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Glucose Estimation: The most suitable blood collection tube for glucose estimation

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Abstract
Introduction: *Patient with high blood sugar depend on accurate diagnosis glucose estimation for correct diagnosis, therefore antiglycolysis are used in collection tube to stabilize the glucose level in the tube.*
Methods: *collecting articles from different databases that related to stability of glucose concentration in blood collection tubes. This study aimed to find out what is the best tube for glucose estimation.*
Conclusion: *The mixture tubes (venosafe, gluco EXACT and Glucomedic) show the best results for glucose stability and the second tube is the citrate tube.*
Keywords: *glucose estimation, antiglcolysis, anticoagulant, blood collection tube, NaF/KOx, citrate, Glucomedics.*

Introduction

Glucose estimation is one of the tests that are done frequently in laboratories. Patients with high blood glucose specifically pregnant female depend on accurate blood glucose measurements to get the proper diagnosis of diabetes and screening. After the sample collection glycolysis may occur in the tube, so the breakdown of glucose should be prevented. This project is to determine the most suitable collection tube for glucose estimation.

Literature Review

According to Medical news today (MNT), hyperglycemia for long period of time referred to diabetes mellitus, it is a human metabolic disorder which has high blood glucose concentration. Diabetes caused by either inadequate insulin production or because the cells in human body unable to response to insulin (Medical News Today, 2018).Notable, the most recent statistics from the World Health Organization (WHO) confirm that, the number of diabetic patients has multiply four times since 1980 until 2014(Roglic, 2016) and the percentage of prevalence of hyperglycemia in 2014 was 8.5% within individuals aged 18 years or old (Roglic, 2016). Moreover, in America country the number of population who are reported to have disease in

2015 was 30.3 million, approximately 9.4% of the population and 1.25 million American children and adults have type 1 diabetes (American Diabetes Association, 2018).

According to statistics (Moh.gov.om, 2018), diabetes disorder spreads between Omanis people around 12-15% from the total percentage of population in Oman. This percentage increased in elders who aged 60 years old or above.

Over time the criteria of diagnosis and controlling diabetes are updated and developed. According to WHO, diabetes recently can be diagnosed if one of this four major criteria is found: hemoglobin A1c -HbA1c- (>6.5%) or ≤48 mmol/mol, a plasma glucose 11.1 mmol/L (200mg/dL) during OGTT test, a fasting plasma glucose 7.0 mmol/L (126mg/dL), or in pregnant women the diabetes should be diagnosed if the woman has fasting plasma glucose level of 5.6mmol/l or more or a 2-hour plasma glucose level of 7.8mmol/l or more(Diabetes.co.uk,2018). The measurement of FPG consider as the most perseverance worldwide because consider as the most least expensive and accessible approach test, especially for low-income countries (G. Lippi et al.,2012).

The measurement of Fasting plasma glucose have unexpected pre analytical issue, for example reduction of glucose level in un-centrifuged blood tubes, due to continues metabolism process because of glycolysis is not immediately stopped or delay separated blood cells from plasma. To troubleshoot this issue, which at the end effect final diagnosis of diabetes due to decrease glucose level, there are some suggestion of solutions (L.M. Mikesh et al.,2008).

The first solution to prevent glycolysis is by stabilizing glucose concentration by adding sodium fluoride (NaF) combination with the anticoagulant potassium oxalate (KOx). The fluoride can inhibit the activity of enzyme called enolase, which is important for glycolysis pathway. The inhibition of enolase enzyme is not immediately stop the glycolysis process, which mean ongoing the metabolize glucose still occur (M.J. Peake et al.,2013). As for this important finding, this clearly explains why inhibition of glycolysis by fluoride can take fourhours to complete stop glycolysis, that can reduce glucose concentration, especially if the specimens are stored at room temperature. notable, this problem can prevented by rapid centrifugation the tube within 10 minutes or stored in ice bage (D.B. Sacks et al.,2011).

On another hand, there are another solution to stabilized the glucose concentration in blood collection tubes which inhibit hexokinase enzyme, this enzyme located in the initial steps of the glycolytic pathway (Fig. 1), hexokinase enzyme usually active at pH 5.9 or higher .However, citrate buffer able to stop glucose metabolism at a much earlier step of glycolysis, and can be more effective by adding fluoride (M. Montagnana et al.,2017).

Manufactures generate a new type of blood tubes has been contained several types additives. One type of additives is citrate which act to decrease the pH of blood and thus prevent activation of the hexokinase enzyme, the other one is NaF which is directly prevent the enolase enzyme , and the third one is EDTA which is prevent coagulation of the blood. Therefore, the manufacturers develop these blood tubes with this new mixture in different physical states and with different size and tubes volume (S. Pasqualetti et al.,2016).

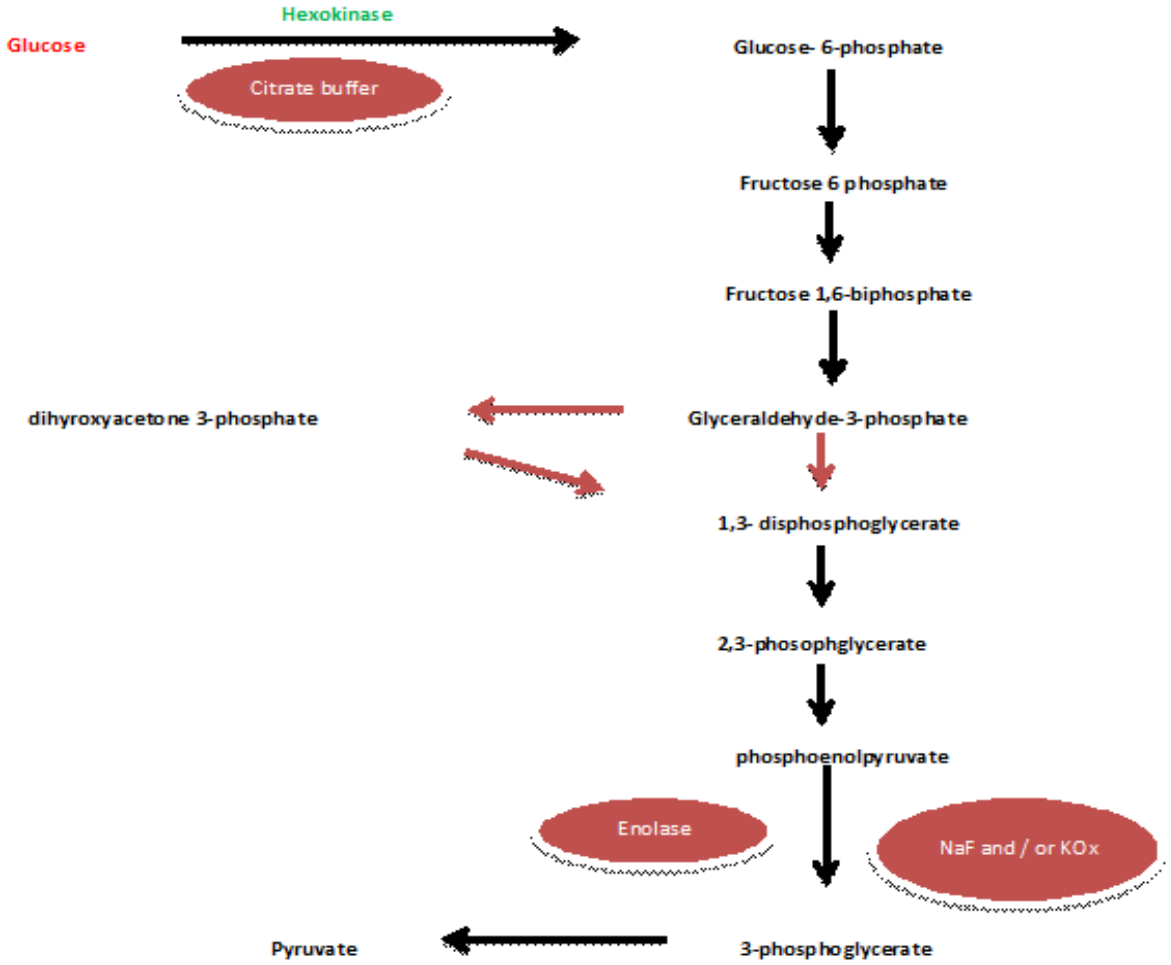

Chart 1: Glycolysis and its inhibitors. KOx, potassium oxalate; NaF, sodium fluoride

There are another ways for preventing glycolysis. For example, immediate separation of plasma from cells and lowering the temperature of the tube to 4 c immediately after collection but, this way cannot be practiced in the lab because it take time to reach room temperature that will waste technician time.

The comparative studies between citrate, sodium fluoride and other collection tubes started from a long time ago. The first study has been done in 1941 by Bueding and Goldfarb found that sodium fluoride cannot save amount of blood sugar in fluoride tubes. Then other studies come to give evidence based on that scientific information. For example a study done by Lawrence, et al in 2008 and found that sodium fluoride can stops the growth of bacteria that consume glucose in tube. Other study done found that when citrate tube used rather than sodium fluoride tube, the researchers found there is an increase in glucose value above the limit for diabetes (Ridefelt et al., 2014).

The aim of this systematic review is evaluate different anticoagulant tube to found suitable blood collection tube for glucose estimation. The objectives of this study are to find articles reported about stability of glucose concentration in blood collection tubes, to exclude articles that did not match with specific criteria and to include articles that match that specific criteria and to extract results from that articles to find suitable blood collection tube for glucose measurement.

Method

The first step was searching about topic question related to clinical chemistry department as the role of the EBP course. There was several topic questions which are related to clinical chemistry laboratory. For instance, troponin as a myocardial infarction marker, the impact of emotional state

on glands secretion, and the influence of anticoagulant substances on glucose test result. On 18/Dec /2017 final decision was to choose the most suitable anticoagulant tube for glucose estimation as a topic question after we met and got approval from our supervisor.

After specified the question, the group started to search about articles associated with this topic. Firstly, the search was initiated from library catalog on nurses building to find chemistry books or journals that contains some information about an anticoagulation tubes and glucose estimation. Subsequently, we visited library of knowledge in Al-Qurum to read local and global healthy newspaper and journals to find articles related to our topic .Also the search was from Google scholar which is professional websites in academic research. We started search by using Google Scholar to look for studies related to specific terms including, blood collection tubes, and glucose estimation which is the key words for our research question .There are several articles about the blood collection tube that measure plasma glucose. Moreover, from magazines database also we found numerous number of articles. There were a lot of articles as well as these articles need to be filtrated in steps depend on criteria as following:

Exclusion Criteria

There are exclusion criteria to remove studies that require there to exclude, For example, we excluded studies which not use English language or not published peer-reviewed journals as well as we excluded long articles with difficult English terms because that will affect our outcome. Also, we exclude articles which are more than five years old or close to our topic but they do not discuss what we decide to search about it or conflict with other articles. Besides that, we exclude studies that did not take informed consent from the participant.

Inclusion criteria

There are some criteria for inclusion articles in our study which is remove the impact of specific confounding variables. For example, full text of the article must use English language and articles should published in peer-reviewed journals. The studies can be from any geographical location. Moreover qualitative and quantitative studies can be included. However, any study it is important to address ethical issues and select studies who obtained their information lawfully, and reported the data accurately. It is important to take informed consent from participant for this reason we include researches that mentioned they got informed consent from their participants.

Table 1: Present main inclusion and exclusion criteria

Criteria	Description of criteria
Exclusion criteria	studies which not use English language
	not published peer-reviewed journals
	articles which are more than five years old.
	long articles with difficult English terms because that will affect our outcome
Inclusion criteria	full text of the article must use English language
	The studies can be from any geographical location
	In studies it is important to take informed consent from the participant

After filtrating the articles we got 4 articles related to our topic and propertied with our criteria. Thereafter, we identified the PICO for each article which is consists of 4 of concepts. Writing a PICO question make the search process easier and help identification best evidence. PICO stand for (P) is normal and diabetic patient, while (I) is unstable glucose concentration in collection tube, no control, and (O) stable glucose concentration in collection tube. However, the aim of this project is to identify the most suitable collection tube for glucose estimation

Strength and Weakness

The strengths of this study are given a summary of four medical articles that talk about stability of glucose concentration in different blood collection tubes and explain them in easy way. Moreover, this study uses a clear method that avoids bias and generate respectable conclusion. Finally, generation of hypothesis about new blood

collection tubes that stop the glycolysis pathway in different stages.

The weaknesses of this study, difficult to find articles that studied the stability of glucose in the blood collection tubes because kind of studies has been little studied in recent years. Furthermore, the p value is not mentioned in this study because the articles used in this study did not mention their p value. Lastly, only four articles were used in this systematic review that only covers four countries.

Related information

In general, several hidden factors can interpreted making accurate result in glucose measurement using anticoagulant tubes including: hemolysis condition in medical cases .For example, gram positive bacteria like Staphylococcus

Enterococcus and Streptococcus can cause. Specimen collected from patients with bacterial infection that able to beak blood cells can impact the accurate of glucose result hemolysis (Dhaliwal G, 2018).

There are some pathogenic condition that can increase blood cell in specimen that lead to consume glucose more rapid resulting in glycolysis including kidney disease, low oxygen levels and other problems other problems (Mayo Clinic, 2018). Furthermore, albumin and goblin level effect PH level in blood. Notable, physical condition in the lab are important for blood enzymes that appear in temperature of the bench and refrigerator store (Worthington-biochem.com, 2018).

In addition, in case of using expired tubes or low cost tubes will disrupted the result, especially in low income countries (Lippi et al.,2012). Also, Carless of staff while processing the test like in case of keeping un-centrifuged tubes more than expected time can influence the glucose level in the tube .On other hand, diabetic statistic and studies results strongly effected with accuracy of tubes and their results.

Result

The diagnosis, the treatment and the assessment of the risk of developing diabetes all require accurate measurement of glucose .The main important of this study is to determine which is the best anticoagulant or antiglycolysis tube that can preserve the level of glucose should be used for glucose estimation to produce accurate results. The following table compare the common characteristics of collection tubes used in glucose estimation:

Table 2: Comparison between studies that evaluating the influence of different preservation on plasma glucose estimation.

Authors	Samples	Sample Matrix	0–1h	0–2h	0–3h	0-4h	0–24 h
(Dimeski et al., 2014)	Samples from healthy volunteers (n 42)	NaF/KOx,Citrate buffer, EDTA (Glucomedics)	-0.8%	-0.9%		-1.5%	
		Sodium Fluoride/KOx plasma	-5.4%	-7.0%		-8.5%	
		Lithium-heparin plasma	-4.2%	-10.5%		-18.5%	
		Serum	-3.1%	-5.5%		-10.1%	
		EDTA	-3.1%	-7.1%		-13.0%	
		Citrate	-2.4%	-3.8%		-10.1%	
(Fobker ., 2014)	Samples from healthy volunteers (n 60)	Sodium fluorid/Citrate buffer/sodium EDTA (Venosafe)					-2.2%
		Sodium fluorid/Citrate buffer (GlucoEXACT)					+0.8%
		Sodium Fluoride/KOx plasma					-3.8%
		K2EDTA					-85.2%
		Sodium fluorid/ sodium heparin					+0.1%
		Sodium fluorid/ sodium EDTA					-9.4%

(Amegashie et al.,2015)	Sample from 75 diabetic patients and 25 healthy patients (n 100)	Fluoride- oxalate	-6.5%	-13%			
		Serum	-8.9%	-16.7%			
(del Pino et al., 2013)	Routine laboratory samples (n 79)	Sodium fluorid/Citrate buffer/sodium EDTA (Venosafe)		+0.2%	-0.1%		
		SodiumFluoride/KOx plasma		-3.8%	-4.6%		

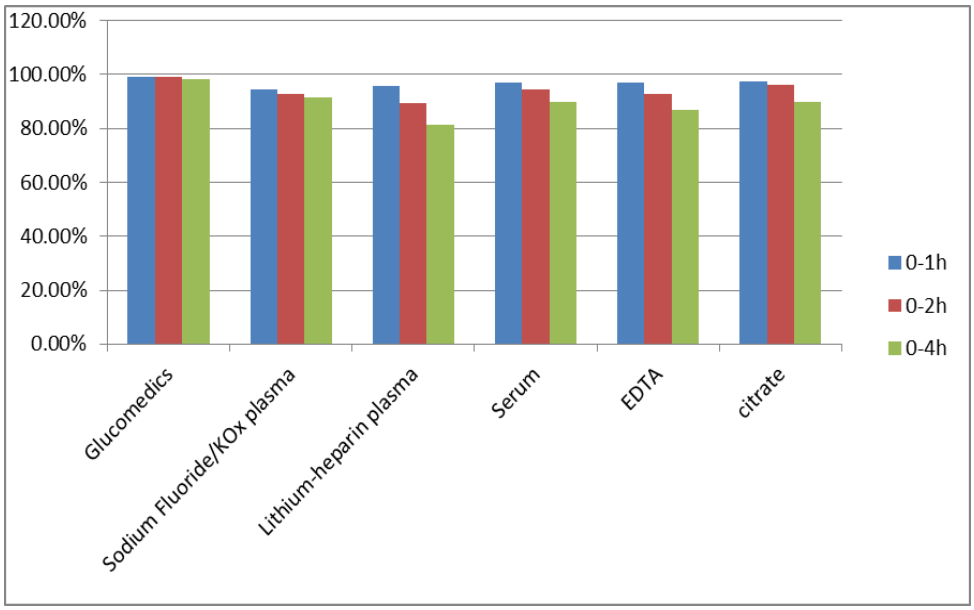

Figure 1: Stability of glucose in the numerous tubes when stored at RT for 1, 2 and 4 h before centrifugation. Shows percentage decrease in glucose con-centration (Dimeski et al., 2014)

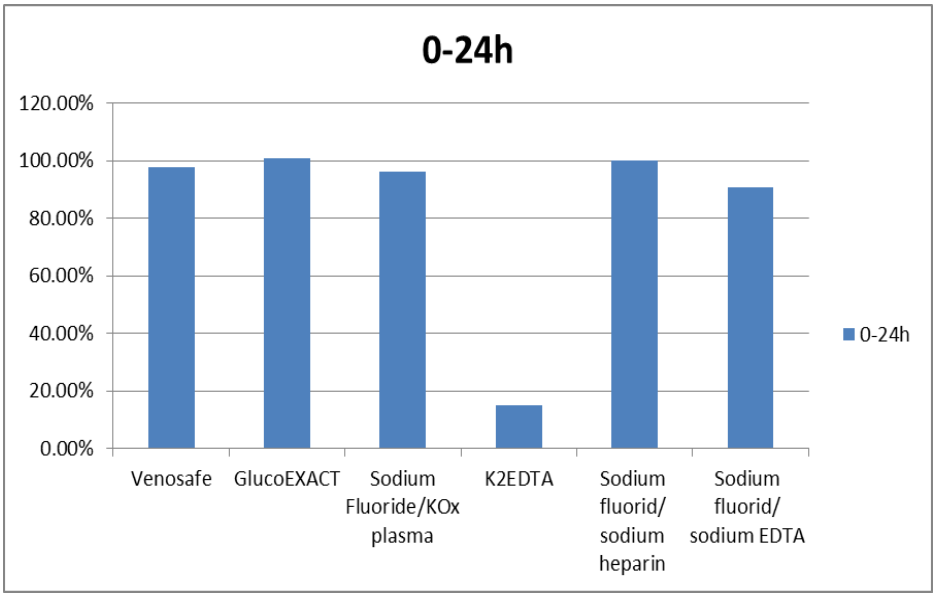

Figure 2: Glucose stability in plasma from blood that was collected with different glycolysis inhibitors and anticoagulants, and stored at room temperature for 24 hours (Fobker., 2014).

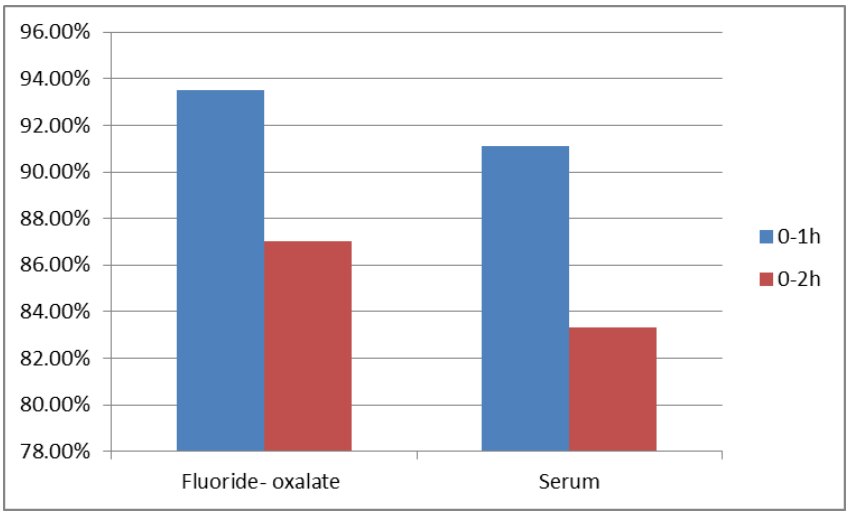

Figure 3: Percentage decrease in glucose con-centration within Sodium Fluoride/KOx plasma and serum tubes at different time interval (Amegashie et al.,2015).

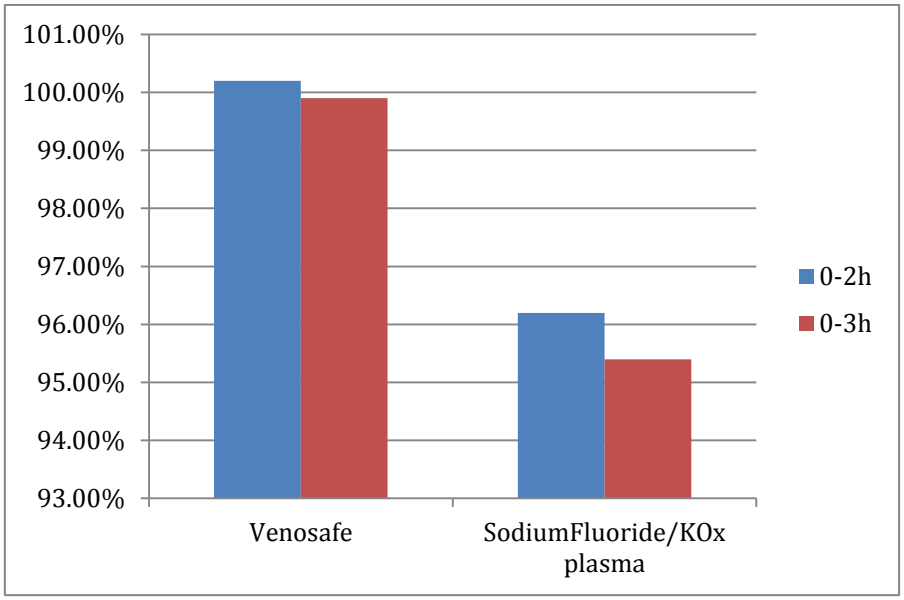

Figure 4: Percentage decrease in glucose concentration within fluoride-oxalate and venosafe (Sodium fluorid/ Citrate buffer/ sodium EDTA) at different time interval del (del Pino et al., 2013).

Evaluating of collecting tubes according to selected studies

To prevent glycolysis, substance needs to be added to stop hexokinase enzyme or enolase enzyme. A lot of studies suggested citrate additive because citrate can inhibit hexokinase enzyme which help to save amount of glucose in un-centrifuged tubes that kept at room temperature. The principle of citrate is lowering the PH (acidification) of the blood; therefore, the hexokinase enzyme will be inactive because the hexokinase is active at PH 5.9 or more (Gupta et al., 2016).

Depend on previous information, many studies done to support using of citrate as anti-glycolysis. In 2014, Dimeski et al found that level of glucose in citrate tube dropped by 2.4% in first hour, 3.8% in second hour and 10.1% in fourth hour from blood collection (Figure1). Their result was the citrate tube is the second chosen tube after mixture tube that called Glucomedics that contain citrate with sodium fluoride, potassium oxalate, and EDTA. When Glucomedics tube kept at room temperature the amount of glucose fell by 0.8% in 1h, 0,9% in 2h and 1.5% in 4h after blood collection refer to figure1 and table2.

In other study, done by fobker in 2014, found that the mixture tube that contain sodium fluoride with citrate buffer (called venosafe) is suitable tube for glucose estimation because the concentration of sugar in the tube decreased by 2.2% in 24h after collection time as shown in table2 and figure 2. Del pino et al in 2013, they support the result of fobker. Their founding was the concentration of glucose dropped by 0.1% in 2h after collection time (figure4 and table2). The last tube that used in the study of (fobker ., 2014) containing citrate is gluco EXACT tube that composed of sodium fluoride with citrate buffer that showed slightly increase around 0.8% in 24h at room temperature (fig2 and table2).

Sodium fluoride stops the glucose pathway by inhibiting enolase enzyme that act in the end of the pathway of glucose (Gupta and Kaur, 2013), therefore, the glucose concentration will decrease in the first 3 to 4h after blood is collected. Dimeski et al, in 2014 found that there is 8.5% drop in an amount of glucose in NaF/KOx tube that kept at room temperature for 4 hours from phlebotomy time, refer to figur1 and table2. Also, other study proved the result of Dimeski et al that done by Del pino et al in2013 found that the NaF/KOx tube shown 3.8% in 2h and 4.6% in 3h of decreasing level of glucose, as shown in figure4 and table2.

Fobker in 2014reported that there is slightly fall of glucose concentration when the blood filled in NaF/KOx tube that stored at room temperature shown decrease of glucose amount around 3.8% of glucose level in 24h because sodium fluoride can lead to hemolysis (figure2 and table2). However, it decreased by 6.5% in first hour and 13% in 2h from collection in the study of Amegashie et al., 2015, refer to figure3 and table2.

Ethylenediaminetetraacetate (EDTA) act as an anticoagulant and does not stop breakdown of glucose in the tube during the duration between collection and analyzing of the sample. A study done by Dimeski et al in 2014decomented that the level of glucose in EDTA tube decreased by 3.1% in a first hour, 7.1%in 2h and 13.1% in 4 hours after phlebotomy time and kept at room temperature (fig1 and table2). Fobker in 204 reported that when blood collected in a tube containing potassium EDTA and stored at room temperature for 24h, the concentration of glucose will drop by 85.2% and that is significant decrease (fig2 and table2).

Once a sample is collected in a serum tube needs to be clotted before centrifugation that process takes around 30 minutes, during that time the cells will contact with the serum that lead to consumption of glucose that caused significant drop in glucose concentration. Gel separator tube provides a wall between serum and the cells that what will inhibit glycolysis. Dimeski et al in 2014 found that the dropping of glucose amount in first hour was 3.1%, in second hour was 5.5% and in fourth hour was 10.1% post phlebotomy time and kept at room temperature as mention in figure1 and table2. Amegashie et al in 2015reported that the falling of glucose level in serum tube was 8.9% in 1h, 16.7 in 2 hour refer to figure3 and table2.

Dimeski et al found that the use of lithium heparin with gel separator can lower the level of glucose by 4.2% in 1h, 10.5% in 2h and -18.5% in 4h when it is stored at room temperature after blood collection (fig1 and table2).

Summary of each study

Dimeski et al, study the stability of glucose level in different anticoagulant and antiglycolysis tubes (Glucomedics, Sodium Fluoride/KOx, plasma Lithium-heparin, plasma Serum, EDTA, and citrate) and found that Glucomedics (NaF/KOx, Citrate buffer, EDTA) is the most suitable tube for glucose estimation among that tubes. The second best tube is citrate tube.

Fobrek found that venosafe (Sodium fluorid/ Citrate buffer/sodium EDTA) and Gluco EXACT (Sodium fluorid/Citrate buffer) have a major advantage in preserving glucose concentration compared with Sodium Fluoride/KOx plasma, K2EDTA, Sodium fluorid/ sodium heparin and Sodium fluorid/ sodium EDTA.

Amegashie et al. study compared between Sodium fluorid/ Potassium Oxalat and serum tube in preserving glucose amount in room temperature and found NaF/KOx tube is better than serum tube.

Venosafe is the suitable tube to prevent glycolysis compared with SodiumFluoride/KOx plasma according to study of del Pino et al.

Discussion

This study aimed to find out what is the best tube for glucose estimation. Sodium fluoride/ potassium oxalat is the most common tube used for glucose estimation in the medical laboratory in the hospitals. According to the systematic review sodium fluoride tube has some disadvantages that described in different studies. First of all, the main material in this tube is fluoride substance that works to stop enolase enzyme which found at the end of the glycolytic pathway, fluoride needs around 4 hours to stop enolase. Approximately 5% to 7% of glucose concentration drop per hour at room temperature because upstream enzymes continue to convert glucose to glucose-6-phosphate (Li G et al., 2013). Secondly, fluoride is known substance to cause hemolysis to red blood cells which will interfere in the concentration of glucose (Fernandez et al., 2013).

In comparison, citrate buffer can be used as antiglycolysis, citrate has a lot of advantages for glucose measurement. First advantage, it has the ability to decrease PH of the blood which lead to inactivation of hexokinase enzyme so glycolytic pathway inhibited from the beginning of the pathway where the level of glucose remain normal until centrifugation time. Second advantage, it has lower impact in causing hemolysis fore red blood cells, thus, the glocuse level in the tube will not change (M.Koseoglu et al., 2011). Citrate tube give the best result if the blood fill in the correct ratio to the citrate (Gracia del Pino I et al., 2013).

Gel separator tube is not suitable tube for glucose estimation because the blood specimen needs around half an hour to be clotted before centrifugation during this time the glycolytic pathway goes on and blood glucose level decrease continually (sacks DB et al., 2011).

After the centrifugation gel separator tube form a gel wall between cells and serum and gives the cleanest specimen, so, the amount of glucose will be the same. In other hand, lithium heparin salt is added to the gel separator tube, there for, the specimen can be centrifuged immediately after collection and the glucose level will be preserved by the gel wall, however some cellular aggregates remain in the plasma which can affect the concentration of the glucose (Li et al.,2013)

The mixture anticoagulant tube contain vary substances that can stop the glycolytic pathway in different ways. Mixture tube contain sodium fluoride that inhibit the function of enolase enzyme and the citrate material which cause change in the PH of the blood in the tube, there for the hexokinase enzyme become in inactive form ,so the glucose pathway will be stop. Sodium EDTA sometime used as anticoagulant along with citrate and sodium fluoride.

For example of mixture tubes are gluco EXACT which composed of sodium fluoride, citrate buffer and sodium EDTA, venosafe tube that contain Sodium fluorid, Citrate buffer, sodium EDTA show better results than other tubes and Glucomedics made up of sodium fluoride, potassium oxalate, Citrate buffer and EDTA . Medical laboratories start using this kind of tubes for daily practice to get suitable results. The European Federation of Clinical Chemistry and Laboratory Medical (EFLM) Working Group for Preamalytical Phase (WG-PRE) start investigating this tube and comparing it with citrate and fluoride tube.

Conclusion

According to the evidence reviewed in this systemic review found that citrate blood tubes is better than sodium fluoride in preserving glucose level in blood collection tubes when stored at room temperature in un-centrifuged condition, also the ability of citrate tube to produce more accurate and stable results and help to minimize

false diagnosis for diabetic patients. Farther more, citrate tube produce less hemolysis compared to sodium fluoride tube. The mixture tubes (venosafe, gluco EXACT and Glucomedic) show the best results for glucose stability more than other tubes. We advise hospitals to use mixture tubes for glucose estimation because the mixture tubes able to preserve plasma glucose concentration and give accurate result. If the mixture tube is not available, the second best tube is the citrate tube.

Recommendation

We suggest the MOH to provide mixture tubes to the hospital laboratories as this tubes are economically available and give more accurate result. Finally, we request from the worlds associations and international organizations such as the WHO and the ADA for additional research and establishment for new anti-glycolytic.

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