The impact of preanalytical factors on glucose concentration measurement

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Glucose is the most commonly ordered test in a clinical chemistry laboratory accounting for about 30-40% of the total laboratory workload. Measurement of glucose concentration is done in all types of samples: capillary and venous whole blood and plasma, serum, pleural fluid, ascites, cerebrovascular fluid and urine. Most of these measurements are included into healthcare of patients with diabetes mellitus. Diabetes mellitus is diagnosed based on the well-established cut-off values originating from the worldwide-accepted guidelines. When performing glucose tolerance tests, changes in glucose concentration indicate the degree of glucose metabolism impairment. Therefore, all factors influencing glucose concentration variability have to be minimized in order to obtain accurate results. Fortunately, nowadays, there are practically no analytical challenges for glucose concentration measurement. Automated methods used in the laboratories fulfil strict criteria with very low analytical variability. However, the biggest pitfall, as for all other laboratory measurements, lies in the preanalytical phase, which is, almost exclusively, responsible for the errors in glucose concentration measurement.

A large number of preanalytical factors like sample type, transport conditions, time from blood sampling, temperature and type of test tube influence glucose concentration. Each of these factors introduces a certain degree of variability. Their cumulative effect can result with the error of the measurement that can wrongly be attributed to the clinical condition.

Glucose can be determined in the venous and in the capillary sample. It is widely accepted that fasting glucose concentration is similar in the venous and capillary blood. However, postprandial glucose and glucose measured after glucose load in tolerance tests differ significantly between venous and capillary sample. Capillary plasma glucose is higher because of the rate of the glucose consumption in the tissues (1,2). This difference can be as high as 19%, as observed in our recently performed study of the quality of the capillary sample in non-fasting healthy volunteers (3). Therefore, currently used recommendations issued by the American Diabetes Association do not support using capillary sample for diagnosing diabetes in non-pregnant adults (4). However, many laboratories still use capillary and venous sample interchangeably for the random glucose concentration measurement, especially in children. To complicate things even further, the practice of alternating between serum and plasma samples, especially in the emergency services, is also widespread. Studies have confirmed that there are significant differences in glucose concentration between serum and plasma (5). Glucose is higher in plasma than in serum because of the lower water content of the plasma. Additionally, some authors speculate that glucose is consumed when the clotting occurs. Interchangeable usage of different samples that significantly differ in glucose concentration, can lead to numerous errors. This practise can cause repeated testing, unnecessary performing of glucose tolerance tests and result in prolonged turn-around-time and delay in diagnosis. Therefore, single sample type, ideally venous plasma, has to be used for glucose concentration measurement. If other types of samples are used, this has to be recorded on the test report, with the explanation of the potential influence on the result.

The critical point in glucose preanalytical variability is the continuation of the *ex vivo* glycolysis in the test tube. The rate of glycolysis is enhanced with the high number of leukocytes, causing large interindividual differences in the degree of loss of glucose. During the years, many efforts have been done in order to minimize this process. Several types of additives have been investigated. Up to recently, tubes containing Iodoacetate have been used. Iodoacetate acts as a glycolysis inhibitor by

inactivating glyceraldehydes-3-phosphate dehydrogenase. D-mannose is a competitive hexokinase inhibitor in the first glycolysis reaction, but this effect of inhibition lasts for a very short period of time. Additionally, mannose interferes with the glucose measurement if glucose-oxidase or hexokinase methods are used. The procedure that effectively inhibits *ex vivo* glycolysis is placing the tube on ice immediately after collection and separating the sample from the cells. However, because of the technical inability, especially of the distant phlebotomy sites to perform this process, test tubes containing sodium fluoride are nowadays routinely used to stop the glycolysis. In the presence of inorganic phosphates, fluoride forms a fluorophosphates ion that is bound to the magnesium in the enolase enzyme. This reaction inhibits the enzyme and stops glycolysis.

Although, this was a gold standard practise used for many years and implemented in all guidelines, numerous studies have confirmed that fluoride does not stop glycolysis in the first two hours after the sampling. Glucose concentration gradually decreases during that period and stabilisation is obtained after 90-120 minutes. When comparing glucose concentration between heparin plasma samples centrifuged at phlebotomy site and sodium fluoride plasma samples centrifuged after transport to the central laboratory, Shi *et al*. discovered that glucose was approximately 0.39 mmol/L (~5%) higher in lithium heparin plasma sample, proving that glycolysis still occurs in fluoride sample (6). Waring *et al*. did a similar experiment using serum separator tubes (SST tubes) (7). They have also confirmed that the glucose concentration was 5% higher in the serum than in the sodium fluoride plasma. Both of these authors, and many others, have concluded that the separation from the cells and refrigeration of the sample are superior to sodium fluoride for stopping glycolysis (8,9).

Fernandez *et al*. have observed another interesting finding concerning sodium fluoride tubes. When comparing NaF tubes with SST tubes, they have discovered that 86.2% of the sodium fluoride samples had free haemoglobin concentration > 0.15 g/L. Interestingly, in the SST tubes that rate was only 2.2%. This very high hemolysis rate can potentially influence glucose concentration, since red blood cells contain lower glucose concentration than serum. Catalase that is released from red blood cells during hemolysis can further lower glucose concentration in hemolysed samples (10).

It has been postulated that the 30-90 minutes delay of inhibition of glycolysis in the sodium fluoride tube can be explained by the period that is necessary for the fluoride ion to enter the red blood cells. However, Mikesh LM and Burns DE have disproven this hypothesis (11). They have investigated influence of the sodium fluoride on the change of lactate and glucose concentration and discovered that fluoride almost instantly inhibits the production of lactate, while the glucose concentration slowly decreases. If there was a delay in fluoride ion influx to the cell, the concentration of the lactate would not be stabilised immediately. Therefore, the authors conclude that fluoride inhibits enolase within of 5 minutes of addition to the blood, while enzymes in the upstream path of glycolysis remain active. Production of lactate is inhibited because piruvate cannot be formed, but glucose is still metabolized into glucose 6-phosphate and other phosphorylated metabolites that are accumulating within the cell. Other pathways may metabolise phosphorylated sugars, until steady state is obtained. Phosphorylation of glucose will continue until there is available supply of ATP in the cell. Supply of ATP is exhausted about 60 minutes after addition of the fluoride and then glucose concentration stabilises in the tube (12).

Based on the study of Mikesh and other authors, it is evident that fluoride alone cannot effectively stop the glycolysis and new solutions have to be implemented. In the year 1986, Uchida K, Okuda S, and Tanaka K by Terumo Corporation, Japan has reported a patent where they have proven that acidification of blood immediately stops *ex-vivo* glycolysis. Low pH value effectively inhibits hexokinase and phosphofructokinase, enzymes that are active early in the Embden–Meyerhof pathway. Glycolysis is inhibited instantly in erythrocytes, leukocytes and platelets when the blood ph of 5.3 and 5.9 is obtained by adding citrate buffer. This inhibition can last up to 10 hours at

room temperature. Their patented additive contained a mixture of citric acid, trisodium citrate, disodium EDTA (to chelate magnesium), and NaF (for inhibition over a longer period of time) in a gravimetric ratio of 3.4:1.6:4.8:0.2 (10 mg/mL blood). This was incorporated into Venosafe® Glycaemia test tubes from the Terumo Corporation.

Recently, test tubes containing citrate buffer have been available on the market and several studies of their performance have been done. Gambino has performed a stability study of the this test tube, and showed that without the separation from the cells, glucose concentration dropped only 0.3% after 2 hours, while after 24 hours the decrease was only 1.2%. In comparison, in fluoride test tube, decrease after two hours was 4.5% and after 24 hours 7% (13,14). Because of the piling evidence on superiority of the citrate tube in comparison with the fluoride tubes, these findings were incorporated into guidelines. In the document issued by the American Association for Clinical Chemistry and American Diabetes Association in the 2011, the authors propose that sample should be put immediately in icy-water slurry, and plasma separated from the cells within 30 minutes. If this cannot be obtained, a tube containing rapid inhibitor of glucose such as citrate buffer should be used. Tubes with only NaF are not enough to prevent glycolysis (15).

Since then this tube was widely implemented, and several studies of the clinical impact of the tube change have been reported. del Pino *et al*. have found an increase of 6% in prevalence of positive results in screening test for gestational diabetes, 3.9% increase in confirmatory test for gestational diabetes and 4.5% increase in positive oral glucose tolerance test for non-pregnant adults, however two latter were not statistically significant (16). Norman M. *et al.* have also investigated clinical impact of change in glucose testing tube. They have compared mean glucose values obtained from the laboratory information system prior, during and after change from fluoride tubes to fluoride/citrate tubes. They have discovered a difference in mean values is +0.80 mmol/L, which represents a 14% increase. Because there were no other changes in laboratory analyzers, reagents or equipment, this difference is attributed solely on inhibition of loss of glucose by glycolysis in the first two hours in the fluoride tube that is now prevented. Based on the distribution of glucose concentration, they have calculated that approximately 18% of patients were wrongly diagnosed based on the glucose fasting concentration (17).

This problem could be expected since all established cut-off values for diabetes diagnosing are determined using sodium fluoride tubes. Even if it is proven that the citrate tube is superior and the concentration of glucose is measured more accurately, the change from fluoride to the citrate tube has a significant clinical impact. All laboratories that introduce the new tube into routine practise should communicate this change with the clinicians and inform them of the expected effect on the laboratory result. It is now necessary to redefine cut-off values for diagnosis and monitoring diabetes patients using the new citrate tube.

Preanalytical variability can significantly influence laboratory results. All factors contributing to the variability have to be carefully monitored to prevent preanalytical errors that can cause harm to the patient.

References

1. *Kuwa K, Nakayama T, Hoshino T, Tominaga M. Relationships of glucose concentrations in capillary whole blood, venous whole blood and venous plasma. Clin Chim Acta 2001;307:187-92.*
2. *Kruijshoop M, Feskens EJ, Blaak EE, de Bruin TW. Validation of capillary glucose measurements to detect glucose intolerance or type 2 diabetes mellitus in the general population. Clin Chim Acta 2004;341:33-40.*
3. *Simundic AM, Nikolac N, Dukic K, Zoricic M. Quality of the capillary sample in non-fasting healthy volunteers. (unpublished results)*
4. *American diabetes Association. Standards of Medical Care in Diabetes 2014. Diabetes Care 2014;37(Suppl 1):S14-80.*
5. *Frank EA, Shubha MC, D'Souza CJ. Blood glucose determination: plasma or serum? J Clin Lab Anal 2012;26:317-20.*
6. *Shi RZ, Seeley ES, Bowen R, Faix JD. Rapid blood separation is superior to fluoride for preventing in vitro reductions in measured blood glucose concentration. J Clin Pathol 2009;62:752-3.*
7. *Waring WS, Evans LE, Kirkpatrick CT. Glycolysis inhibitors negatively bias blood glucose measurements: potential impact on the reported prevalence of diabetes mellitus. J Clin Pathol 2007;60:820-3.*
8. *Gambino R, Bruns DE. Stabilization of glucose in blood samples: out with the old, in with the new. Clin Chem Lab Med 2013;51:1883-5.*
9. *Gambino R. Sodium fluoride: an ineffective inhibitor of glycolysis. Ann Clin Biochem 2013;50:3-5.*
10. *Fernandez L, Jee P, Klein MJ, Fischer P, Perkins SL, Brooks SP. A comparison of glucose concentration in paired specimens collected in serum separator and fluoride/potassium oxalate blood collection tubes under survey 'field' conditions. Clin Biochem 2013;46:285-8.*
11. *Mikesh LM, Bruns DE. Stabilization of glucose in blood specimens: mechanism of delay in fluoride inhibition of glycolysis. Clin Chem 2008;54:930-2.*
12. *Mikesh LM, Bruns DE. Stabilization of glucose in blood specimens: mechanism of delay in fluoride inhibition of glycolysis. Erratum. Clin Chem 2008;54:1261.*
13. *Gambino R, Piscitelli J, Ackattupathil TA, Theriault JL, Andrin RD, Sanfilippo ML, Etienne M. Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. Clin Chem 2009;55:1019-21.*
14. *Bruns DE. Are fluoride-containing blood tubes still needed for glucose testing? Clin Biochem 2013;46:289-90.*
15. *Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Executive summary: guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2011;57:793-8.*
16. *del Pino IG, Constanso I, Mourín LV, Safont CB, Vázquez PR. Citric/citrate buffer: an effective antiglycolytic agent. Clin Chem Lab Med 2013;51:1943-9.*
17. *Norman M, Jones I. The shift from fluoride/oxalate to acid citrate/fluoride blood collection tubes for glucose testing - the impact upon patient results. Clin Biochem 2014;47:683-5.*