associated with several complications such as retinopathy, nephropathy, neuropathy and cardiovascular disease. Reduced patient compliance or inadequate treatment regimes may contribute to a faster deterioration of patient's health. Upon diagnosis, patients must frequently visit the doctor in order to reach an adequate treatment plan. Periodic appointments follow this initial phase approximately every 6 months. During those periods, patients follow a set of written rules given by the doctor to reach or maintain glycemic target and manage glycemic levels.

Patients with diabetes mellitus are faced with a daily cumbersome routine. They must pay attention to what and how much to eat, what dose of insulin to take

and how much exercise to practice. Patients need help and information to make those decisions. But not only patients struggle. Physicians often have a short amount of time with each patient and need to analyze all the information that a patient brings to consults and reevaluate treatment if necessary. There is an apparent need for clear and accessible information.

Tools that enable easier contact between patient and HCP can improve treatment adjustment and assist in making prompt alterations when necessary.

Many apps are available which mostly offer simple functionalities. The main benefit to users of such apps for regulating blood glucose levels is the availability of quick information which gives patients greater level of autonomy.

One solution comes in the form of software for computers, smartphones or tablets, capable of receiving and storing patients' necessary data (carbohydrate intake, insulin dose, FPG, BG, HbA1c) and do calculations in order to give feedback on the development of a given parameter or treatment suggestions. Apps for cell phones have increasingly been promoted for patient use in day-to-day care. Ultimately, patients are able to make informed lifestyle choices and at the same time, if treatment is effective, see improvements in metabolic control. The visual feedback provided by these apps acts as an incentive to behavioral changes. If a patient is able to see his daily achievements and that each step brings him closer to goal, he will be more motivated to continue treatment.

Storing data is not the only important component of these apps. Sharing it with the right parties also plays a significant role in managing the patients' health. For example, HCP should have access to selected information, ensuring better and safer care. Stored and shared data of blood glucose levels, insulin doses, hypoglycemic events and pertinent graphs can give HCP a fast way to evaluate treatment evolution and eventually act remotely if some value is cause of concern.

Likewise, the market of SMBG has been evolving to bring new functionalities to the user, in the form of smarter BGMs that can store BG values, calculate insulin

doses and store time and amount of previous doses.

It is important to note that these new advances in managing diabetes give freedom to the patient with less medical interaction. These tools should be used with caution in particular if they are designed to give treatment suggestions, as for example insulin doses. HCPs should prescribe with caution and only to trained and informed patients.

Regardless of these newer Smartphone applications and the added functionalities they provide, an accurate measurement of blood glucose is still the most fundamental piece of information for a proper management of diabetes. Therefore, BGMs' system accuracy must be guaranteed to ensure safety for the patient.

Studies have suggested that SMBG frequency as an important role in metabolic control (HbA1c) [**65**] [**66**]. Yet it is important to emphasize that SMBG in itself is not an intervention. It is a tool to assist treatment decision and ultimately achieve glycemic targets. Quality of measurement is nonetheless linked with metabolic outcomes. Quality of measurement does not refer exclusively to analytical accuracy, but its assurance is important if only to prevent propagation of error if BGM is mishandled.

Although SMBG is paramount to ensure proper insulin dosage, literature has shown that there are other factors which may contribute to errors. For patients on fixed insulin regimens, dose calculation is obviously not necessary. For these patients, meal planning with comparable carbohydrate portions is more important. A more adaptable regimen, however, may be preferable to some patients who want to have choice on type of meal and exercise. In such cases, education on insulin calculation and carbohydrate counting is extremely important.

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Appendix

Appendix has been removed due to confidentiality