Comparison Of Alternate Glycolysis Inhibitors With Fluoride For Preservation Of Blood For Glucose And Other Common Clinical Chemistry Examinations

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Abstract : Aims: The study aims at comparing observed values of glucose and various common clinical chemistry examinations in sample containers with different glycolysis inhibitor – Glyceraldehyde. Materials And Methods: Prepare sample conainers with alternate glycolysis inhibitors.Collect blood samples in prepared sample containers (systematic sampling of 100 consenting patients admitted at various wards of New civil Hospital,Surat for each perservative).Observe values of various examinations in collected samples. Statistical Analysis Used: Perform two-sample Student's t-test on observed sets of data. Results: Average Glucose concentration in 5 mmol/L DL-Glyceraldehyde, Fluoride and Plain tube each was 118.78 mg/dl, 121.84 mg/dl and 100.24 mg/dl respectively after 8 hours. P-value for glyceraldehyde tube vs. Fluoride tube was 0.64737, P-value for glyceraldehyde tube vs. Plain tube was 0.00796 and P-value for Fluoride tube vs. Plain tube was 0.00187. P-value for Bilirubin, ALT,ALP, Amylase,Electrolytes, Triglycerides, Cholesterol, HDL Cholesterol, Urea, Uric Acid, Albumin, Total Proteins were unaffected by DL-Glyceraldehyde, while there was negative interferance with Creatinine measurement by Alkaline Picrate method. Conclusions: Use of DL-Glyceraldehyde containers for preservation of blood for Glucose measurement is as good as fluoride containers. Because Glyceraldehyde interfere with creatinine assay by Alkaline Picrate method and pottasium assay by ISE , Glyceraldehyde containers can not replace Plain tube for common routine clinical chemistry analysis. [Mangukiya K et al NJIRM 2013; 4(3) : 97-102]

Key Words: Glucose Measurement, Glycolysis Inhibitor, Fluoride, DL-Glyceraldehyde

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Introduction: Blood for glucose examination is generally stored in [fluoride + (Oxalate/EDTA)] containing containers to inhibit glycolysis during period between sample collection and glucose examination. Interference with various examinations by fluoride, EDTA or oxalate present in blood containers for glucose examination makes it necessary to collect blood sample in other tubes with no preservative or other preservatives for additional examinations. Finding a glycolytic inhibitor with clinically negligible interference with additional examinations can reduce cost of blood collection containers, needles, phlebotomist payment, patient pain, sample data entry in computers, centrifugation time, wear and tear, analyzer sample positions, technologist payment, waste disposal resources and reporting stationary. Fluoride action starts only 1 hour after sample collection in the tube1.Fluoride samples can not be used for other investigations. Several approaches have been proposed to minimize glucose loss, including centrifugation/decantation of plasma immediately after specimen collection, Refrigeration/cooling on ice during transport2, addition of antiglycolytic agents such as iodoacetate3 ,fluoride4, mannose to the collection tubes,Use of glucose analyzers designed for near-patient testing, at the bedside. All of these approaches makes incomplete inhibition of glycolysis. Disadvantages of use of fluoride is interference in testing for co-analytes (e.g., electrolytes, creatinine,and urea), disturbance of cellular integrity (e.g., hemolysis) and promotion of leakage of intracellular potassium.

The ideal approach for eliminating glucose loss would provide reasonably stable glucose concentrations for the period needed for transport to a centralized laboratory, avoid costly near-patient analysis, and yield a specimen that was suitable for analysis of many other common analytes so that separate collection of specimen for those analytes was not necessary. From a practical standpoint, the best way to achieve this goal is discovery of an antiglycolytic agent that could be added to collection tubes but did not alter cellular integrity or interfere in common analytical methodologies. Such an agent should also be effective at low concentrations (minimizing volume addition to avoid dilution errors), dissolve rapidly

during the collection process, be nontoxic, be stable in the room-temperature storage environment of blood collection devices, and be inexpensive. Considerable effort has been expended in the past to find such preservative.

While fluoride is the single most common preservative used for blood glucose measurement, many other strategies are in use. Various preservatives in use for Blood glucose measurement are as follows:

- fluoride
- Iodoacetate
- D-Mannose
- Glyceraldehyde

1)Sodium Fluoride: Sodium fluoride acts as a weak anticoagulant and is usually considered the preservative of choice for blood glucose levels. It exerts its preservative action by inhibiting the enzyme involved in glycolysis.
Mechanism: Fluoride acts primarily by inhibiting enolase in the glycolytic pathway. Fluoride strongly inhibits the enzyme in the presence of organic phosphate. The inhibitory species is the fluorophosphate ion,which when bound to magnesium forms a complex with enolase and inactivates the enzyme.
Advantage: Concentration of glucose remain stable up to a period of a 10 days.
Disadvantage: Fluoride acts as an inhibitor on serum enzymes and urease, which is used in measuring urea.

Specimens collected in standard fluoride/oxalate tubes are invariably hemolyzed, which makes them unsuitable for analysis of other important analytes that are frequently requested in conjunction with glucose, such as potassium.The useful concentration of Fluoride also cause ICF to ECF fluid shift in blood, causing preanalytical error.

Concentration required:1-2 mg/ml of blood

The delay in fluoride's prevention of glucose loss in blood samples is sometimes attributed to a postulated delay in the entry of fluoride ion into the blood cells in which the glycolytic enzymes reside. Several observations cast doubt on this explanation, however. Fluoride does enter blood cells rapidly, and inhibit enolase, but upstream enzymes are not inhibited, causing metabolism of intracellular Glucose till upstream metabolites are accumulated. That is the reason for immediate inhibition of production of lactate, which is produced from pyruvate, the final product of glycolysis. With the glycolytic pathway blocked, other pathways may also metabolize phosphorylated sugars. Such metabolism will continue until equilibrium states are reached for the several reactions involved. In particular, the rate of phosphorylation of glucose to glucose 6-phosphate will decrease because this rate depends on the supply of ATP, the concentration of which decreases in erythrocytes by almost 90% at 60 min after addition of fluoride[5].

The proposed explanation for delay in the action of fluoride does not require postulating the existence of a barrier to the movement of fluoride into erythrocytes and leukocytes. In fact, studies of lactate transport into human erythrocytes indicate that fluoride exchange across the erythrocyte membrane is rapid[6]. We conclude that the delay in fluoride's ability to stop the use of glucose reflects continuing metabolism of glucose despite inhibition of the downstream target enzymes inhibited by fluoride.

2) Glyceraldehyde: There are two form of Glyceraldehyde: L- Glyceraldehyde and D-Glyceraldehyde. Commercially available glyceraldehyde may be pure L- Glyceraldehyde and D- Glyceraldehyde or recemic mixture DL-Glyceraldehyde.
Mechanism: The mechanism of L-Glyceraldehyde inhibition ofglycolysis in erythrocytes has not been fully established. L-GA is poorly metabolized[7,8], through conversion to glycerol[9]. Hexokinase has been proposed as the site of inhibition by L-GA[9] through condensation with dihydroxyacetone phosphate to form sorbose-1-phosphate, which is an inhibitor of hexokinase.
Advantages: Glyceraldehyde does not cause hemolysis. potassium concentrations in plasma from Glyceraldehyde treated specimens remain suitable for assessment for up to 8 h.
Disadvantage: interference in alkaline picrate method for creatinine.

Concentration required: 5 mmol/l of D,L-glyceraldehyde

Specimens containing Glyceraldehyde were suitable for most common clinical chemical determinations, . From the standpoint of stability and solubility, GA seems ideal as an additive because it is highly soluble (30 g/L)[10] and, according to the source, Sigma Chemical, D,L-Glyceraldehyde can be stored at room temperature in the crystalline form. Because L-Glyceraldehyde is fully effective at concentrations as low as 2.5 mmol/L, the actual amount of L-Glyceraldehyde needed for the standard evacuated 7-mL collection tube is 1.58 mg. This small dose limits the expense of use of Glyceraldehyde and eliminates the potential that volume dilution of the specimen by the additive might decrease glucose and other determinations, in contrast to the equivalent fluoride/oxalate 7-mL tube, which contains >31 mg of additive. Volume dilution of specimen by additive becomes more important when collection tubes are only partially filled.

The antiglycolytic effect of L- Glyceraldehyde has been noted previously, but L- Glyceraldehyde has never been tested as an additive for preservation of blood specimens for glucose analysis[7]. The ability of L- Glyceraldehyde to inhibit the formation of glucose from D- Glyceraldehyde in slices of rat kidney cortex-mix was documented in 1966[8]. Thornalley and Stern [9] noted L-Glyceraldehyde inhibition of lactate/pyruvate formation from radioactive glucose in erythrocytes but did not measure glucose concentrations in blood or plasma in their experiments. Glyceraldehyde has been investigated as an agent to promote insulin secretion from isolated pancreatic islets [7]; both D- Glyceraldehyde and L-Glyceraldehyde were effective in promoting secretion. The action of D- and L- Glyceraldehyde was related to auto-oxidation to methylglyoxal, which is a highly reactive inhibitor of many cellular function[11]

While previous study[12]compared 8 hrs. Glyceraldehyde incubation with immediate Glucose analysis, this study aims at real life scenario of glucose preservation in laboratory by comparing glucose concentration among Glyceraldehyde, Fluoride and Plain tubes in indoor hospital patient. The study also aims at comparing other commonly done clinical chemistry analytes between plain tube and glyceraldehyde tube, to find weather glyceraldehyde adversely affects other clinical chemistry analytes of not.

Subject: Whole blood specimens was collected in vaccutainer tubes containing 5 mmol/L D,L-glyceraldehyde. admitted an indoor ward of New civil hospital,Surat after obtaining written consent . D,L-glyceraldehyde was obtained from HI media. Fluoride and plain vaccutainer tubes of 4 ml capacity was obtain from BD.

Preparation of D,L-Glyceraldehyde Tubes: D,L-Glyceraldehyde Tubes was prepared by adding 60 micro liter of D,L-glyceraldehyde of 5 mmol/l after removing vacuum. Tube was allowed to dry for a period of 48 hours at 37 ´C. after complete drying vacuum tube its cap was reapplied. Final concentration of D,L-glyceraldehyde was 5 mmol/l.

Stock solution of D,L-Glyceraldehyde : 3% stock solution was prepared by adding 3 gm of D,L-glyceraldehyde in 100 ml of water.

Patient selection: Samples were taken from consenting indoor patients of New Civil Hospital.

Sample collection: 10 ml of venous blood was collected from consenting indoor patients of New Civil Hospital admitted at various ward of New civil hospital,Surat. Sample was collected in supine position under full aseptic precaution after taking written consent and giving complete information regarding to study. Blood was collected from Median cubital vein. After collection of 10 ml blood, it was distributed in a 3 separate vaccutainer Tube; 2 ml blood in fluoride vaccutainer tube,4 ml blood in plain vaccutainer(with clot activator) and remaining 4 ml is in D,L-glyceraldehyde Vaccutainer Tube. Dummy identity number was given to each participant involve in study. Same identity number also given on vaccutainer tube.

All above samples were put at room temperature at 25´C for a period of 8 hr. in clinical

biochemistry laboratory of New Civil Hospital. After 8 hr ,all above sample was centrifuged at 3000 RPM in R-8C BL Bench top Remi centrifuge For a period of 10 minutes. Aliquot ware prepared from serum/plasma separated from above samples.

Samples were analyzed at biochemistry laboratory of New civil Hospital,Surat in fully auto analyzer ERBA XL 640. along with quality control sera of normal and abnormal range. Following parameters were analyzed.

Table 1

Parameters analyzed from various containers

Sr No	Vaccutainer Tube	Parameters
1	D;L Glyceraldehyde	Glucose, SGPT, Creatinine, Urea, Total and Direct Bilirubin, albumin, TG, Cholesterol, HDL, Amylase,Total protein, Uric acid, electrolytes,
2	Flouride	Glucose
3	Plain	Glucose, SGPT, Creatinine, Urea,Total and Direct Bilirubin ,Amylase, Albumin, TG, Cholesterol, HDL, Total protein, Uric acid, electrolytes

All results were entered in computer spreadsheet and statistical analysis was performed.

Results And Discussion: After analysis of various biochemical parameter, P-value is calculated by using online student t-test calculator. In case of glucose, comparison was done between Glyceraldehyde vs Fluoride, Glyceraldehyde vs. plain and Fluoride vs. plain (Table-2). If P-value is <0.05, then the difference between them is consider significant and if It is >0.05, then it is consider as a non-significant.While rest of other common clinical parameters,comparision was done between plain tube and Glyceraldehyde containing tube.(Table-3)

Table-2

Glucose results from Fluoride,Plain and Glyceraldehyde tube

Parameter name	Preservative pair compared	Preservative	Sample No.	Average	p-Value
Glucose (mg/dl)	Glyceraldehyde vs. Fluoride	Glyceraldehyde	100	118.78	0.64737
		Fluoride	100	121.84	
	Glyceraldehyde vs. plain	Glyceraldehyde	100	118.78	0.00796
		plain	100	100.24	
	Fluoride vs. plain	Fluoride	100	121.84	0.00187
		plain	100	100..4	

Table-3A

Various biochemical parameters from Plain and Glyceraldehyde tube

Parameter	Preservative pair compared	Preservative	Sample No.	Average	p-Value
Triglyceride (mg/dl)	Glyceraldehyde vs. plain	Glyceraldehyde	100	137.14	0.1459
		Plain	100	121.82	
Total cholesterol (mg/dl)	Glyceraldehyde vs. plain	Glyceraldehyde	100	150.41	0.9565
		Plain	100	150.73	
Albumin (gm/dl)	Glyceraldehyde vs. plain	Glyceraldehyde	100	3.719	0.4202
		Plain	100	3.553	
Total protein (gm/dl)	Glyceraldehyde vs. plain	Glyceraldehyde	100	7.676	0.8780
		Plain	100	7.496	

The P-value of Glucose between Fluoride and Glyceraldehyde containing tube is 0.64, that is non significant. It indicate that Glyceraldehyde prevents glycolysis in blood for at least up to 8 hours at room temperature as efficiently as Fluoride. The P-value of Glucose between Fluoride containing tube and plain tube as well as between Glyceraldehyde containing tube and plain tube is significant because glycolysis remain continuous in plain tube due to absence of glycolytic inhibitior like Fluoride

or Glyceraldehyde. Unless, turnaround time for measurement of glucose (from sample collection to analysis of sample is as low as 1 hour, laboratory may not use plain tube for measurement of glucose.

The p-value of serum creatinine by alkaline picrate method for Glyceraldehyde vs. Plain tube is <0.0001, so the difference between creatinine of Glyceraldehyde vs Plain tube is considered significant.

The bias caused by Glyceraldehyde is positive. It is likely that Glyceraldehyde, like acetoacetate, pyruvate, protein etc. reacts with alkaline picrate causing positive interference in creatinine measurement. Further study is required to find time-window during which glyceraldehyde react with alkaline picrate, weather any change in reagent, incubation period and measurement period can decrease Glyceraldehyde interference to creatinine measurement.

Table-3B

Various biochemical parameters from Plain and Glyceraldehyde tube

Parameter	Preservative pair compared	Preservative	Sample No.	Average	p-Value
ALT (U/L)	Glyceraldehyde vs. plain	Glyceraldehyde	100	30.391	0.8318
		Plain	100	31.85	
Alkline phosph-atase (U/L)	Glyceraldehyde vs. plain	Glyceraldehyde	100	115.33	0.9565
		Plain	100	114.79	
Amylase (U/L)	Glyceraldehyde vs. plain	Glyceraldehyde	100	79.28	0.8550
		Plain	100	77.08	
Sodium (mmol/l)	Glyceraldehyde vs. plain	Glyceraldehyde	100	137.04	0.5620
		Plain	100	138.418	
Pottasium (mmol/l)	Glyceraldehyde vs. plain	Glyceraldehyde	100	4.59657	0.0196
		Plain	100	4.32616	
Creatinine (mg/dl	Glyceraldehyde vs. plain	Glyceraldehyde		1.622	<0.001
		Plain		1.171	

Table-3C

Various biochemical parameters from Plain and Glyceraldehyde tube

Parameter	Preservative pair compared	Preservative	Sample No.	Average	p-Value
Uric acid (mg/dl)	Glyceraldehyde vs. plain	Glyceraldehyde	100	3.284	0.3013
		Plain	100	3.471	
Total bilirubin (mg/dl)	Glyceraldehyde vs. plain	Glyceraldehyde	100	0.9	0.9882
		Plain	100	0.9	
Direct bilirubin (mg/dl)	Glyceraldehyde vs. plain	Glyceraldehyde	100	0.3	0.6238
		Plain	100	0.3	
Calcium (mg/dl)	Glyceraldehyde vs. plain	Glyceraldehyde	100	8.471	0.9721
		Plain	100	8.467	
HDL cholesterol (mg/dl)	Glyceraldehyde vs. plain	Glyceraldehyde	100	26.7	0.8368
		Plain	100	27.07	

This difference can be avoided by estimating s. creatinine by enzymatic method[9,12]. The study done by Michael Landt[12] using DL-Glyceraldehyde by using enzymatic kit for Measurement of serum creatinine in (Vitros 250 and Hitachi 747)fully automated biochemical analyzer does not show significant difference between serum creatinine value of plain tube(without additive) and DL-Glyceraldehyde tube. Measurement of serum creatinine in(Dade Behring RXL)fully automated biochemical analyzer by using Alkaline Picrate method shows positive interference by any form of Glyceraldehyde.

The p-value of serum potassium is also <0.019, so that difference of serum potassium between

Glyceraldehyde Vs Plain tube is also consider as a significant[9,12]. The bias caused by glyceraldehyde in measurement of potassium is positive. It is likely that, during 8 hours, there was net efflux of potassium from RBC due to unavailability of ATP, resulting from glycolysis inhibition.The study by Michael Landt[12] done using DL-Glyceraldehyde found no interference by DL-Glyceraldehyde on

ISE measurement of serum potassium. Thus, this study do not come to the same conclusion as that of Michael Landt[12]. The study of effect of glyceraldehyde as preservative for glucose and other clinical chemistry analyte needs to be repeated to resolve the discordance among these studies.

Conclusion: Glyceraldehyde & Fluoride tubes show no significant difference in their glucose concentration.

- DL-Glyceraldehyde at 5 mmol/L of blood, preserves blood for glucose measurement for at least 8 hours.
- DL-Glyceraldehyde can be used as an alternative to Fluoride for preserving blood for measurement of Glucose in serum.
- Serum Creatinine shows positive interference by DL-Glyceraldehyde in alkaline picrate method .
- Serum Electrolyte (s. potassium) also shows positive interference by DL-Glyceraldehyde. This conclusion is at discordance with other similar studies done in past.
- DL-Glyceraldehyde containing vial for many biochemistry related parameter analysis is better option as it is save additional use of vaccutte, except for serum potassium and serum creatinine (by alkaline picrate method).
- This study needs to be extended for looking in to alternative methods for measurement of creatinine in Glyceraldehyde preserved serum. One candidate method for such study can be enzymatic method for measurement of serum creatinine.

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