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Preparation of a Quality Sample: Effect of Centrifugation Time on Stat Clinical Chemistry Testing

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Abstract

Background: Centrifugation time is a major bottleneck in laboratory specimen throughput. In most cases, 10 minutes is suggested to centrifuge samples on a swing-out rotor centrifuge, at room temperature, with a relative centrifugal force of 1,200±100 *g*; and 1,300 *g* or centrifugation times longer than 10 to 15 minutes may be advisable to get a better platelet clearance. Nevertheless, there is little evidence supporting the influence of different centrifuge times for primary lithium-heparin tubes with plasma separator on stat clinical chemistry testing.

Methods: Five evacuated tubes collected from 10 consecutive subjects were centrifuged on an identical swing bucket centrifuge at 1,200 *g* for 1, 2, 5, 10, and 15 minutes respectively and tested for clinical chemistry stat analytes.

Results: Statistically significant variations from the 15-minute centrifuge reference specimen were observed for ALT, calcium, glucose, potassium, urea nitrogen, and CK-MB (1 minute centrifugation), ALT, glucose, urea nitrogen, and CK-MB (2 minutes centrifugation), and glucose (5 and 10 minutes centrifugation). Meaningful biases according to the analytical quality specifi-cations for clinically-allowable variance were recorded for ALT, calcium, glucose, potassium (1 minute centrifugation), and for ALT, glucose, and potassium (2 minutes centrifugation).

Conclusion: We confirm that a 10-minute centrifugation time at 1,200 *g* on a swing bucket centrifuge for samples collected in plastic tubes with a plasma separator may be suitable for stat clinical chemistry testing.

The turnaround time (TAT), conventionally defined as the time elapsed from specimen collection to the caregiver results reporting, is a critical parameter of quality and effectiveness, particularly in clinical laboratories performing stat analyses.[1] Along with specimen transport, sample analysis, and results reporting, centrifugation time is a major bottleneck in laboratory specimen throughput. About 38.8% of the delays in the preanalytic and analytic phases of the entire testing process are caused by technical issues, including specimen processing and preparation, difficulty with the instrument, and specimen-associated delays.[2] This issue should be regarded with special concern, as results delivery outside the specified time is increasingly integrated among the quality indicators and specifications of the extra-analytical phase, and it often causes deterioration in stakeholders' satisfaction with laboratory services.[2-4]

Clinical decisions based on laboratory test values are correctly made when each process of the pre-analytical stage is properly performed. Although reduced centrifuge time would contribute to abbreviate the batch processing procedure, it may result in an incomplete gel barrier, and the content of serum and plasma separators may produce significant analytical interference.[5-9] At variance with coagulation testing, which requires the analyses to be performed on platelet-poor plasma (PPP) (platelet count $<10\times10^9$/L),[10] the current laboratory practice on the minimal centrifuge time necessary to obtain complete separation of plasma from blood cells for clinical chemistry testing is heterogeneous among clinical laboratories and mostly based on local empirical observations. When using primary lithium-heparin tubes with plasma separator, the manufacturer's literature provides indicative guidelines. In most cases it is suggested to centrifuge the samples on a swing-out rotor centrifuge, at room temperature, for at least 10 minutes and with a relative centrifugal force (RCF) of 1,200±100 *g*. Relative centrifugal forces higher than 1,300 *g,* or centrifugation times longer than 10 to 15 minutes may be advisable to get a better platelet clearance.[11,12] Nevertheless, there is little evidence so far on the influence of different centrifuge times for primary lithium-heparin tubes with plasma separator on stat clinical chemistry testing.[4]

Materials and Methods

A single practiced phlebotomist collected blood from 10 healthy volunteer physicians using a 20-gauge, 0.80 × 19 mm Venoject multisample straight needle (Terumo Europe, NV) directly into 5 sequential 3.5 mL evacuated tubes containing Gel + 52.5 USP Lithium Heparin (Terumo Europe, Haasrode, Belgium). Venipunctures were performed in the morning of the same day on fasting subjects, who had given informed consent. Venous accesses were straightforward in all cases, and no hemolyzed or lipemic specimens were encountered. The 5 tubes collected from each subject were gently inverted 6 to 8 times and immediately centrifuged at 1,200 *g*, at room temperature (21°C), for 1, 2, 5, 10, and 15 minutes, respectively. The 5 centrifugation procedures were performed sequentially on an identical swing bucket centrifuge (Varifuge 3.2RS, Heraeus Instruments). After centrifugation, plasma was separated and immediately analyzed. The concentrations of alanine aminotransferase (ALT), albumin, α-amylase pancreatic, total bilirubin, calcium, creatinine, glucose, and urea nitrogen were determined by enzymatic procedures on a Roche/Hitachi Modular System P (Roche Diagnostics

GmbH, Mannheim, Germany), according to the manufacturer's specifications and using proprietary reagents. Sodium, chloride, and potassium were measured on a Roche/Hitachi Modular System using indirect ion-selective electrode methods. Cardiospecific troponin T (cTnT) and creatine kinase isoenzyme MB (CK-MB) were assayed on the Elecsys 2010 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Hematological measurements on plasma were performed on the Advia 120 automated hematology analyzer (Bayer Diagnostics, Newbury, Berkshire, UK). Total imprecision, as expressed by the coefficient of variation (CV), is less than 2.5% for the analytes tested on the Modular System P and less than 10% for cTnT and CK-MB. All measurements were performed in duplicate within a single analytical session, and results were finally reported as the mean of paired measurements. Results were further compared with the current available analytical quality specifications for clinically allowable variance, as expressed by the desirable bias[13]. The significance of differences was assessed by paired Student t-test; the level of statistical significance was set at $P<0.05$.

Results and Discussion

Results of the present investigation are shown in **Table 1** and **Figure 1**. The 15-minute centrifuge specimen was considered as the reference, as currently suggested by the manufacturer. The centrifugation time was inversely associated with the residual blood cell elements measured in the plasma. The amount of platelets in plasma increased significantly in specimens centrifuged for 10 minutes or less, whereas red blood cell and white blood cell counts increased significantly in samples centrifuged for 2 minutes or less and for 1 minute, respectively. Statistically-significant variations from the 15-minute centrifuge specimens were observed for ALT, calcium, glucose, potassium, urea nitrogen, and CK-MB in the 1-minute centrifugation samples; ALT, glucose, urea nitrogen, and CK-MB in the 2-minute centrifugation samples; and glucose in either the 5-minute or 10-minute centrifuged samples. Meaningful biases, as related to the analytical quality specifications for clinically-allowable variance,[13] were observed for ALT, calcium, glucose, and potassium in the 1-minute centrifugation specimens, and for ALT, glucose, and potassium in the 2-minute centrifugation specimens. No false positive results were observed for cTnT, as no plasma specimen displayed concentrations exceeding the analytical sensitivity of the assay (0.01 µg/L).

Laboratory TAT for stat testing has remained unchanged for a long time and emergency department physicians are not always satisfied with this type of laboratory service.[14] Most of the delays attributed to the stat laboratory do not occur in the testing phase, but rather during those preanalytical processes that have a queue. Accordingly, specimen preparation, when required, is a well-recognized cause of prolonged TAT, which may contribute to lessen stakeholders' satisfaction with the laboratory service.[14,15] Centrifuges are commonly used to separate components of a mixture on the basis of particle size or density. The most frequent laboratory application is the separation of blood into cells and a serum or plasma supernatant. Each application requires specific centrifugal forces and defined time periods.[4] The introduction of plastic separator tubes, including a gel that serves as a barrier between plasma and the cellular elements, has provided several practical advantages, such as reduced centrifuge time, the use of primary collection tubes for testing, increased sample stability, decreased breakage hazard, and suitability for disposal by incineration.[7] Besides the manufacturer's suggestions,[11,12] there is no definitive information on the minimal centrifuge time on a conventional centrifuge required to obtain suitable plasma specimens for stat clinical chemistry testing using primary gel plasma tubes.[4]

Hypothetically, there are 4 major solutions that may be pursued to reduce the burden of the centrifuge time on the TAT for stat clinical chemistry testing: increase the speed, reduce the time, introduce innovative separation techniques, or abolish this critical preanalytical step.

Table 1_Stat Clinical Chemistry and Hematological Testing on Lithium Heparin + Gel Primary Collection Tubes (n = 10) Centrifuged at 1,200 *g* for 15, 10, 5, 2 and 1 Minute in a Conventional Swing Bucket Centrifuge.

	Clinically-Allowable Variance[13]	Centrifuge Time				
		15 min	10 min	5 min	2 min	1 min
White blood cells, 10^9/L		0.03±0.01	0.04±0.01	0.04±0.01	0.04±0.02	1.06±1.12†
Red blood cells, 10^{12}/L		0.01±0.01	0.01±0.01	0.01±0.01	0.03±0.01‡	0.06±0.02‡
Platelets, 10^9/L		20±9	39±14‡	127±52‡	316±112‡	434±149‡
Alanine aminotransferase, U/L	±12%	21±7	21±7	20±7	18±8‡	15±8‡
Albumin, g/L	±1.3%	44.3±1.6	44.3±1.6	44.3±1.7	44.3±1.6	44.0±1.6
α-amylase pancreatic, U/L	±8.0%	22.4±12.0	22.2±11.9	22.3±12.0	22.3±11.8	22.5±12.1
Bilirubin total, µmol/L	±10%	9.0±2.8	9.0±2.8	9.0±2.8	9.0±2.8	8.8±2.8
Calcium, mmol/L	±0.8%	2.31±0.06	2.31±0.06	2.31±0.06	2.32±0.07	2.34±0.06†
Chloride, mmol/L	±0.5%	103.8±1.8	103.8±1.8	103.8±1.8	103.8±1.8	103.8±2.0
Creatinine, µmol/L	±3.4%	66±8	67±8	67±8	67±8	67±8
Glucose, mmol/L	±2.2%	4.75±0.44	4.79±0.44†	4.83±0.43‡	4.92±0.40‡	5.00±0.38‡
Potassium, mmol/L	±1.8%	3.96±0.25	3.96±0.25	3.97±0.23	3.98±0.22	4.02±0.22†
Sodium, mmol/L	±0.3%	104.3±2.1	104.3±2.1	104.3±1.9	104.3±2.0	104.3±1.9
Urea nitrogen, mmol/L	±5.5%	4.75±0.89	4.76±0.96	4.75±0.92	4.86±0.88†	4.94±0.88‡
Creatine kinase MB, µg/L	±16%	3.46±1.21	3.45±1.22	3.28±1.17	3.17±1.13†	3.04±1.10†
Cardiac troponin T, µg/L	—	<0.01	<0.01	<0.01	<0.01	<0.01

Results are expressed and mean ± standard deviation. Differences from the reference 15-minute centrifuge specimens are evaluated by paired Student t-test († P <0.05, ‡ P <0.01).

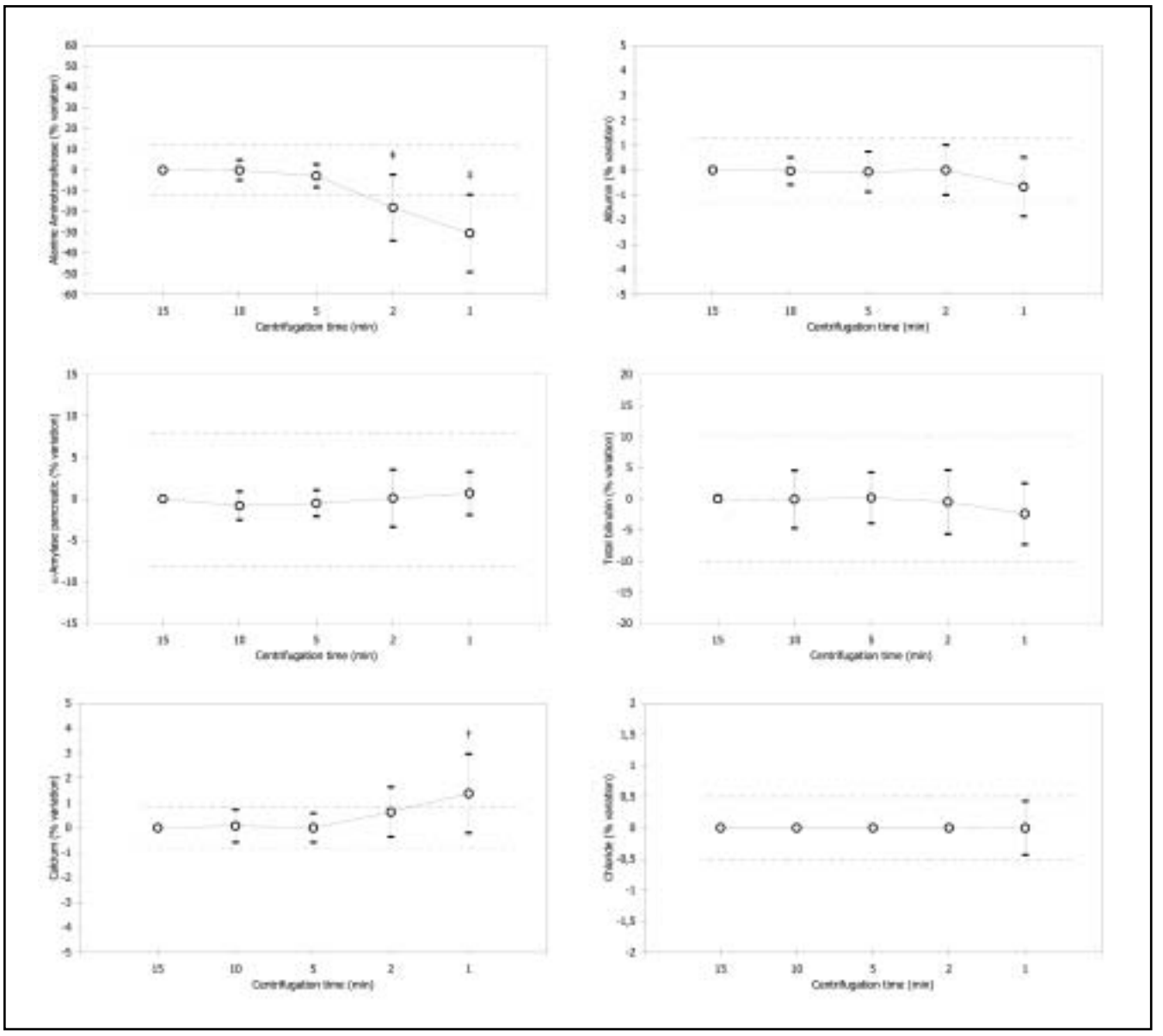

Figure 1_Stat clinical chemistry testing on lithium heparin + gel primary collection tubes (n=10) centrifuged at 1,200 *g* for 15, 10, 5, 2 and 1 mininute in a conventional swing bucket centrifuge. Results are expressed as percentage differences (mean ± standard deviation) from the reference 15-minute centrifuge samples. Differences are evaluated by paired Student t-test (†P <0.05, ‡P <0.01) and compared with the current analytical quality specifications for clinically-allowable variance derived from biological variation[13] (dotted lines).

Although it has been demonstrated that centrifugation periods shorter than 3 minutes at 13,600 *g* effectively clear plasma of cellular components at high plasma yield,[16] RCFs over 1,300 *g* are not recommended as they may be associated with analytical and biological interferences from hemolysis, leukocyte, and platelet activation.[11,12,17] Axial separation is an innovative, cost-effective alternative to conventional centrifugation; however, it is incompatible with the wide variety of specimen containers available in the market and allows centrifugation of specimens 1 at a time.[15] In the future, advanced integrated preanalytical units, combined technologies based on innovative centrifugation methods coupled with analytic platforms may offer substantial time-saving improvements, enabling sample separation within a few minutes.[18,19] Nevertheless, the present investigation was designed to establish a minimal suitable centrifuge time for samples designed for stat clinical chemistry testing, with the aim to improve the TAT thresholds. Our results confirm that, when using a swing bucket centrifuge, a 10-minute centrifugation time at 1,200 *g* for samples collected in plastic tubes with a plasma separator may be suitable for stat clinical chemistry testing.[4] Although shorter procedures, from 2 to 5 minutes at 1,200 *g,* may be sufficient for enabling the reliable testing of some stat analytes (albumin, α-amylase pancreatic, total bilirubin, calcium, chloride, creatinine, and sodium), they cannot be widely recommended, due to the observed biases occurring from interference of residual blood cell elements or content of the plasma separator[8,9] in the measurement of ALT, glucose, potassium, urea nitrogen, and CK-MB. Although these findings have practical applications in our experimental conditions (lithium heparin + gel Terumo tubes and Roche Modular P clinical chemistry analyzer), they

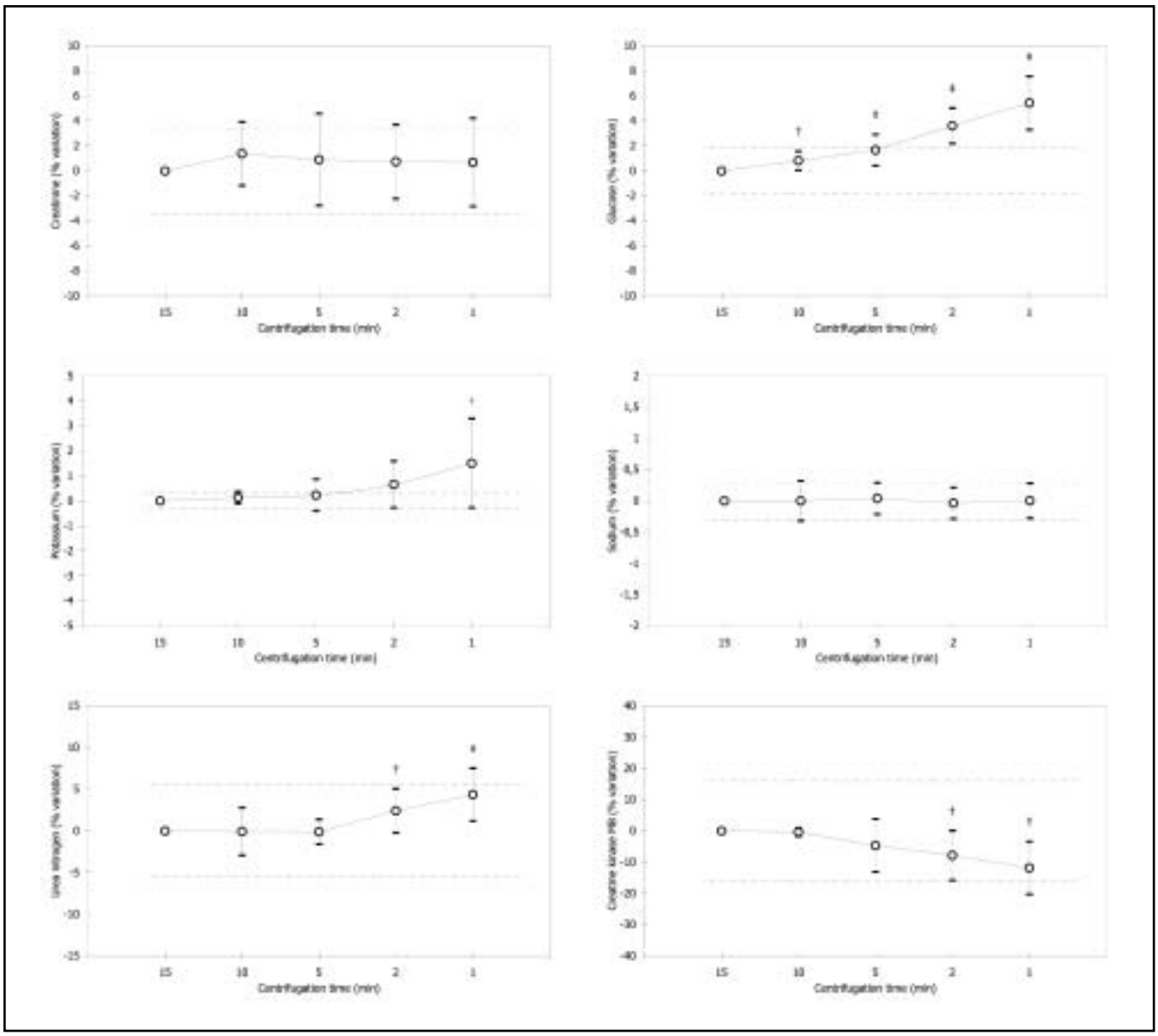

Figure 1_Continued.

may also serve as a rational basis for additional investigations aimed to establish the most suited local centrifugation procedures for the preparation of quality specimens.

Several activities of the preanalytical phase are critical for the containment of the TAT, especially for stat analyses.[2] A further contraction of these processes would improve the global laboratory efficiency, though assay interferences resulting from unsuitable handling of blood specimens, including an inappropriate centrifuge time, can present challenges to clinical laboratories.[9] Emerging whole-blood analyzers fully compatible with the conventional laboratory technology[20,21] will have the potential to abolish the time required for specimen clotting and centrifugation, providing a substantial improvement in the TAT when compared with the traditional centralized laboratory testing. LM

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