Article published online: 2020-02-19

Short Paper

Access this article online
Quick Response Code:

Website: www.jlponline.org
DOI: 10.4103/JLP.JLP_151_17

Comparisons of metabolite profile from paired serum and ethylenediaminetetraacetic acid–plasma samples using dry chemistry technology: An emergency department perspective

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Abstract:

BACKGROUND: No data is available evaluating the difference in serum versus plasma sample assay of commonly tested parameters in the emergency department, where the sample processing time can be significantly reduced if plasma is used for analysis instead of conventionally used serum. Hence, this study aimed to evaluate the differences in serum versus plasma sample estimation of commonly evaluated biochemical parameters using dry chemistry technology.

MATERIALS AND METHODS: Paired blood samples were collected from a single venipuncture of 405 patients admitted to the emergency department. Dry chemistry autoanalyzer (Vitros-350, Ortho Clinical Diagnostics) was used to process all the samples.

RESULTS: Data from 401 patients were analyzed. Percentage differences between serum versus plasma samples for all analytes ranged from 0.0% to 57.44% and were <±4% for a majority of parameters, except uric acid (–6.25%), albumin (+11.90%), chloride (–5.05%), phosphorus (–6.06%), creatine phosphokinase (CPK) total (–57.44%), amylase (–37.53%), lipase (–42.74%), lactate dehydrogenase (LDH) (–8.53%), and C-reactive protein (–7.44%). For albumin, CPK total, amylase, and lipase, the difference between serum and plasma samples was more than the accepted upper range recommended by College of American Pathologists.

CONCLUSION: Glucose, urea, creatinine, bilirubin, total protein, serum glutamate-pyruvate transaminase, total cholesterol, high-density lipoprotein cholesterol, triglycerides, sodium, and CPK-mb can be reliably assayed from either serum or plasma samples in emergency/routine practice. CPK total, amylase, and lipase should always be assayed from serum and not plasma due to significant variations. Uric acid, chloride, phosphorous, and LDH only in emergency situations should be assayed from plasma. For routine assays, serum should be preferred.

Key words:

Dry chemistry technology, emergency department, plasma, serum

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Submission: 04-10-2017
Accepted: 14-01-2018

Introduction

Conventionally, serum is the most commonly used matrix for biochemical assays in the clinical laboratory. The principle advantage of using serum as matrix is that reference ranges are readily available for all the different biochemical systems and platforms used for assay. However, serum separation requires at least 20–30 min standby time, which may be further increased in cooler temperatures.

How to cite this article: Sharma LK, Dutta D, Sharma N, Thakur B. Comparisons of metabolite profile from paired serum and ethylenediaminetetraacetic acid–plasma samples using dry chemistry technology: An emergency department perspective. J Lab Physicians 2018;10:346-50.

This leads to a significant increase in the turnaround time for an investigation (time taken from blood sample collection, processing, analysis, and the final report reaching the clinician). Although it may not be important for elective investigations, this increased turnover time can lead to a significant delay in the clinical decision-making by the treating doctor in the Emergency Department, which can have an adverse impact on patient morbidity and mortality. As a consensus, <60 min is considered to be an ideal acceptable turnover time for laboratory investigations (sample registration to result reporting) for common laboratory tests.[1] Advantages of ethylenediaminetetraacetic acid (EDTA) anticoagulated plasma for biochemical analysis include reduced time for sample processing (as no time lost for serum generation). Also for certain investigations such as hemogram, only EDTA anticoagulated blood sample is used. If all the biochemical investigations can be done from EDTA anticoagulated plasma, it will reduce the need for collection of blood samples in different vials at the time of sample collection, thus reducing the volume of blood sample needed for biochemical investigations. Sometimes, there may be a restriction to the volume of blood sample that can be collected from a patient based on the clinical status of the patient.

However, there are a few reports to suggest that significant difference may be observed when the same biochemical parameter is measured in serum and plasma samples based on the platform used for analysis.[2,3] Incorrect reporting may lead to diagnosis of nonexistent disorders (labomas), which can lead to significant patient morbidity and mortality.[4,5]

Dry chemistry technology is increasingly being used in large laboratory setups. Advantages of dry chemistry technology include the ability to process a large number of samples in a relatively shorter duration of time along with a good accuracy of testing. However, till date, no data is available evaluating the difference in serum versus plasma sample assay of commonly tested biochemical parameters in clinical practice. Hence, the aim of this study was to evaluate the differences in serum versus plasma sample estimation of commonly evaluated biochemical parameters using dry chemistry technology in a large tertiary care center of India.

Materials and Methods

Paired blood samples were collected from a single venipuncture of 405 patients admitted to the emergency department of a large tertiary care center in India. Blood samples were collected in plain as well as K2-EDTA vacutainer tubes (Becton Dickinson). K2-EDTA plasma and serum were separated by centrifugation using standard guidelines and processed for estimation of glucose, urea, creatinine, uric acid, total bilirubin (TBil), direct bilirubin (DBil), aspartate transaminase, alanine transaminase, total protein, albumin, total cholesterol (TC), direct high-density lipoprotein (HDL), triglycerides (TGs), sodium, chloride, phosphorus, creatine phosphokinase total, CPK-MB (CPKmb), lactate dehydrogenase (LDH), amylase, lipase, and C-reactive protein (CRP). Dry chemistry autoanalyzer (Vitros-350, Ortho Clinical Diagnostics) was used to process all the samples. The Institute Ethics Committee approved the study protocol. The study protocol was explained and informed written consent was taken from all patients before sample collection for this study.

Dry chemistry technology

The main principle of dry chemistry is based on the reflectance spectrophotometry in which the reflectance of the materials is measured to give a reference standard for the comparison of the color of the different samples. In dry chemistry technology, the slides are dry, multilayered analytical elements coated onto polyester support. A small amount of patient sample is deposited onto slide and evenly distributed to all the layers. The spreading layer consists of appropriate substance and other components needed for the chemical reaction to occur. The analyte in the sample catalyzes the reaction sequence to yield products which absorb light at wavelengths in various regions (340–680 nm), diffuses into underlying layer, and is monitored by reflectance spectrophotometry. The tests performed were colorimetric, enzymatic endpoint, two-point or multipoint rate, or potentiometric analysis depending on the analysate. The CRP was measured by immunoturbidimetric assay. Advantages of DCT include the extremely small amount of sample needed for analysis (10 μL), accuracy, and high volumes of patient samples being analyzed over a shorter period.

The methods used for analysis, the assay range, and intra- and inter-assay coefficient of variation for each of the biochemical parameter evaluated have been elaborated in Table 1.

Statistical analysis

Normality of the distribution of variables was assessed using the Kolmogrov–Smirnov test. Parametric and nonparametric tests were used for analysis for normally distributed and skewed variables, respectively. Pearson's (r) or Spearman's (σ) correlation coefficient was calculated for normally distributed and skewed variables, respectively. SPSS version 20 (Chicago, IL, USA) was used for data analysis.

Results

For the collected samples from 405 patients, data from 401 patients were analyzed. Four pairs of samples

Table 1: Details of the methods used for biochemical analysis of different evaluated parameters in this study with their assay range, minimal detectable concentration, and their inter- and intra-assay coefficient of variations

Parameter	Method	MDC	L1-concentration	Intra-assay CV (%)	Inter-assay CV (%)	L2 concentration	Intra-assay CV (%)	Inter-assay CV (%)
Glucose (mg/dl)	GOD-POD	20	83	0.48	1.5	292	0.38	1.2
Urea (mg/dl)	Urease	4.29	42.9	1	1.5	107	0.8	1.6
Creatinine (mg/dl)	Creatinine amidohydrolase	0.05	0.41	2.93	4.8	13.58	0.72	1
Uric acid (mg/dl)	Uricase	0.5	4.4	0.91	1.7	10.3	0.68	1.1
Bilirubin (mg/dl)	Dyphylline	0.1	1.2	1.7	2.4	15.2	0.66	1.7
SGPT (U/L)	LDH-PLP	3 U/L	44	3.63	6.6	187	1.02	1.9
Total protein (g/dl)	Biuret	2 g/dL	4	1	2	7.4	0.81	1.2
Albumin (g/dl)	BCG	1	2.4	0.83	1.7	4.6	0.65	0.9
Total cholesterol (mg/dl)	Cholesterol ester hydrolase	50	147	0.68	1.8	259	0.7	1.5
dHDL-C (mg/dl)	PTA/$MgCl_2$	5	37.5	2.7	2.9	65.7	2.3	3
Triglyceride (mg/dl)	Glycerol kinase	10	104	0.96	1.1	464	1.12	1.4
Sodium (mmol/L)	Direct potentiometry	75	119	0.42	0.6	152	0.4	0.6
Chloride (mmol/L)	Direct potentiometry	50	84	0.5	0.7	122	0.41	0.6
Phosphorus (mg/dl)	Ammonium molybdate	0.5	3.6	0.83	2.4	7.1	0.7	1.5
CPK total (U/L)	NAC-Mg+2	20	145	1.4	3.7	769	1.65	3.2
CPK-MB	Anti CK-M, NAC-Mg+2	2.7	22	2.3	3.4	47	1.3	1.9
LDH	Pyruvate-NADH	100	441	2.6	3.3	1455	1.5	2.1
Amylase	Amylopectin	30	74	4.3	5.2	313	2.1	2.5
Lipase	Colipase	10	159	1.7	2.1	674	0.9	1.4
C-reactive protein	Immuno-rate assay	5	21	4.8	8.4	69	3.5	3.8

MDC = Minimal detectable concentration, CV = Coefficient of variation, SGOT = Serum glutamic oxaloacetic transaminase, SGPT = Serum glutamate-pyruvate transaminase, dHDL-C = Direct high-density lipoprotein cholesterol, CPK = Creatine phosphokinase, LDH = Lactate dehydrogenase, CRP = C-reactive protein, GOD = Glucose oxidase, POD = Peroxidase, PLP = Pyridoxal phosphate, NAC = N-acetyl cysteine, NADH = Nicotinamide adenine dinucleotide, CPK-MB = Creatine phosphokinase-MB, CK-M = Creatine kinase-M

were excluded from the analysis due to the presence of significant hemolysis. Percentage differences between serum versus anticoagulated plasma samples for all analytes ranged from 0.0% to 57.44% and were <±4% for a majority of parameters, except for uric acid (−6.25%), albumin (+11.90%), chloride (−5.05%), phosphorus (−6.06%), CPK total (−57.44%), amylase (−37.53%), lipase (−42.74%), LDH (−8.53%), and CRP (−7.44%) [Table 2]. A significant positive correlation was observed between serum and plasma values of all the biochemical parameters evaluated.

When we compare the results of our study with the maximum allowable error from College of American Pathologists (CAP) Chemistry Survey reports,[3] we observe that albumin, CPK total, amylase, and lipase – the biochemical parameters where the difference between serum and plasma samples – were more than the accepted upper range recommended by CAP.

Correlation between serum and plasma values for all the parameters evaluated in this study was statistically significant ($P < 0.001$), with the correlation coefficient for glucose, urea, creatinine, uric acid, TBil, DBil, indirect bilirubin, serum glutamate-pyruvate transaminase (SGPT), total protein, albumin, TC, HDL-C, TGs, sodium, chloride, phosphorus, CPK total, CPK-MB, LDH, amylase, lipase, and CRP being 0.973, 0.979, 0.980, 0.960, 0.927, 0.806, 0.919, 0.996, 0.908, 0.928, 0.952, 0.867, 0.739, 0.350, 0.655, 0.404, 0.308, 0.263, 0.911, 0.885, 0.994, and 0.996, respectively.

Discussion

Significant data are available in literature highlighting the impact of the nature of blood sample used for a biochemical analysis on the final result. A study published in 1990 highlighted that when finger prick blood was used for serum cholesterol measurement, it resulted in increased measurement of serum cholesterol with an average positive bias +2.4% as compared to serum levels, resulting in a substantial larger number of patients being labeled with hypercholesterolemia.[6] In a small study of 24 volunteers in 1994 revealed significant variance in serum and plasma levels of TC, TGs, and HDL-C on different assay platforms.[7]

Studies have demonstrated that the nature of the blood sample collected (serum vs. plasma) may have an impact even on epigenetic studies, due to their potential interference with biological processes such as DNA methylation during the sample collection.[8] Matrix metalloproteinases (MMPs) have been used as useful diagnostic or prognostic markers in different malignancies. Studies have shown that there is a significant difference in MMP levels depending on when plasma or serum samples were used for analysis, and plasma samples, in general, are preferred for

Table 2: Difference between serum and plasma values of selected biochemical tests using dry chemistry autoanalyzer (Vitros-350, Ortho Clinical Diagnostics) from blood samples obtained from emergency department (*n*=401)

Parameter (units) (normal range)	Specimen analyzed		Absolute difference (P–S)	Percentage difference (median)	MAE (%)	*P*#
	Serum (S)	Plasma (P)				
Glucose (mg/dl) (70-100)	107.8±40.29	110.24±41.46	3 (1-4)	2.43	10	<0.001
Urea (mg/dl) (15-45)	24.17±14.35	23.24±14.62	−1.0 (−1.1-0.0)	−3.74	9	<0.001
Creatinine (mg/dl) (0.6-1.2)	0.80±0.55	0.78±0.55	0.00 (−0.1-0.0)	0.00	15	<0.001
Uric acid (mg/dl) (2.5-6.5)	5.31±1.33	4.97±1.25	−0.3 (−0.5-−0.2)	−6.25	17	<0.001
Total bilirubin (mg/dl) (0.2-1.3)	0.76±0.35	0.72±0.34	0.0 (−0.1-0.0)	0.00	20	<0.001
Direct bilirubin (mg/dl)	0.26±0.16	0.26±0.15	0.0 (0.0-0.1)	0.00	20	0.582
Indirect bilirubin (mg/dl)	0.49±0.30	0.45±0.29	0.0 (−0.1-0.0)	0.00	20	<0.001
SGPT (U/L) (10-50)*	36 (27-49)	37 (29-51)	1.0 (−1.0-3.0)	2.63	20	0.043
Total protein (mg/dl) (6.3-8.2)	7.48±0.59	7.61±0.59	0.10 (0.0-0.3)	1.42	10	<0.001
Albumin (mg/dl) (3.5-5.0)	4.32±0.47	4.82±0.50	0.5 (0.4-0.6)	11.90	10	<0.001
Total cholesterol (mg/dl) (<200)	168.86±39.93	165.27±39.93	−3.0 (−7.0-1.0)	−1.99	10	<0.001
HDL-C (mg/dl) (>40)	44.62±12.52	45.38±14.46	1.0 (−1.0-2.0)	1.81	30	<0.001
Triglycerides (mg/dl) (<150)*	131 (91-184)	127 (86-179)	−3.0 (−6.0-1.0)	−2.75	25	0.043
Sodium (mmol/L) (137-145)	141.89±7.73	139.94±3.83	−2.0 (−4.0-−1.0)	−1.41	2.9	<0.001
Chloride (mmol/L) (95-110)	104.11±4.54	98.83±7.66	−5.0 (−7.0-−4.0)	−5.05	5	<0.001
Phosphorus (mg/dl) (2.5-4.5)	3.87±1.62	3.55±0.67	−0.2 (−0.3-−0.2)	−6.06	10	<0.001
CPK total (U/L) (50-170)*	83 (55-117)	25 (20-49)	−39 (−76-−17)	−57.44	30	<0.001
CPK-mb (U/L) (10-50)*	13 (9-19)	13 (9.0-18)	0.0 (−2.0-2.0)	0.00	30	0.368
LDH (U/L) (200-600)	499 (426-584)	442 (394-501)	−43 (−77-−20)	−8.53	20	<0.001
Amylase (U/L) (30-110)	70 (55-87)	42 (34-52)	−27 (−39.5-−17)	−37.53	30	<0.001
Lipase (U/L) (23-300)	101 (67-161)	59 (38-93)	−44 (−69-−28)	−42.74	30	<0.001
CRP (mg/L) (<1.0)	0.4 (0.1-0.7)	0.33 (0.10-0.6)	−0.05 (−0.11-0.0)	−7.44	14.3	<0.001

*All nonnormally distributed variables expressed as median (25th-75th percentile), *P*<0.05 considered statistically significant, #Measure of difference between the absolute serum and plasma values, percentage difference=[(P−S)/S]×100. Normally distributed variables expressed as mean±SD. MAE = Maximum allowable error from CAP chemistry survey reports, SGOT = Serum glutamic oxaloacetic transaminase, SGPT = Serum glutamate-pyruvate transaminase, HDL-C = High-density lipoprotein cholesterol, CPK = Creatine phosphokinase, LDH = Lactate dehydrogenase, CRP = C-reactive protein, SD = Standard deviation, CAP = College of American Pathologists, CPK-mb = Creatine phosphokinase-mb, CK-M = Creatine kinase-M

analysis due to the higher levels reported from plasma samples.[9]

This is the largest ever study reported, comparing the serum and plasma levels of commonly used anaylates in the biochemistry department. The study highlights that for most of the biochemical parameters which are evaluated in the emergency department, the testing can interchangeably be done on serum or plasma samples without any significant difference in the laboratory results. These parameters, which can be freely assayed on serum or plasma samples using dry chemistry technology, include glucose, urea, creatinine, bilirubin, total protein, SGPT, total cholesterol, HDL-C, TGs, sodium, and CPK-mb. This is because the percentage difference between serum and plasma samples was consistently <±4%. Hence, even in routine clinical practice, either serum or plasma can interchangeably be used for analysis, without any impact on the outcome result.

It must be highlighted that the parameters which should be assayed preferably on serum samples, and not plasma samples due to significant associated differences, include CPK total, amylase, and lipase, as the percentage differences for this parameters were more than the cutoffs provided by CAP. For parameters such as uric acid, chloride, phosphorous, and LDH, although the difference between serum and plasma samples was more than ±4%, it was less than the cutoffs provided by CAP. Hence, only in real emergency scenarios should these parameters be evaluated from plasma samples. In routine clinical practice, we should continue to evaluate these parameters from serum samples preferably.

Conclusion

It may be said that said that serum versus plasma samples should not be interchangeably be used in routine clinical practice unless specific data for the same are available depending on the assay platform used for analysis.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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