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Research Article

A Systematic Review on the use of Serum and Plasma for Glucose Determination between 2011 to 2022

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ABSTRACT

Background: Diabetes Mellitus (DM) is a chronic disease affecting carbohydrate, protein and fat metabolism. It can be a result of a deficiency or a nonfunctional hormone of insulin. Moreover, DM can be diagnosed by measuring the level of blood glucose. Different laboratory procedures can be used such as fasting blood sugar, hemoglobin A1C determination, and post-prandial test. The primary objective of this study is to compare the result obtained from using plasma as compared to serum.

Methodology: Electronic sources online were accessed for published articles between 2011 to 2022 related to the use of serum and plasma for glucose determination. The following databases such as Google Scholar, Proquest, EBSCO, PubMed, MEDLINE, Science Direct and other open access journals were utilized in the conduct of systematic review. A total of twenty seven (27) studies were used out of the initial one hundred thirty-six (136) studies screened. It is not limited to the sample population or participant category with or without pre-existing health conditions that have undergone testing.

Results: Based on the findings of the study, the most frequently utilized specimen in blood glucose determination is the plasma. Various additives had been used over the years to aid in the most ideal for glucose analysis such as the gel separator for serum, sodium fluoride/potassium oxalate, citrate, lithium heparin and ethylenediaminetetraacetic acid. Moreover, the mainly common procedure to measure blood glucose is the enzyme technique either the glucose oxidase or the hexokinase method. Both procedures are highly sensitive and specific tests for a more accurate analysis.

Conclusion: Based on the findings of the study, the most commonly used specimen in fasting blood glucose determination is plasma. The use of plasma is preferred over serum for glucose determination, particularly with the immediate separation of the plasma from red blood cells through centrifugation within thirty (30) minutes of specimen collection.

Keywords: Diabetes mellitus; Plasma; Serum; Fasting blood glucose

INTRODUCTION

Back in 2002, Diabetes Mellitus (DM) is diagnosed from the patient through the accurate measurement of plasma glucose [1]. A chronic disease affecting carbohydrate, protein and fat metabolism, DM is primarily caused by the lack of insulin secretion as a result of either the progressive or a marked inability of pancreatic Langerhans islet cells to produce insulin or defects in insulin uptake in peripheral tissue. Diabetes is further classified into type 1 and 2 [2]. In 2012, it was estimated by the Centers for Disease Control and Prevention (CDC) that 29.1 million people were diagnosed with the condition. Moreover, prediabetes was also recognized as a period where individuals had high glucose levels without meeting the other criteria for diabetes yet [3]. Determination of glucose levels in the blood has become one of the regularly tested analytes in diagnostic laboratories [4]. The capability of blood sample to undergo in vitro glycolysis must be given attention to allow accurate glucose measurement in the

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laboratory [5]. The use of blood collection tubes containing various anticoagulants such as lithium heparin, sodium fluoride, potassium oxalate, sodium citrate, Ethylenediamine Tetraacetic Acid (EDTA) are among the few that had been utilized over the years to prevent glycolysis [6].

In the DM glycemic regulation, overnight fasting plasma glucose determination is a core component. Unfortunately, there is no known accepted uniform definition of a fasting state; the World Health Organization (WHO) recommends 8-14 hours of fasting while the American Diabetes Association defines fasting as no caloric consumption for at least eight hours [7].

Blood from the patient is drawn to a test tube containing citrate buffer, a glycolysis inhibitor. It is instantly placed in a container with ice to minimize glycolysis within 30 minutes from the time of collection [4]. It was determined that the blood collection procedure for glucose is very crucial to attain truthful values. The characteristics of glucose to be easily consumed by blood cells and further broken down by glycolysis when left in room temperature without a preservative eventually results to a drastic decrease in measured glucose values [8].

METHODOLOGY

Various laboratory methods are currently used as a standard to diagnose diabetes and measure glucose values in the blood such as Fasting Blood Glucose (FBG), 2-hour plasma glucose, Oral Glucose Tolerance Test (OGTT) and Hemoglobin A1C (HBA1C) [9]. According to Garg, HbA1C remains the gold standard test for the assessment of glycemic control because it reflects the glucose values in the previous 3-month period [10].

Studies show that glucose is best measured for the diagnosis of diabetes when plasma is examined from venous blood [11]. Furthermore, other laboratory tests such as Capillary Blood Glucose (CBG), random plasma glucose and fructosamine can also be utilized for measurement but must be accompanied by other standard tests to confirm diagnosis.

The review encapsulates the strengths and weaknesses on the use of serum and plasma for glucose determination, alongside with other blood samples used for laboratory analysis. Moreover, the results of different researches may serve as a valuable reference for researchers, clinicians and other professionals in the healthcare field that may associate the use of serum and plasma in their own respective specializations.

Objectives of the study

General objective: To compare the result obtained from serum and plasma for glucose determination. The following are the specific objectives:

1. To determine the most commonly used specimen in the measurement of blood glucose
2. To determine the common procedure in the measurement of blood glucose level
3. To establish the most accurate specimen in the determination of blood glucose level

This systematic review paper includes publications on the use of serum and plasma for glucose determination in the past decade. It concentrates on the similarities, advantages and convenience of one sample over the other with different types of tests currently conducted in the laboratory. A total of twenty-seven (27) studies were used out of the initial one hundred thirty-six (136) studies screened. It is not limited to the sample population or participant category with or without pre-existing health conditions that have undergone testing. The data analysis is comprised of studies performed from 2011 to 2022. Utilization of electronic databases from Google Scholar, Proquest, EBSCO, PubMed, MEDLINE, Science Direct and other open access journals to generate significant findings is applied on this study. Moreover, published articles with title inclusions of serum and plasma for glucose were specifically screened. Only full-text articles accessible online are included in the study.

RESULTS AND DISCUSSION

Glucose and diabetes mellitus

Diabetes mellitus is renowned to be one of the earliest illnesses affecting humans as described around 3000 years ago in an Egyptian scripture. The distinction between DM type 1 and 2 was defined initially in 1936 while the evident identification of DM type 2 was made in 1988 as a constituent of a metabolic syndrome [12]. In 2012, there is an establishment of prediabetic stage where individuals classified are those with high normal glucose levels but did not meet the other parameters consistent with diabetic patients. However, it is regarded as a risk factor for diabetes and other significant conditions such as cardiac diseases. It was also observed that there is a higher rate to develop other complications for those who were undiagnosed with prediabetes and diabetes. This condition may likewise progress into kidney failure, amputation of lower limbs and blindness in adults [3]. Based on statistics in 2014, 9% of the worldwide population were affected with diabetes. Seventy-seven (77) percent were residing in low- and middle-class countries. Gestational diabetes was also known for affecting more than 21 million mothers and newborns as well [13]. Additionally in 2020, the World Health Organization (WHO) reported that DM was part of the top 10 diseases leading to death worldwide. Characterized as a chronic metabolic disease due to insufficiency of insulin production, DM can be further classified into three (3) types, namely: Type 1 Diabetes Mellitus, Type 2 Diabetes Mellitus and Gestational Diabetes [14]. According to the American Diabetes Association (ADA), type 1 Diabetes is caused by autoimmune beta cell destruction resulting to absolute insulin deficiency [9]. Type 2 Diabetes is usually caused by gradual loss of adequate beta cell insulin secretion. Gestational Diabetes is detected in the second and/or third trimester of pregnancy that was not evidently overt diabetes proceeding to gestation [9]. Considered as one of the most frequent metabolic conditions globally, type 2 DM is mainly due to the combination of malfunctioning insulin excretion by pancreatic beta cells and the failure of the individual's insulin-reliant tissues to react to insulin. The secretion of insulin and its accurate activity must meet the individual's metabolic requirement. Therefore, the regulation of molecular processes implicated in synthesis and release of insulin with its specific response in tissues must be monitored. Hence, a defect in any of the involved mechanisms could lead to metabolic imbalance that leads to the pathogenesis of type 2 DM [15]. Since the pathophysiology of type 2 DM remains complicated, it has been observed in previous decades that the disturbance of circadian rhythm owing to changes in body clock increases the progress of the condition and the probability to develop obesity at the same time. This rhythmicity in the body is widely affected by various areas of physiology including movement/rest, feeding/fasting and release of hormones. Additionally, glucose is known

to be affected by the mechanisms of the biological rhythms most especially in homeostasis [16].

In 2022, thirty-seven (37) million cases were recorded for diabetes in the US, while there is an estimated 422 million people affected globally between low- and middle-class countries. With these data, the prevalence of diabetes continuously rises attributing to around 1.5 million casualties annually. The reported figures of diabetes in the past three (3) decades multiplied evidently in huge numbers.

Misdiagnosis between type 1 and type 2 diabetes are common. The old concept that type 1 diabetes mellitus arises only in children while the type 2 arises only in adult individual is already inaccurate since both types can occur in both ages [9]. Type 1 in children is usually with polyuria, polydipsia, and some Demonstrates Ketoacidosis (DKA). Type 1 in adult is more variable. Rarely, type 2 diabetes patient may demonstrate DKA5.

As mentioned by the ADA, diabetes mellitus may be detected using plasma glucose levels. This plasma glucose may be values of Fasting Blood Glucose (FPG), 2-hour plasma glucose during a 75-gram oral glucose tolerance test or Hemoglobin A1C [9]. Table 1 shows the values of the different tests to diagnose diabetes mellitus.

Serum data demonstrates that the recommended clotting time of thirty (30) minutes was enough to lead to considerable glucose level changes primarily due to prolonged contact with cells [5]. As mentioned by Kim, et al. the tube should be placed in ice-slurry water to minimize glycolysis [4]. This was likewise the same for a previous study done by Turchiano in 2013 where samples for glucose testing be immediately placed in ice slurry or the rapid separation of plasma from blood cells must be done for more accurate glucose determination [17]. Gel separator can offer a physical wall between the cells and the serum or lithium heparin to prevent glycolysis [5]. In the study of Bhargava, et al. they found out that serum can be used in blood glucose measurement instead of using NaF since there is only minimal difference between the two specimens. This will be reached if the serum will be separated within 2 hours of collection [18]. According to the study of Almomin, et al. serum is free from any blood cells; hence, it is suitable for the measurement of blood glucose levels [7]. However, in the study of Kim, et al. plasma glucose levels are increased as compared to the serum glucose levels [1]. This claim is also proven in other studies that glucose concentrations are slightly increased in plasma rather in the serum [11]. Due to this, standard serum tubes are not recommended for accurate glucose determination [5].

Specimen for fasting plasma glucose should be collected in the morning instead in the afternoon since glucose levels demonstrate diurnal variations [1]. According to Sacks, et al. plasma glucose levels are higher in the morning than in the afternoon [11].

The rate of decrease in glucose level determination in the first hour after specimen collection are almost identical for tubes with or without NaF, and the decrease of glucose in NaF continues for up to four (4) hours. After which the glucose level in NaF remains stable for 72 hours at room temperature [11]. Additionally, as mentioned by Dimenski, et al. both EDTA and Sodium Fluoride (NaF)/Potassium Oxalate (KOx) tubes demonstrated a significant decrease in glucose level determination proving the results to inaccurate glucose determination [5].

According to the study performed by Bonetti, et al. the mixture of NaF and citrate buffer in Sarsted GlucoEXACT tube is the only blood collection tube that can preserve blood glucose concentration stored at room temperature for up to four (4) hours [4]. As mentioned by Sacks, the use of citrate buffer inhibits glycolysis way better as compared to NaF [11]. If individual tubes such as sodium fluoride, lithium-heparin or Sarsted tubes will be used alone, this tube will miss the analytical goal for the desired bias [4]. The addition of a glycolytic inhibitor like the NaF in tubes with Na2EDTA resulted to a decrease of mean blood glucose level by 3.6% after 2 hours and 9.4% after 24 hours for glucose hexokinase test and 4.1% after 2 hours and 9.7% after 24 hours for the glucose oxidase test [8]. Likewise, the addition of NaF with K2 Oxalate resulted to a decrease of 3.8% for the glucose hexokinase test and 3.3% for the glucose oxidase test after 24 hours at room temperature [8].

In the study of Fobker, the Terumo VENOSAFE Glycemia tubes that contained NaF/citrate buffer/Na2EDTA resulted to a decrease of 0.3% after 2 hours and 1.2% after 24 hours. Thus, the Terumo VENOSAFE Glycemia tubes and Sarstedt S-Monovette GlucoEXACT blood collection tube systems are appropriate for transporting venous blood samples to the clinical laboratory for glucose testing within twenty-four (24) hours preventing significant glycolysis [8].

Though it was also demonstrated that there is similarity between the separation of serum and plasma from red cells within half an hour, it has also been noted that the timing of processing the samples due to various factors such as transport of specimen from collection area to the laboratory may also take a significant amount of time which primarily affects the glucose level results [19].

In the study by Montagnana, the use of three (3) additives such as NaF, citrate and/or EDTA was demonstrated and appeared better in stabilizing blood glucose levels compared to the traditional mixture of NaF and/or KOx10. The mixtures of the three additives act on the early (hexokinase) and late (Enolase) stage in the glycolytic pathway [20].

A study of Frank demonstrated that the use of fluoride in plasma glucose determination showed the least decrease while EDTA plasma showed the most decrease after eight (8) hours at room temperature [6]. Additionally, the determined fluoride plasma glucose correlated linearly with serum blood glucose.

Table 1: Criteria for the diagnosis of diabetes mellitus.

	Fasting blood glucose level	2-hour plasma glucose (75-g OGTT)	Hemoglobin A1C	Random plasma glucose	Remarks
American Diabetes Association (ADA)	≥ 126 mg/dL (7.0 mmol/L)	≥ 200 mg/dL (11.1 mmol/L)	≥ 6.5% (49 mmol/L)	≥ 200 mg/dL (11.1 mmol/L)	Fasting is defined as no caloric intake for at least eight (8) hours

In locations without readily access to laboratory equipment that would determine a more accurate glucose level, Point-Of-Care Testing (POCT) is widely used as a diagnostic test due to its efficiency in results. This could immediately produce results that would aid in the diagnosis and treatment to reduce the patient anxiety by the decrease in blood sample collection and by providing faster turnaround time for clinicians. Although the use of plasma or serum is extensively used in the central laboratory, glucose levels from POCT remains consistently dependable when done in patients' bedside. This highly facilitates glycemic fluctuations in hospitalized patients since results are found stable that could provide immediate response and therapy [21]. The use of glucose meters for blood sugar monitoring is recommended for patients treated with insulin for diabetes. This is highly useful for the general population, coupled with the different laboratory tests used in conjunction with other established glucose tests [11].

Another laboratory test that aids in the evaluation of blood sugar is known as the hemoglobin A1C (HbA1C). It is highly standardized which demonstrates the ability to obtain low intra-individual variation at any time without requiring the patient to prepare oneself prior to blood collection. Reasonably, HbA1c is stable at room temperature after specimen acquisition [22].

Methods and principles on glucose analysis

Table 2 summarizes the different procedures and specimen utilized in the different studies included in this research. Most glucose measurement is exclusively measured using enzymatic procedures. As mentioned by Sacks, the use of glucose hexokinase and oxidase methods generally produces the same result [11].

Point-Of-Care (POC) glucose measurements use capillary or arterial blood as specimens. It was developed for the management of outpatient with diabetes [23]. POC is based on enzymatic reactions.

Another innovation was studied as an alternative to common invasive procedures of blood collection for glucose testing that would eliminate the need to continuously prick the skin for monitoring purposes. This would utilize electrochemical and electromagnetic non-invasive procedures. However, further studies should still support the possibility of including the method for routine usage of patients [14].

Additionally, Zhang conducted a study using Isotope Dilution Liquid Chromatography-Tandem Mass Spectrometry (UD LC-MS/MS) to measure blood glucose. This procedure is said to be simple, accurate and can serve as a candidate Reference Measurement Procedure (RMP) [24].

Furthermore, most of the studies preferred the use of plasma instead of using serum specimen in the measurement of blood glucose.

Table 2: Summary of glucose determination in the previous study.

Study	Procedure	Reference Sample	Best specimen	Remarks
Almomin, et al. (2022)	COBAS, Integra 400 plus	-	Serum	No significant difference in five (5) to six (6) hours fasting and eight (8) hours fasting for individuals with or without having type 2 diabetes mellitus
Bhargava, et al. (2018)	-	-	Serum	There is no need to use sodium fluoride as an anticoagulant if analysis will be achieved within two (2) hours
Bonetti, et al. (2016)	Hexokinase method	-	Mixture of sodium fluoride and citrate buffer in Sarsted GlucoEXACT tube	Preserve blood glucose concentration stored at room temperature for up to four (4) hours
Dimeski, et al. (2014)	Glucose oxidase method	Lithium Heparin	Glucomedics	After two (2) and four (4) hours, only the glucomedics anticoagulant combination maintained the desired stability
Eerdekens, et al. (2020)	Point-of-Care Test (POC)	-	Capillary/ arterial blood	Utilized to monitor outpatients with diabetes
Fobker (2014)	Glucose hexokinase and oxidase method	-	Terumo VENOSAFE Glycemia and Sarstedt S-Monovette GlucoEXACT collections tube	Suitable for the shipping of blood samples to the clinical laboratory within 24 hours at room temperature preventing significant glycolysis
Frank, et al. (2012)	Glucose oxidase method	-	Serum	Clearer serum was observed due to lesser proteins present in the samples after an 8-hour preservation and comparison with plasma after the delay in laboratory processing
Kim (2016)	-		Plasma	Upon analyzing plasma glucose following centrifugation after fifteen (15) minutes of collection, 20.9% of participants had been diagnosed with diabetes
Montagnana, et al. (2017)	-	Citrated-NaF-EDTA	Plasma	Critical issues include the need to accurately fill the collection tube and urgent mixing of blood and additives
Pant, et al. (2021)	-	-	Serum	Serum is advantageous once centrifugation is accomplished within half an hour
Sacks, et al. (2011)	Glucose hexokinase and oxidase method	-	Plasma	Tubes with sodium fluoride should not be used as a sole basis on the glycolysis inhibition

Turchiano, et al. (2013)	-	Clot activator	Serum	Serum samples with clot activator must be centrifuged within twenty (20) minutes of blood extraction
Xue, et al. (2022)	Electrochemical and electromagnetic non-invasive blood glucose monitoring	-	-	The use of scientific sensor systems provides a promising alternative to invasive glucose monitoring
Zhang, et al. (2016)	Isotope dilution liquid chromatography-tandem mass spectrometry	-	Serum	Technique is simple, accurate, and can be used as s reference measurement procedure (RMP)

Laboratory errors

According to Dimeski, et al. the decrease in the citrate plasma decrease was caused by the dilutional effect from the liquid anticoagulant. Citrate and glucomedics tubes are the only tubes used for glucose level determination that is affected by dilutional effect if underfilled. The primary reason for this is that the mentioned tubes contain liquid anticoagulants [5]. It is very important to fill the tubes up to the correct fill volumes. When the dilutional effect was corrected, the result is comparable with the standard lithium heparin values [5].

Furthermore, Sarstedt tube is also affected by dilution factor. Thus, may result to inaccurate glucose result [8]. The decline in the glucose serum tube was due to the 30-minute contact of the serum with the red cells, leading to glucose utilization [5].

On the other hand, POC glucose determination is sensitive to pH fluctuations and may be affected by drugs such as ascorbic acid, acetaminophen, dopamine and mannitol [23]. Inaccurate glucose determination can be a result of hemolysis due to vigorous shaking [8]. Moreover, hemolysis has a greater risk in Terumo products as compared to GlucoEXACT tube. Hemolysis affects the glucose oxidase and hexokinase methodologies [8].

Additionally, plasma still contains platelets and thus, will affect the glucose determination [6]. Platelets consume glucose over time [7]. Plasma contains more proteins as compared to the serum. These proteins may occasionally interfere in the glucose determination [6].

Test sensitivity/stability

In the stability test performed by Dimeski, et al. only the citrated plasma and Glucomedics plasma were stable and were less than the analytically allowable error of <2.5% [5]. After two (2) and four (4) hours, only the Glucomedics anticoagulant combination maintained the desired stability. It prevented glycolysis in the mentioned time [5].

CONCLUSION

Based on the findings of the study, the most commonly used specimen in blood glucose determination is plasma. The use of plasma is preferred over serum for glucose determination, particularly with the immediate separation of the plasma from red blood cells through centrifugation within thirty (30) minutes of specimen collection. Majority of the reviewed studies visibly showed similar results. Various additives had been used over the years to aid in the most ideal for glucose analysis such as the gel separator for serum, sodium fluoride/potassium oxalate, citrate, lithium heparin and EDTA. Moreover, the most common procedure to measure blood glucose is the enzyme technique either the glucose oxidase or the hexokinase method. Both procedures are highly sensitive and specific tests. However, it is highly recommended that regardless of the additive, the timing of specimen processing must be considered to ensure the release of accurate results in the laboratory.

CONFLICT OF INTEREST

None of the authors have any relevant conflict of interest to disclose.

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