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IS IT TIME TO SWITCH OVER TO HEPARIN PLASMA FOR BIOCHEMISTRY ANALYSIS OVER TIME TESTED SERUM SAMPLES?

Clinical Biochemistry

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ABSTRACT

Introduction- Lab investigations are essential in patient management and qualities of the tests reports are emphasized. Rapid turnaround time(TAT) of laboratory tests is therefore very important to achieve early diagnosis and treatment as well as to decrease emergency room or hospital in- patient services for longer stay. Serum, most often used sample type in testing, is a clear fluid obtained from blood that remains after solid element s of WBC, RBC and platelets form a clot. **Objective-** In this study it was aimed to investigate the differences between result of lithium heparin plasma and serum tube for 28 biochemical parameters, for shortening of turnaround time and to check the volume difference between plasma and serum. **Material & Methods-** A prospective study of 100 samples (comprising total 5600 tests) was done on 28 biochemistry parameters with both serum samples from clot activator plain tube (5ml VACUTTE Serum clot activator tube) and plasma from lithium heparin tube (5ml VACUTTE Lithium heparin tube) on biochemistry analyzer Siemens Dimension XL200. The statistical significance of the differences between results obtained from serum and plasma samples was assessed, by regression analysis and bias % was calculated. P values < 0.5 were considered to be statistically significant. To determine clinically significant difference, total error (TE) between 2 samples for each test was estimated and then compared with Total allowable error (TEa) limits. If TE was higher than TEa , it was considered as a clinically significant difference. **Results-** All 28 biochemical parameters showed good correlation (correlation =/> 0.9393) with R value >/= 0.9039, p values > 0.05. Parameters with Highest Bias% were, Total Bilirubin (2.81), Potassium (2.202), CRP (-2.507). Parameters with lowest Bias% were, Albumin (0.1), cholesterol (-0.015), LDL (0.124). All the parameters where within the Total Allowable Error limit. Average TAT (from sample collection to reporting) for serum sample was 35 minutes for renal function test & for plasma sample was 20 minutes for renal function test. Fibrin clots or strands were observed in 9 serum samples (9/100) i.e. 9% and none in plasma samples. On comparing the volume of serum and heparin plasma sample in 4 ml vaccutainer tube volume of plasma recovered is more than serum i.e by 8-10 % . **Conclusion-** This study has shown that most assays in clinical biochemistry are compatible with both serum and lithium heparin plasma. The findings of the study are of great importance especially in case of urgent samples received from ICU or emergency and transplant units where delay in reporting can be detrimental to patient care and Lithium Plasma samples can help to reduce TAT by skipping clotting process.

KEYWORDS

Turnaround time (TAT), clot activator plain tube, lithium heparin tube

INTRODUCTION

In the era of evidence based medicine the diagnosis of disease depends on quality laboratory reports from right patient, right sampling, and right analysis done at right time to an extent that 70% of decisions rely upon laboratory reports [1]. The quicker, accurate the laboratory results the prompt is the diagnosis and ultimately treatment and hence the patient care. The growing development in medicine with more and more 'evidence based' over past few decades have revolutionized laboratory working where every effort is made on improving turnaround time (TAT) , accuracy, high through output and automation [1]. The expectation of clinicians is high now from laboratory. In spite of all efforts the literature studies have pointed out that laboratories strive to meet out what is expected from them [2].TAT is the most noticeable sign of laboratory service[3] to such as extent that clinicians are ready to sacrifice analytical quality of testing[4] and even opt for point of care testing (POCT)[5]. Any delay in laboratory test results or in other words prolonged TAT elicit complaints from users [6].

Lundberg described 'Brain to Brain' total testing cycle as a nine step process- test order, sample collection, identification, transportation, preparation, analysis, reporting, interpretation and action [7].

The College of American Pathologists (CAP) Q probe studies in different pints of time 1989 [6], 1996 [8], 1998[9], 2002[10], 2004[11] and 2016 [2] have highlighted the dissatisfaction among clinicians from laboratories in particular to TAT.

Serum is the key sample requirement for performing biochemical tests [12] however blood sample needs to be kept for 30 minutes for complete clotting before it can be centrifuged and analyzed. The samples with anticoagulant do not require blood clotting and can be processed quickly resulting in shorter TAT. The plasma also has less interference by fibrin clot, high sample yield from given volume of whole blood. The trend now a day is to use plasma in serum biochemistry [13].

The present study was aimed to investigate the differences between the results obtained for biochemical parameters by lithium heparin and serum gel tube in patients.

MATERIAL & METHODS

The observational study was conducted at a tertiary care hospital in India. There were 100 patients enrolled in the study after institutional ethics committee approval and after taking written informed consent by participants. All the participants were adults. The blood sample was drawn from ante cubital vein using 22 G needle and collected in two vials – Clot activator plain tube red top vial (4 ml serum clot activator tube, Becton Dickinson BD) and Lithium Heparin dark green top vial (

4 ml green anticoagulant tube, Becton Dickinson BD). Serum tube were allowed to clot for 20minutes at room temperature and then centrifuged at 4000rpm for 10 minutes using normal centrifuge. Plasma tubes were centrifuged immediately at 4000 rpm for 10 minutes. 28 routine clinical biochemistry parameters were measured on Siemens Dimension XL200 analyzer according to manufacture instructions. The analytes assayed in the study were Urea, Uric acid, Creatinine, Total Protein, Albumin, Alkaline phosphatase, Total Bilirubin , Direct Bilirubin, Aspartate aminotransferase, Alanine aminotransferase, Calcium, Magnesium, Phosphorus, Iron, Total iron binding capacity(TIBC), Glucose, C-reactive protein, Cholesterol, High density lipoprotein(HDL), Triglyceride, Low density lipoprotein (LDL), Lactate dehydrogenase, sodium, potassium, chloride, Creatinine kinase (CKI), Amylase, and Lipase.

The statistical analysis was done using online free statistical software {Medcalc}. The statistical significance of the differences between results obtained from serum and plasma samples was assessed, by regression analysis and bias % was calculated. P values < 0.5 were considered to be statistically significant.

To determine clinically significant difference, total error (TE) between 2 samples for each test was estimated and then compared with Total allowable error (TEa) limits. If TE was higher than TEa, it was considered as a clinically significant difference.

Bias between the results from 2 samples for each test was computed as follows
BIAS%= {(plasma result- serum result)/ Serum result} x 100
TE calculated from following equation, which was generated by a combination of imprecision and bias
TE= Bias% + 1.65 x CV %

Imprecision for each test was calculated from result of internal quality control (two Different Levels). First the coefficient of variation (CV) for different levels of control samples was separately calculated and then CV of higher value was chosen for TE calculation [14].

TEa goals were selected from multiple sources CLIA, Biological variation (BV), American Association of Bio analysts (AAB) [13]. Correlation analysis was also conducted to define relationship between results from 2 sample types. In correlation analysis, if both test results from serum and plasma were normally distributed, Pearson`s correlation coefficient was used.

RESULTS

Table -1 Summary of comparison for two vials for 28 biochemistry tests

S No	Parameters	Correlation	CV%	BIAS%	TE	TE LIMITS +/- %	Total error reference
1	UREA	0.9847	3.8	0.75	7.02	9	CLIA
2	UA	0.9921	4.5	-0.945	6.48	17	CLIA
3	CRE	0.9927	4.5	-1.42	6.005	16	CLIA
4	TP	0.9791	3.5	0.72	6.495	10	CLIA
5	ALB	0.9471	3.5	0.1	5.875	10	CLIA
6	ALK	0.9986	6.6	-0.69	10.2	30	CLIA
7	T BIL	0.9912	5.6	2.81	12.05	20	CLIA
8	SGOT	0.9991	6.7	-2	9.055	20	CLIA
9	SGPT	0.9995	6.5	-1.47	9.255	20	CLIA
10	CA	0.9727	3.5	0.57	6.345	10	CLIA
11	MG	0.9724	4.5	-0.39	7.035	25	CLIA
12	PH	0.9855	3.3	0.81	6.255	12	CFX
13	CRP	0.9981	3	-2.507	2.443	3	AAB
14	SODIUM	0.9256	1.7	0.3461	3.1511	4	CLIA
15	POTASSIUM	0.9671	0.8	2.202	3.522	5.8	BV
16	CHLORIDE	0.9393	3.5	0.2987	6.0737	5	CLIA
17	CHO	0.9671	4.5	-0.015	7.41	9	CLIA
18	TG	0.9949	4	-1.28	5.32	25	CLIA
19	HDL	0.9773	4.5	-2.094	5.331	30	CLIA
20	LDL	0.9703	4	0.124	6.724	12	CLIA
21	CKI	0.9955	4.5	-1.133	6.292	30	CLIA
22	AMYLASE	0.9966	5	-1.82	6.43	30	CLIA
23	LIPASE	0.9921	5	-0.353	7.897	30	CLIA
24	LDH	0.9921	5.6	-1.9	7.34	20	CLIA
25	IRON	0.9903	8	-2.26	10.94	20	CLIA
26	TIBC	0.9803	8	-2.47	10.73	20	CLIA
27	GLUCOSE	0.9931	3.8	0.013	6.283	10	CLIA
28	D BIL	0.9871	5.1	-2.322	6.093	20	CLIA

CLIA- Clinical Laboratory Improvement Act, CFX-Canadian Fixed limits from the College of Physicians and Surgeons of Saskatchewan, BV- Desirable Quality Specifications based on Biological Variation, AAB- American Association of Bioanalysts

All 28 biochemical parameters showed good correlation (correlation =/> 0.9393) with R value >/= 0.9039, p values > 0.05 (Table-1). Majority of parameters by both sampling mode had concordanr results shown in regression plots – Fig 1-7. The parameter with highest Bias% were- Total Bilirubin (2.81), Potassium (2.202), CRP (-2.507) and with lowest bias were- Albumin (0.1), cholesterol (-0.015), LDL (0.124). The Total Allowable Error limit (TE) for Iron, Total iron binding capacity (TIBC), Total bilirubin & alkaline phosphatase was high but within acceptable range as per CLIA. It was also observed that for renal function tests (urea, Creatinine & electrolytes) the average TAT (from sample collection to reporting) for serum sample was 35 minutes and for plasma sample was 20 minutes. The issue of fibrin clot was not observed in any of the plasma (lithium heparin tube) while it was noticed in 9% (9/100) samples in plain vial. The volume of recovered sample was higher in plasma (lithium heparin) tube than serum (plain) tube.

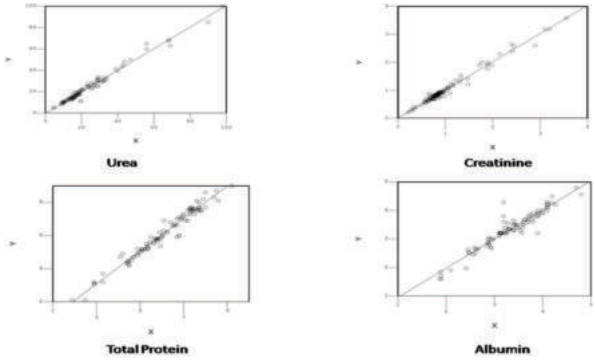

Fig -1 Regression analysis

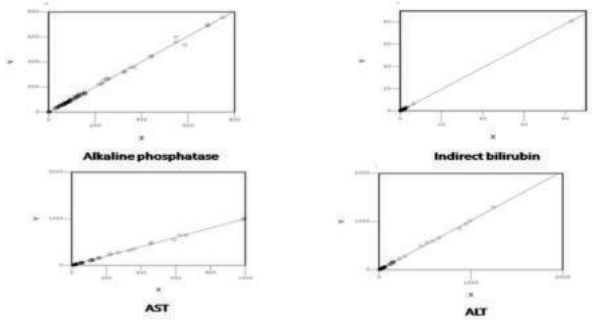

Fig 2 Regression analysis

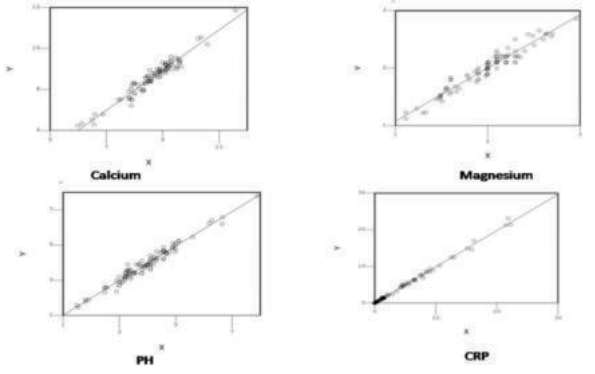

Fig -3 Regression analysis

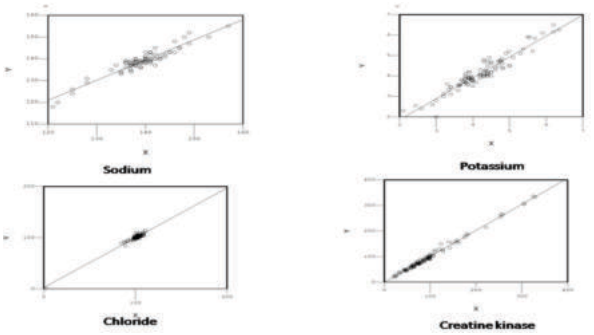

Fig-4 Regression analysis

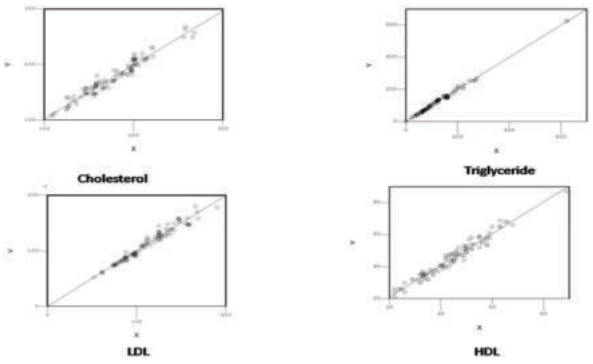

Fig -5 Regression analysis

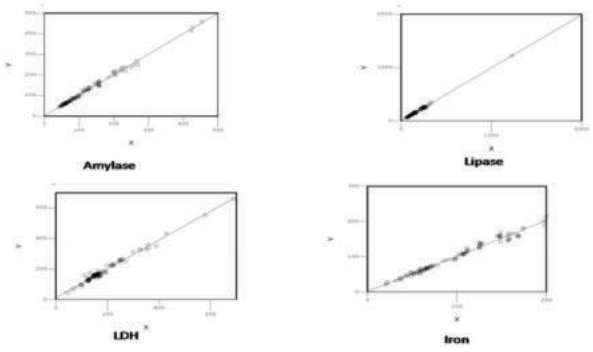

Fig – 6 Regression analysis

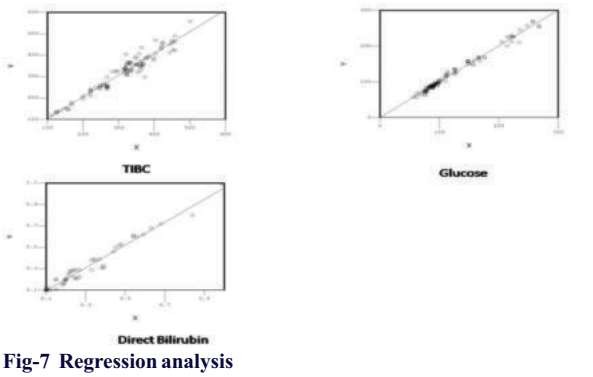

Fig-7 Regression analysis

DISCUSSION

There is a worldwide increase use of plasma as an alternative to serum for use as sample for biochemical analysis [15]. There were 100 test samples for each analyte which were sufficient enough so that the two vials can be compared. The present study gives an opportunity to compare the test results for biochemistry analysis using two different sampling tubes- plain verse lithium heparin anticoagulant. The results of the study show good correlation between testing results obtained by these two different sampling tubes.

In the present study there was reduction in TAT observed for renal function test when lithium heparin was used in comparison to plain tube – 20 minutes & 35 minutes respectively. The findings of the present study are similar to those by O Keane & Cunningham who performed similar analysis for renal function test and lipid profile [16]. Similarly, for lipid profile concordant results were obtained between both vials when samples were run for cholesterol, triglyceride, LDL and HDL, with correlation > 0.9 and TE within acceptable limits as set by CLIA [12]. Herzum et al [17] in their study found Heparin vial to be more suitable for LDH measurement.

The present study findings are similar to studies by Arslanet et al [18], Wei et a[19] and Miles e al[20] who also found good correlation between testing results obtained from two different vials by using patient sample and further estimated bias and compared it with limits of acceptance as per CLIA guidelines like our study.

It is pertinent to mention that in the present study for all 28 biochemistry analytes the total allowable error was within acceptable range as prescribed by CLIA. Whether for analytes with narrow range (<5%) for TE like – CRP, Sodium & chloride or with high TE limits (>10%) like urea, Creatinine , alkaline phosphatase, total bilirubin, SGOT, SGPT, Magnesium, HDL, LDL, LDH, Amylase, Lipase, Iron & TIBC.

Our findings give evidence that assay in clinical biochemistry will be compatible with serum & lithium heparin plasma and can be considered equivalent to plasma for these analytes.

In study by Carey et al [21] the specimen of Lithium Heparin were analyzed after overnight transportation causing variation in results whereas in present study the plasma and serum samples were run immediately within 2 hours and therefore the efficacy of lithium heparin beyond that time could not be ascertained.

The samples collected in plasma have two major issues-insufficient volume and interference by fibrin clot stands. We observed that lithium heparin vial such pitfalls were not seen and yield of sample was higher by 8-10%.

CONCLUSION

If the quality of laboratory results are jeopardized by TAT failures, fibrin issues or sample volume issues, lithium heparin plasma can be considered for collection of sample. This study has shown that most assays in clinical biochemistry are compatible with both serum and Lithuim Heparin plasma. The findings of this study are of great importance especially in case of urgent sample received from ICU, casualty and transplant units where delay in reporting can be detrimental to patient care and Lithium Plasma samples can help to reduce TAT by skipping clotting process.

Conflict of Interest- Nil

Sources of funding –Nil

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