

Comparitive Study on the Effect of Serum and Plasma on Glucose and Cholesterol Estimation

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Objective is to study if there is any clinically significant difference between EDTA plasma and serum for glucose and cholesterol estimation. A general problem faced by the clinical laboratories is the integrity of uncentrifuged specimens for chemical analyses. Because prolonged contact of plasma or serum with cell is a common cause of spurious test results, plasma and serum should ideally be separated from cells as quickly as possible to prevent ongoing metabolism of cellular constituents as well as active and passive movement of analytes between the plasma or serum and cellular compartments. (Goodman JR, Vincent J 1954).

Key words: Serum glucose, Plasma glucose, Serum cholesterol, Plasma cholesterol, EDTA plasma.

Human blood is composed of two parts, cellular components containing the RBC, WBC and Platelets and straw colour fluid called plasma. Blood cells are suspended in plasma which accounts for 50-55% of blood volume, with blood cells accounting for the remaining portion (Fox SI 1999). Serum and plasma are not the same. The word "serum" is a Latin word meaning "whey." when whole blood is allowed to clot, the serum is the remaining liquid component, excluding the fibrin clot (Venues. D(ED) 2001).

Plasma is the cell-free liquid remaining after whole blood is centrifuged. The major difference from serum is that plasma contains the

clotting proteins, but serum does not. Plasma is obtained from blood samples by introducing any type of anticoagulant, and centrifuging, which decants the most buoyant (non-cellular portion). Serum is obtained from blood that has coagulated fibrin clots formed during coagulation along with blood cells and related coagulation factors, are separated from serum by centrifugation.

The primary differences between the plasma and serum lie in the compound involved in the clotting process. If no anticoagulant is added, the blood is allowed to clot, and the supernatant fluid is called serum which is less viscous than plasma and lacks fibrinogen, prothrombin and others clotting proteins. (West JB, 1985) Both plasma and serum are aqueous solutions (95% water) containing variety of substances including proteins and peptides (such as albumin, globulin, lipoproteins enzymes & hormones. Nutrients, such as carbohydrates, lipids and amino acids),

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Electrolytes, organic molecules are suspended or dissolved in them.(Beheshti, 1994)

Plasma can be separated from whole blood by addition of anticoagulant. Anticoagulants are additives that inhibit the clotting of blood and or plasma, thereby ensuring that the concentration of the substance to be measured is changed as little as possible before the analytical process.

The harvest of serum requires 15-30 minutes, should wait for coagulation completion before centrifugation, the use of plasma expedites analysis in emergency situations, further more plasma yield from a given volume of whole blood is always greater than the yield of serum (*Young and Bermes 1999*) also additional biochemical analysis not previously indicated for the initial required hemogram are often required, in this situation it is better to obtain another sample for serum harvesting but this is not always possible for all patients especially cattle. Thus analysis must be performed on plasma anticoagulated with various types of anticoagulants most commonly EDTA. (*M.mohari 2007*).

EDTA is the common anticoagulant used for hematological examination like glycosylated hemoglobin assay etc. The quickness of separation of red blood cells from the plasma by centrifugation is a critical element, because it is estimated that plasma glucose levels are reduced 10mg/dl per hour by consumption of glucose in the red blood cells glycolytic pathway. (*Sacks D. Bruns. D 2002*). Anticoagulation is achieved either by the binding of calcium ions (EDTA, citrate and fluoride) or by the inhibition of thrombin (heparin).

Materials & Methodology

Sample Procurement

50 blood samples were collected from patients and aliquoted in 2 tubes each containing plain tube and an EDTA tube. *Glucose Estimation Principle:* This test is based upon the enzymatic glucose oxidase/ peroxidase (GOD/POD) method. Glucose is oxidised to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose present in the sample.

Total Cholesterol Estimation Principle

The cholesterol estimation is based on the cholesterol oxidase/ peroxidase (CHO/POD) method. Here the cholesterol esters are hydrolysed by enzyme cholesterol esterase to give free cholesterol and fatty acid molecules. This free cholesterol gets oxidised in presence of cholesterol oxidase to liberate cholesterol 4-en-3-one and H₂O₂. Liberated H₂O₂ by this reaction combines with phenol and 4-aminoantipyrine in presence of peroxidase to form red colour quinoneimine complex, the intensity of which is measured at 530nm (490 – 530nm). It is directly proportional to cholesterol concentration present in the sample.

Test Procedure for Glucose & Cholesterol Estimation

Three test tubes were taken and marked as Standard (S), Test (T) and Blank (B) for glucose & cholesterol separately. (Table 4.0) 1000µl of glucose and cholesterol reagent for respective tests were added to all the tubes. 10µl of glucose standard and cholesterol standard were added to standard 'S' tubes, 10µl of sample was added to tube 'T' and 10µl distilled water was added to tube 'B' for their respective tests. The tubes for glucose test were mixed well and incubated at 37°C for 15 minutes. The tubes for cholesterol test were mixed well and incubated at 37°C for 5 minutes. The absorbance was read with the help of colorimeter at 530 nm (green filter) within 10 minutes for both glucose and cholesterol.

RESULTS

Totally 50 samples were collected from male and female patients collectively. Then the samples were aliquoted equally into plain tubes and EDTA tubes for glucose and cholesterol estimation for statistical analysis.

DISCUSSION

Statistical Review on Glucose Estimation

Primarily mean and standard deviation was determined between the 50 plain serum samples and 50 EDTA plasma samples from the estimated glucose results and they were found to be 138.6 and 48.81 for serum sample and 140.4 and 50.24 for plasma samples respectively. Then the

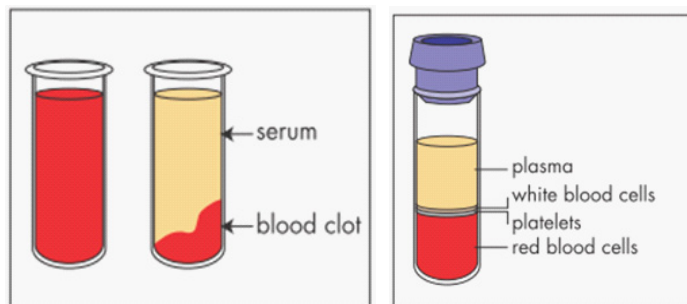
comparison between the glucose results of serum and EDTA plasma was carried out statistically and the exact value was determined to be not >0.08 , irrespective of the standard deviation which shows 7.0, thereby explaining that there is no vast and significant difference between the glucose values derived from serum sample and EDTA sample. Thus when the scatter diagram (graph 1) was plotted, there was no major deviation between the values of serum and EDTA plasma of the glucose samples from the line of identity.

Statistical Review for Cholesterol Estimation

Similar to that of glucose estimation statistical review, for cholesterol also the mean and the standard deviation calculated for the results

of the 50 serum and 50 EDTA samples where the mean was found to be 166 for serum and 170.1 for EDTA sample and the standard deviation was found to be 42.12 for serum and 42.97 for EDTA samples. Then the results of serum and EDTA samples were compared statistically and the exact value was derived to be 0.0003 which is not >0.0005 and it signifies that there is no comparable difference between the results derived from serum and the EDTA plasma sample. The scatter diagram (graph 2) was plotted diagrammatically representing that there is no significant difference between the values obtained from serum and EDTA plasma.

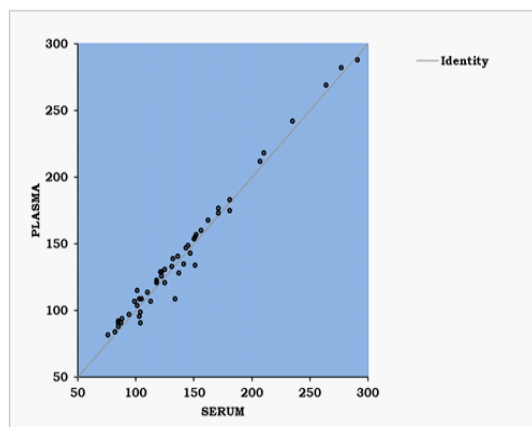
There were also some suggestion of an inherent difference between serum glucose and plasma glucose; serum glucose has been found to



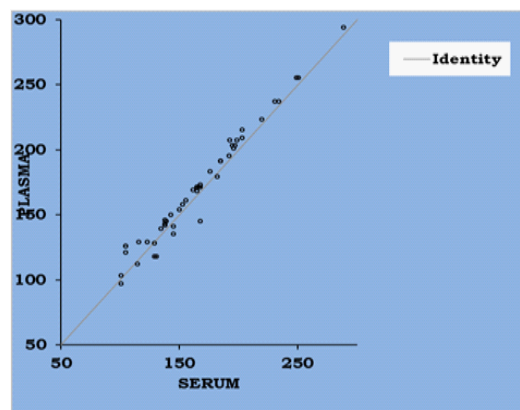
Significant differences between serum and plasma

Calculation of Standard Deviation for Glucose (Graph 1)

	n	mean	95% CI	SE	SD
Serum	n=50	138.6	124.5 – 152.8	7.04	49.81
Plasma	n=50	140.4	126.1- 154.7	7.10	50.24
n	50				
Correlation - absolute difference v average	-0.06				
Bias	1.8				
95% CI	-0.2		to 3.8		
SE	0.99				
t statistic	1.79				
DF	49				
p	0.0789				
SD of differences	7				
		95% Limits of agreement	between single measurements	95% CI	
Lower		-12	-15.4		to -8.5
Upper		15.5	12.1		to 19.0



Graph 1: Scatter plot



Graph 2. Scatter plot

	n	mean	95% CI	SE	SD
Serum	n=50	166.0	154.0 – 177.9	5.96	412.12
Plasma	n=50	170.1	157.9 – 182.4	6.08	42.97
n	50				
Correlation - absolute difference v average	-0.11				
Bias	4.2				
95% CI	2		to 6.3		
SE	1.07				
t statistic	3.91				
DF	49				
p	0.0003				
SD of differences	7.6		between single measurements		
	95% Limits of agreement		95% CI		
Lower	-10.6		-14.3		to -6.9

be 2-5% higher than plasma glucose (**R Gambino, unpublished observations**). Either hexokinase or glucokinase enzyme analysis is used in ~99% of all laboratories for glucose measurement. These two tests have an intra-and interassay coefficient of variation with a 95% confidence interval <4%. Based on results obtained in this study, it is determined that there is no significant difference between the glucose and cholesterol values tested from a serum and an EDTA plasma which means that EDTA plasma tube could also be used for sample collection for testing of glucose and total cholesterol.

CONCLUSION

Therefore test estimation of glucose and total cholesterol can be performed using the plasma sample collected in an EDTA tube also as there are no significant variation between the values of glucose and cholesterol obtained from a serum sample and an EDTA plasma sample.

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