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Original article:

Effect of temperature and serum–clot contact time on the clinical chemistry laboratory results

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Abstract

Introduction- Clot contact time and temperature have variable effect on the stability of laboratory investigations. This study is conducted to establish the maximum time delay acceptable between sample collection and separation of serum and the optimum temperature which should be maintained during this time delay.

Methods and materials- six morning samples from 30 healthy volunteers are collected.3 samples from each volunteer is stored for 0hrs, 3hrs and 6hrs at 230C and another 3 samples at 0 hrs, 3hrs and 6hrs at 320C. stability of electrolytes is analyzed by repeated measure anova.

Results and discussion- stability of electrolytes (sodium and potassium) is not altered when samples are stored at 23^0C. The maximum clot contact times which have no effect the stability of electrolytes is 3hrs.

Keywords- electrolytes, clot contact time, preanalytical variables

Introduction

For most routine assays in clinical chemistry laboratory, serum is the sample (1). Changes in the tests results can be induced by preanalytical, analytical or post analytical variations. To detect the real pathological changes in patients, the preanalytical, analytical and post analytical variations must be reduced to acceptable levels at which they cause no impact on the clinical interpretation of the results. As analytical and post analytical variation have decreased with the advancement of new technologies, automation, standardization and improved laboratory information system the relative contribution of preanalytical variation becomes an important element of overall test reliability (2). Pre analytical phase starts with request for investigation, patient and specimen identification, blood drawing, sample collection and handling and ends with the transportation of specimens to the laboratory.

The time interval between blood collection and sample processing in analyzer is one of the most error prone areas and also the bottleneck of

the turnaround time of the laboratory. The two important time delay processes that occur in this phase is clot contact time and centrifugation delay. Clot contact time is defined as- 'Optimum time interval between sample collection and separation of serum from the clot'. This period should be long enough to allow complete clot formation but should be shorter than the time in which a significant change in the test result is induced by serum –clot contact. A minimum time interval of 20-30mins between blood collection and serum separation is considered acceptable according to Tietz Textbook of Clinical Chemistry (3). Along with this optimum temperature which should be maintained during this period is also an important variable as suggested by different authors (4,5). Among the investigations performed in the laboratory, the parameters which are mostly affected by these preanalytical variables are the serum electrolytes (6-8). Concentration of Sodium, the major extracellular cation is altered in renal injury, brain injury, severe dehydration, diarrhea and excessive urine loss. Similarly potassium the major intracellular cation is altered in electrolyte imbalance, cardiac arrhythmia, diabetes mellitus and hepatic encephalopathy. Therefore in this Endeavour we sought to determine the optimum storage temperature and acceptable maximum delay in serum-clot separation with respect to serum electrolytes.

Materials and methods

This study is conducted in central clinical laboratory of the department of biochemistry of a tertiary care hospital in India. Venupuncture is carried in the morning on 30 healthy volunteers who are on overnight fast by a trained phlebotomist. Resting time of 5 mins and tourniquet placement time of 30sec is employed for each collection. A set of six samples are collected from each volunteer. Samples are collected with needle and plastic adaptor into vacutainers. All the samples are collected in vacutainers of single lot (Becton Dikinson, Franklin Lakes, NJ, USA). The vacutainers from each participant are differentiated based on clot contact period of 30mins, 3 hours and 6 hours and stored for the time at two different temperatures 32^0 C and 23^0C during that time period. All the tubes are centrifuged at 1500RPM for 10mins. All the samples are analyzed on automated clinical chemistry analyzer, vitros 5600 (Ortho Clinical Diagnostics, Inc, Rochester, NY, USA). Dry chemistry is utilized for analysis of electrolytes in this analyzer. The slides for electrolytes are multilayered analytical element coated on a polyester support that uses direct potentiometry for measurement of different ions(9). The slide consists of two ion selective electrodes, each containing a reference layer, silver and a silver chloride layer coated on a polyester support. For potassium the slide contains valinomycin and for sodium methyl monensin. A drop of patient sample and a drop of vitros reference fluid on separate halves of the slide results in migration of both fluid towards the centre of the paper bridge. The potential difference between the two electrodes is proportional to the electrolyte concentration in the sample.

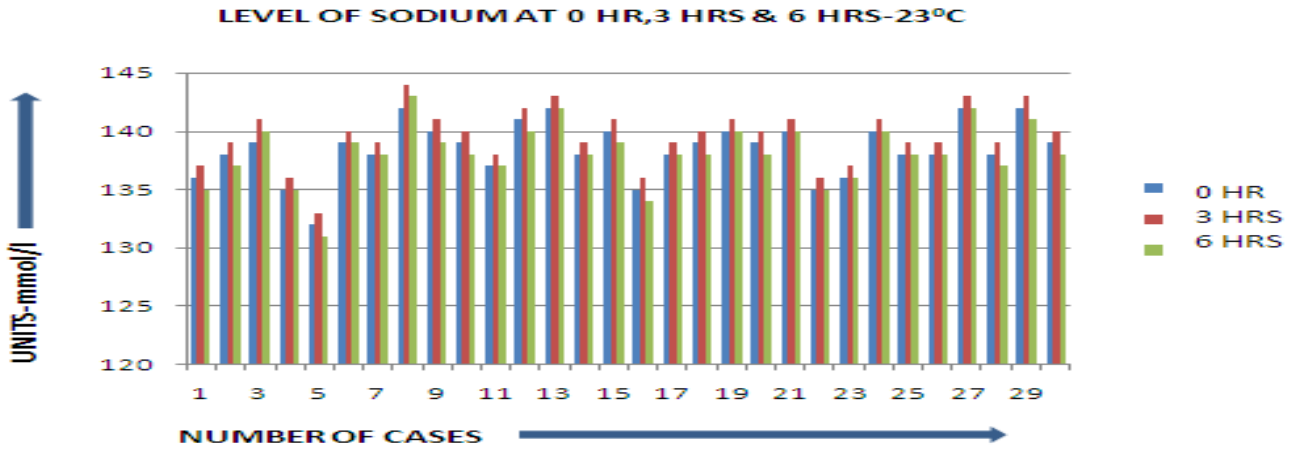
Statistical analysis

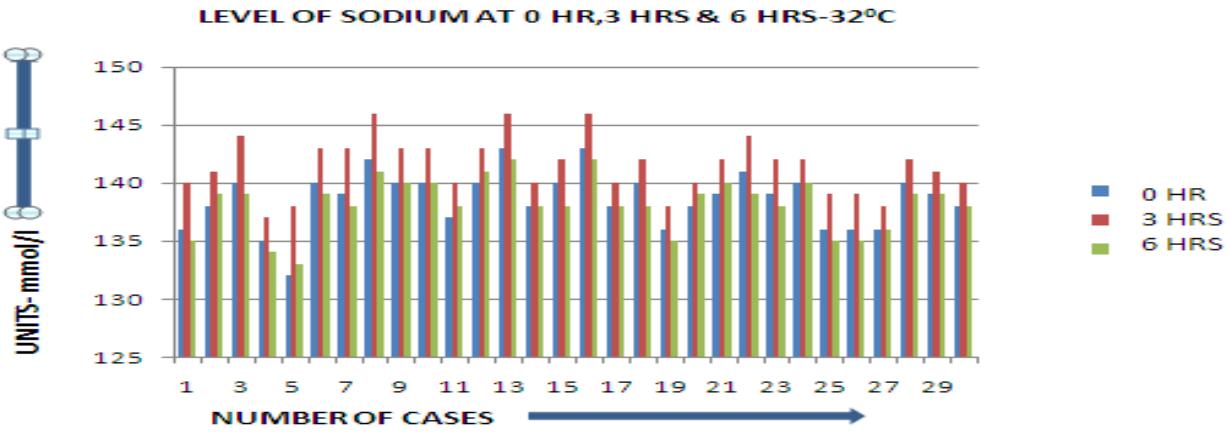
SPSS 19.0 for windows (SPSS Inc, Chicago, IL, USA) is used for statistical analysis. The change in concentration is measured by repeated measure ANOVA. The significance level is set up at $p<0.05$. The mean percentage deviation is compared to the acceptable change limit (ACL) according to ISO 5725-6 (10). The CV was obtained from in house

routine mean data of QC value over 6 months duration

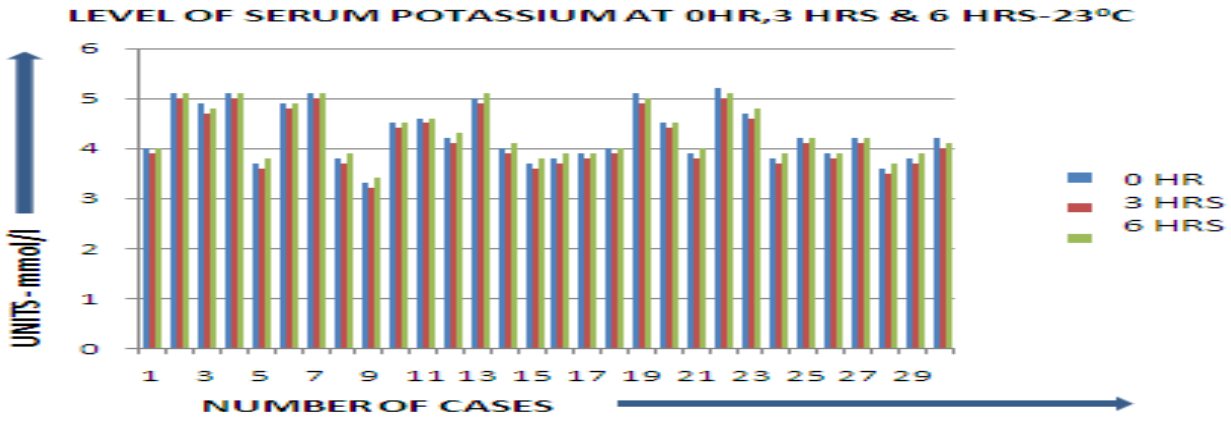
Results and observations

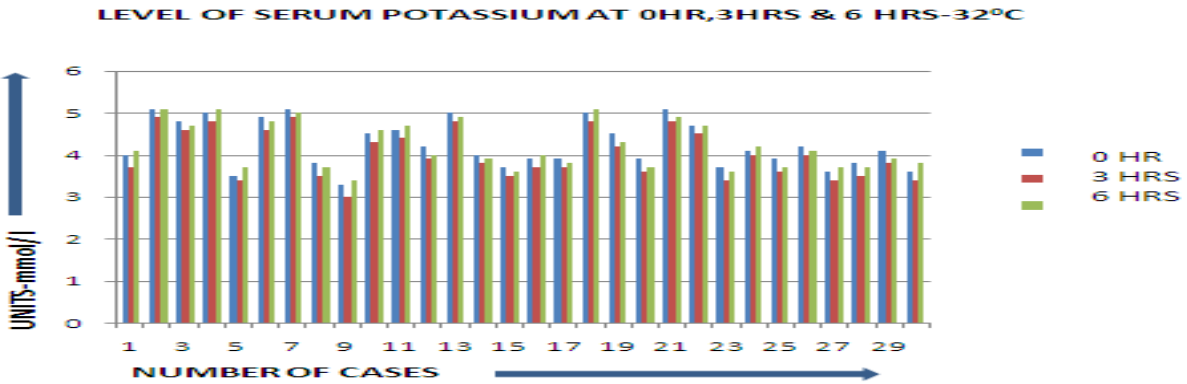
Analysis of variance is done on the values of the serum electrolytes (sodium and potassium) at three different time in hours (0hr,3hrs,6hrs) at each temperature (23°C and 32°C).The difference in the values obtained at different durations at each temperature is found to be statistically significant ($p<0.001$) for each analyte.





SODIUM (137-145 mmol/l)											
UNITS	ANALYTICAL CV% (QC1)	ANALYTICALLY ACCEPTABLE LIMIT (QC1)	ANALYTICAL CV% (QC2)	ANALYTICALLY ACCEPTABLE LIMIT (QC2)	SODIUM MEAN					
					AT 23^{O}C			AT 32^{O}C		
					0H	3H	6H	0H	3H	6H
mmol/l	5.06%	133-163	5.03%	116-142	138.5 ± 0.43.	139.5 ± 0.44	138.1 ± 0.46	138.6 ± 0.44	141.4 ± 0.44	138.2 ± 0.41





POTASSIUM (3.5-5 mmol/l)											
UNITS	ANALYTICAL CV%(QC1)	ANALYTICALLY ACCEPTABLE LIMIT (QC1)	ANALYTICAL CV% (QC2)	ANALYTICALLY ACCEPTABLE LIMIT (QC2)	POTASSIUM MEAN						
					AT 23^{O}C			AT 32^{O}C			
mmol/l	4.93%	3.65-54.6	5%	5.54-6.77	0H	3H	6H	0H	3H	6H	
					4.2 ± 0.1	4.1 ± 0.09	4.3 ± 0.09	4.2 ± 0.1	4.0 ± 0.1	4.2 ± 0.1	

Student's paired t- test is done to substantiate the significance of the difference in values obtained for each analyte at different temperature and time in hours.

For sodium, the effect of storage at 23^{o}C and 32^{o} C is found to be similar in the first 6 hours. The difference in the initial 0 hour values at both the temperatures is found to be statistically insignificant ($p>0.05$). At 32^{o}C, the difference in the sodium values at 0 hour and 3 hours is found to be statistically significant (** $p<0.01$) while the difference in the 0 hour and 3 hours value at 23^{o}C is negligible and statistically insignificant ($p>0.05$).

For potassium, the 0 hour values at both the temperatures is found to be statistically insignificant ($p>0.05$). At 23^{o}C ,the values of potassium obtained at 3 hours and 6 hours when compared to the 0 hour value is not significant ($p>0.05$ for each) while the 3 hours value obtained at 32^{o}C is statistically significant ($p<0.05$). The difference between 3 hour and 6 hour value is also significant at 32^{0} C ($p< 0.05$).

Discussion

The adverse effect on laboratory results of prolonged contact between cells and serum has long been recognized, and immediate separation of serum from cells has always been considered essential to accurately report laboratory tests results. But due to collection of samples in remote location and time delay during the transportation to the laboratory, stability of electrolytes often gets compromised. This study is conducted to estimate the maximum acceptable time delay between sample collection and serum separation as well as to analyze the effect of different temperature on electrolyte stability. During a prolonged contact time between the serum and the clot, both biological activity of the cells and transmembrane diffusion can change the concentration of the analytes in the serum (11).The current recommendation of an acceptable time interval between sample collection and serum separation is 2 hours , a recommendation based on the report of Laessig et al(4). Review of literature reveals many

experimental works done in this field, but because of a variety of experimental designs, the interpretation of information on specimen stability is still not conclusive.

In this experimental study, we tried to find the effect of temperature and duration of serum-clot time on the measured concentration of serum sodium and potassium, determine an optimum temperature for storage of the samples as well as work out an acceptable maximum delay in serum-clot separation with respect to each electrolyte in our laboratory settings.

In estimation of potassium levels, an initial decrease at 3 hours followed by increase at 6 hours is noticed at both the temperatures (23^{O}C and 32^{O}C) though the difference is negligible for the samples stored at 23^{o}C.The difference is significant for the samples stored at 32^{o}C at 3 hours. The change in the potassium levels is the net effect of glycolysis which moves the potassium into the cells, and passive diffusion which allows potassium to diffuse out of the cells (13,14). Initially glycolysis is very fast, thus the serum potassium concentration decreased significantly. But as time went on, glucose in the serum is depleted, and passive diffusion of potassium from cells became dominant producing an increase in the levels after long serum –clot contact. This finding is in agreement with the report by Zhang et al(16).The stability of potassium is also found to be sensitive to temperature in accordance to different studies(6,7,15).

For sodium ,the effects of storage at 23^{O}Cand 32^{O}C is found to be similar in the first 6 hours , though the changes at 32^{o}C is found to be more significant than at 23^{O}C.An initial increase at 3 hours duration is noticed followed by decrease in the levels at 6 hours duration. This probably may be due to the activity of the sodium potassium pump which is active while glycolysis is dominant and is inhibited after the glucose in the serum is depleted. The decrease in sodium levels at 6 hours may be due to the effect of passive diffusion into the cells. As found in our study the changes in the sodium levels is sensitive to temperature change in accordance to the report by Ono et.al.(5).

In conclusion, the stability of both the analytes (sodium and potassium) is found to be sensitive to temperature. At 32^{o}C, the stability of both the analytes is found to be significantly sensitive to the duration of storage of the samples as well. Thus it is advisable to separate the serum from the clot preferably before 3 hours and maintain the temperature at 23^{o}C when prolonged storage occurs inadvertently or is unavoidable. We also suggest that further studies with large sample size are needed in this field to conclusively set up guidelines that would assist the laboratories in identifying the specimens that are unsuitable for analysis because of prolonged storage.

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