

# Evaluation of an Abbreviated Centrifugation Protocol for Chemistry Testing

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## Abstract

**Background:** Processing blood specimens when they arrive in the laboratory is a necessary step for performing most chemistry tests that adds significantly to the total turnaround time (TAT).

**Methods:** We investigated the analytical effects of an abbreviated 4-minute centrifugation on common analytes. We also

assessed the effects of different centrifugation times on the TAT of results.

**Results:** No significant differences in analytical accuracy or precision were observed when centrifugation time was reduced. Reduced centrifugation time resulted in significant improvements in TAT, with a median TAT for STAT results decreasing from 38 minutes to 28 minutes.

**Conclusion:** Our results demonstrate that a shorter centrifugation time does not adversely affect the analytical accuracy or precision of a variety of assays. Furthermore, marked reductions in the TAT of chemistry results are achieved by using a shorter centrifugation time.

**Keywords:** centrifugation, automation, turnaround time

Most clinical laboratories are constantly pressured to deliver results more quickly. A recent College of American Pathologists Q-probes study<sup>1</sup> found that most physician dissatisfaction with laboratory services resulted from the turnaround time (TAT) of results. Of note, the preanalytical phase of testing often contributes significantly more to the total TAT than either the analytical or postanalytical phases.<sup>2</sup> Unfortunately, many steps in the preanalytical phase, such as specimen collection and transport to the laboratory, often are beyond the laboratory's direct control.

Specimen preparation is a preanalytical process that can be controlled by the laboratory. A viable option to improve sample handling in the laboratory is automation. Installation of total laboratory automation systems has been shown to dramatically improve laboratory TAT and clinical throughput.<sup>3,4</sup> However, even when using automation, centrifugation is still a lengthy step in specimen processing that can take as long or longer than the analysis of the specimen.

Manufacturers of blood collection tubes typically recommend centrifugation for 10 to 15 minutes.<sup>5,6</sup> Hence, the goal of a total TAT of less than 1 hour from order to result is difficult to meet with critical analytes because as much as 25% of the allotted time is consumed by centrifugation.<sup>7</sup>

A few studies<sup>8-10</sup> have noted equivalent analytical accuracy for chemistry assays when using shorter centrifugation times

when specimens are processed manually. In this study, we report the effect of varying centrifugation times on the accuracy and precision of chemistry assays, TAT, and specimen stability using an automated specimen processing system for chemistry testing.

## Materials and Methods

Two pairs of venous blood samples were collected from 20 healthy volunteers into serum separator tubes (SSTs) and plasma separator tubes (PSTs) (Becton Dickinson [BD], Franklin Lakes, NJ) and immediately mixed by proper inversion. The SST specimens were allowed to clot for 30 minutes before processing. One tube from each pair was randomly assigned to the standard or the abbreviated centrifugation protocol. The standard protocol called for centrifugation for 10 minutes at 1600 × g, using a Drucker 642VES tabletop centrifuge (The Drucker Company, Port Matilda, PA). The specimens were then manually loaded onto the appropriate instruments. The abbreviated centrifugation protocol was performed by loading tubes onto the automation line where they were then centrifuged for 4 minutes at 1900g by the Power Processor Sample-Handling System (Beckman Coulter, Brea, CA).

One pair of tubes was used to assess analytical accuracy by performing a panel of tests, including a variety of testing methods and manufacturers used in the chemistry section. Routine chemistry assays were performed using the UniCel DxC 800, a SYNCHRON clinical system (Beckman Coulter), and immunoassay by the UniCel DxI 800 (Beckman Coulter) and ADVIA Centaur XP (Siemens Healthcare Diagnostics, Deerfield, IL) systems. This panel included serum tests performed by the DxI 800 (prostate-specific antigen [PSA], thyroglobulin, and cortisol) and the Centaur XP (free thyroxine [T4] and thyroglobulin antibodies) systems as well as plasma tests performed by the DxI 800 (ferritin and transferrin), Centaur XP (troponin) and DxC 800 (sodium, potassium, chloride, total calcium, CO<sub>2</sub>, glucose, blood urea nitrogen, creatinine, iron, magnesium, phosphorus, and lactate dehydrogenase) systems.

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## Abbreviations

TAT, turnaround time; SST, serum separator tube; PST, plasma separator tube; PSA, prostate-specific antigen; T4, thyroxine; C3, complement component 3; LDH, lactate dehydrogenase

A small amount of plasma from each PST remained after the testing was performed. To assess the stability of the gel barrier, these tubes were stored refrigerated (2°-8° C) for 48 hours and then re-assayed for potassium, glucose, and lactate dehydrogenase.

A more limited panel of tests was performed on the contents of the second pair of tubes to determine the effect of centrifugation on analytical precision. This panel included serum tests performed by the DxI 800 (PSA, thyroglobulin, and cortisol) and Centaur XP (free T4), as well as plasma assays performed on the DxI 800 (TSH), Centaur XP (troponin), and DxC 800 (C-reactive protein, potassium, lactate dehydrogenase, complement component 3 [C3]). Due to limited specimen volume, each assay could be repeated only six times.

The DxC 800 platform is used to perform a spectrophotometric analysis of each specimen to determine serum indices for hemolysis, icterus, and lipemia. Although the indices are not rigorously calibrated, a result of zero for hemolysis suggests the presence of less than 50 mg/dL of free hemoglobin, according to the manufacturer. The equivalence of analytical accuracy was assessed by the paired *t* test; the equivalence of precision was assessed by the *f* test using Microsoft Excel, version 2010 (Microsoft, Redmond, WA).

The TAT for each specimen was calculated as the time from receipt in the laboratory until a result was verified. The TAT of sodium results was selected to represent overall TAT. During the study period, nearly all chemistry samples (95%-97%) were processed by the automation line with auto-verification of the results. No significant changes in procedures or serious instrument issues occurred during the periods in which TAT was calculated. Outliers were defined as results that took longer than 45 minutes to obtain.

The typical time for centrifugation was 10 minutes for samples processed using the automated chemistry system. The default centrifugation time for the automation line was increased to 12 minutes for a 2-week trial period to evaluate the effect on immunoassay results. No appreciable analytical effect on these results was observed (data not shown), so the centrifugation time was reverted to 10 minutes. Soon thereafter, centrifugation time was decreased to 8 minutes for a 2-week trial period to observe any effect on TAT but a thorough evaluation of analytical equivalence was not performed. Based on favorable TAT results at 8 minutes, a rigorous evaluation of the analytical equivalence of a 4-minute centrifugation was undertaken.

## Results

Paired samples centrifuged for 4 minutes yielded statistically equivalent results to those from samples centrifuged for 10 minutes for all analytes tested ( $P > .05$  for all). The average difference between samples was less than 1% for 16 of 20 analytes and less than 3% for the others, with a balance of positive and negative differences (Table 1). After 48 hours of storage, no significant differences were noted for potassium, glucose, or lactate dehydrogenase [LDH] between the groups, with the average of all differences less than 3% ( $P > .05$  for all; data not shown). Similar and clinically acceptable analytical precision was found for all analytes regardless of centrifugation time ( $P > .05$  for all; Table 2). The hemolytic index for all specimens was zero.

Changes in centrifugation time affected the median TAT and the percentage of TAT outliers. The observed change in median TAT was always greater than the concurrent change in centrifugation time (Table 3). Furthermore, the relationship between change in median TAT and centrifugation time was not linear, with larger relative changes occurring when the centrifugation time was longer.

**Table 1 Differences in Paired Results of Selected Analytes Using 4- or 10-Minute Centrifugation**

Test	Sample	Method	Paired <i>t</i> -test	Avg. diff
Cortisol	serum	IA	0.34	2.1%
Free T4	serum	IA	0.31	-2.7%
PSA	serum	IA	0.71	0.1%
Thyroglobulin	serum	IA	0.92	-0.6%
Tg antibody	serum	IA	0.39	-0.2%
Ferritin	plasma	IA	0.52	-0.6%
Transferrin	plasma	IA	0.59	-0.4%
Troponin	plasma	IA	1.00	0.0%
CO <sub>2</sub>	plasma	ISE	0.68	-0.4%
Chloride	plasma	ISE	0.42	0.4%
Potassium	plasma	ISE	0.38	1.5%
Sodium	plasma	ISE	0.51	-0.3%
BUN	plasma	enz.	0.68	-0.1%
Glucose	plasma	enz.	0.70	0.5%
LDH	plasma	enz.	0.31	1.6%
Calcium	plasma	color	0.27	-0.5%
Creatinine	plasma	color	0.94	0.5%
Iron	plasma	color	0.68	-0.2%
Magnesium	plasma	color	0.59	-0.4%
Phosphorus	plasma	color	0.34	0.2%

IA, immunoassay; ISE, ion-specific electrode; enz., enzymatic; color, colorimetric.

**Table 2 Differences in Precision for Selected Analytes Using 4- or 10-Minute Centrifugation**

Test	Sample	Method	<i>f</i> -test	CV% 4 / 10 min
Cortisol	serum	IA	0.47	2.5 / 3.7
Free T4	serum	IA	0.63	1.8 / 2.4
PSA	serum	IA	0.30	1.2 / 1.4
Thyroglobulin	serum	IA	0.21	1.2 / 2.3
TSH	plasma	IA	0.84	2.7 / 3.0
Troponin	plasma	IA	1.00	0.0 / 0.0
C3	plasma	turbid	0.70	0.9 / 0.8
CRP	plasma	turbid	0.73	1.2 / 1.3
LDH	plasma	enz.	0.64	2.1 / 1.7
Potassium	plasma	ISE	1.00	1.0 / 1.0

IA, immunoassay; ISE, ion-specific electrode; enz., enzymatic; color, colorimetric.

**Table 3 Effects of Changing Centrifugation Times on Chemistry Turnaround Time Performance**

Time	Routine TAT			STAT TAT		
	Median	TAT/cfuge*	Outliers	Median	TAT/cfuge*	Outliers
12 min	45	2.50	52%	42	2.00	47%
10 min	40	—	48%	38	—	35%
8 min	37	1.50	37%	34	2.00	28%
4 min	32	1.33	30%	28	1.67	12%

\*Change in median TAT (min) / change in centrifugation (min) compared to 10-minute condition.

Outliers = results taking > 45 min from received to verified.

## Discussion

This study examined the potential effects of alternate centrifugation conditions when using BD PSTs and SSTs and performing a variety of assays on common chemistry and immunochemistry platforms. We found no significant difference in analytical accuracy, precision, or stability of specimens processed with a shorter, slightly higher-force centrifugation compared to the standard centrifugation protocol.

Unlike those in previous studies, specimens in this study were centrifuged for 4 minutes using the automation line, just as patient specimens are processed. This allowed for a real-world comparison of shorter centrifugation times to the theoretically ideal condition of longer centrifugation times occurring off the automation line. Despite all these potential differences in processing, we could not demonstrate any analytical difference in the results obtained using different centrifugation conditions.

These findings are consistent with those from other studies<sup>8-10</sup> that previously demonstrated equivalent analytical accuracy using alternate centrifugation with other collection tubes and instruments. The distinction between collection tube manufacturers is an important consideration when evaluating alternate centrifugation conditions. For example, 2 commonly used collection tubes in the United States utilize chemically distinct formulations of separation gel and recommend different centrifugation conditions (10 minutes at 1000-1300 × *g* for the BD tubes; and 15 minutes at 1800-2200 × *g* for the tubes manufactured by Greiner Bio One International AG [Kremsmuenster, Austria]). Given the large disparity in recommended centrifugation conditions, results from studies using a particular type of collection tube may not be generalizable to collection tubes from different manufacturers.

One previous study<sup>8</sup> examined the effect of a 3-minute, 4400*g* centrifugation protocol compared with a 20-minute, 1500 × *g* centrifugation protocol on a small number of immunoassays performed using the Siemens ACS:180 SE (Siemens Healthcare Diagnostics). Paired venous blood samples were collected from healthy volunteers into BD PSTs and spiked with desired amounts of analytes before centrifugation. No significant difference was found in recovery between the specimens processed using the different centrifugation protocols.

Another study<sup>10</sup> examined the difference between 3 different centrifugation protocols: 15 minutes, 2180 × *g*; 10 minutes, 2180 × *g*; and 7 minutes, 1870 × *g*. Specimens were collected from inpatients, placed into Greiner Bio One lithium heparin with gel tubes, and extensively tested using a Cobas 6000 analyzer (F. Hoffmann-La Roche, Rotkreuz, Switzerland), including many general chemistry tests and immunoassays. The authors concluded there were no differences in results between the centrifugation conditions.

A third study<sup>9</sup> examined the effect of an even broader range of centrifugation conditions, namely, 1, 2, 5, 10, or 15 minutes at 1200 × *g*. This study was unique in that, unlike the other studies cited in this report, the centrifugation time was varied while centrifugal force remained constant. Specimens were collected in Terumo lithium heparin with gel tubes (Terumo Corporation, Tokyo, Japan), and analyzed for a limited number of general chemistry and immunoassay analytes using a Roche Modular P system and a Roche Elecsys 2010 (F. Hoffmann-La Roche). Seven of 13 analytes showed no statistical difference in results even when specimens were

centrifuged for as little as 1 minute. Statistically significant differences between alanine aminotransferase (ALT), calcium, glucose, potassium, urea nitrogen, and creatine kinase-MB results were noted in specimens centrifuged for less than 5 minutes. Of interest, although statistically significant, the magnitude of these differences was surprisingly small for calcium, glucose, potassium, and urea nitrogen even when comparing a 1-minute to 15-minute centrifugation time: 1.3%, 5.1%, 1.5%, and 3.9%, respectively.

A potential concern when performing higher-force centrifugation is red cell hemolysis. In this study, the hemolytic index for all specimens was zero, equivalent to less than 50 mg/dL of free hemoglobin. This finding suggests that no significant amount of *in vitro* hemolysis occurred with the increased force. A limitation of this study is that only healthy volunteers were used. It is possible that the more fragile red cells of seriously ill patients may be hemolyzed to a greater extent at increased force. However, a study using specimens collected from patients using the BD Vacutainer SST II Advance collection tube (BD) showed no difference in measured free plasma hemoglobin using a 13-minute, 1700*g* or a 5-minute, 3000*g* centrifugation protocol.<sup>11</sup> It seems unlikely that increased hemolysis would occur with patient specimens using our abbreviated protocol.

Another potential concern with shorter centrifugation is that it may compromise analytical precision overall by increasing the number of outlier results. Despite current technology, unreproducible, spuriously elevated results can occur, especially when using immunoassays.<sup>12</sup> In this study, no difference was observed in the variability of results between centrifugation protocols. Furthermore, no outlier results were observed for any of the more than 2000 assays performed using the shorter-time, higher-force tubes.

Sample stability and integrity is a consistent concern with storage of processed tubes for later add-on testing. Nearly all tubes sent to our laboratory contain a gel separator; however, a gel layer that is improperly formed during centrifugation may fail to maintain an effective barrier between the supernatant serum or plasma and underlying red cells. Also, improperly centrifuged specimens may have cellular elements above the gel, contaminating the serum or plasma.<sup>9</sup>

In this study, centrifuged BD PSTs stored for 48 hours showed equivalent stability of potassium, glucose, and LDH results regardless of the centrifugation protocol. This does not imply that changes in potassium, glucose, or LDH do not occur during 48 hours of refrigerated storage; it has been well documented by others<sup>13</sup> that small but statistically significant changes occur during storage for these analytes. Rather, our data suggest that the changes, if any, are equivalent regardless of the centrifugation protocol. This study was not designed to detect the small, possibly clinically insignificant, changes that occur during storage. Differences may have become evident if the tubes were stored for a longer period of time, but at our institution more than 90% of requests for add-on chemistry tests are received within the 24 hours of specimen collection.

To our knowledge, the effect of varying centrifugation time on the TAT of laboratory results when using an automated specimen processing system has not been previously reported. The results of our study indicate that, when using an automated system, a linear relationship between centrifugation time and median TAT does not exist. This outcome was **not** expected given the first-in-first-out queuing system used by most automated systems.

Using 10-minute centrifugation as the baseline, a 2-minute decrease in centrifugation time reduced the TAT by an amount varying from 1.33 to 2.50 minutes (**Table 3**). In our laboratory, reducing centrifugation time from 10 minutes to 4 minutes resulted in an 8-minute and 10-minute improvement in TAT for routine and STAT orders, respectively. Furthermore, decreasing centrifugation time to 4 minutes had an even greater effect on the percentage of TAT outliers for STAT results, reducing it from 35% to 12%. The reduction in the number of outliers may be of greater clinical benefit than changes in median TAT.<sup>3-4</sup>

As would be expected from these data, we have heard praise from our clinical colleagues about the timeliness of chemistry results since implementing the shorter centrifugation time. Furthermore, we have had no complaints from health care professionals that could be traced back to preanalytical issues related to centrifugation conditions. Reduced centrifugation time at higher force is an option that similar laboratories should consider to improve the TAT for chemistry tests. LM



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1. Jones BA, Bekeris LG, Nakhleh RE, et al. Physician satisfaction with clinical laboratory services: a College of American Pathologists Q-probes study of 138 institutions. *Arch Pathol Lab Med.* 2009;133:38-43.
2. Chung H-J, Lee W, Chun S, et al. Analysis of turnaround time by subdividing three phases for outpatient chemistry specimens. *Ann Clin Lab Sci.* 2009;39:144-149.
3. Holland LL, Smith LL, Blick KE. Total laboratory automation can help eliminate the laboratory as a factor in emergency department length of stay. *Am J Clin Pathol.* 2006;125:765-770.
4. Holland LL, Smith LL, Blick KE. Reducing laboratory turnaround time outliers can reduce emergency department patient length of stay: an 11-hospital study. *Am J Clin Pathol.* 2005;124:672-674.
5. BD Vacutainer® Evacuated Blood Collection System. Available at [www.bd.com/vacutainer/pdfs/blood\\_collection\\_tubes\\_product\\_insert\\_VDP40035.pdf](http://www.bd.com/vacutainer/pdfs/blood_collection_tubes_product_insert_VDP40035.pdf). Accessed August 8, 2011.
6. Vacuette® Evacuated Blood Collection System. Grenier Bio One International AG Available at [www.gbo.com/documents/980200B\\_0722001R2\\_IFU\\_VenousBloodCollection\\_11-12-10\\_US.pdf](http://www.gbo.com/documents/980200B_0722001R2_IFU_VenousBloodCollection_11-12-10_US.pdf). Accessed August 8, 2011.
7. Novis DA, Jones BA, Dale JC, et al. Biochemical markers of myocardial injury test turnaround time: a College of American Pathologists Q-Probes study of 7020 troponin and 4368 creatine kinase-MB determinations in 159 institutions. *Arch Pathol Lab Med.* 2004;128:158-164.
8. Foster K, Datta P, Orswell M, et al. Evaluation of a centrifuge with rapid turnaround time for the preparation of plasma samples for measurement of common STAT markers on the ACS: 180 system. *Clin Lab.* 2000;46:157-160.
9. Lippi G, Salvagno GL, Montagnana M, et al. Preparation of a quality sample: effect of centrifugation time on STAT clinical chemistry testing. *Lab Med.* 2007;38:172-176.
10. Minder EI, Schibli A, Mahrer D, et al. Effects of different centrifugation conditions on clinical chemistry and Immunology test results. *BMC Clin Pathol.* 2011;11:6.
11. Mensel B, Wenzel U, Roser M, et al. Considerably reduced centrifugation time without increased hemolysis: evaluation of the new BD Vacutainer SSTTMII Advance. *Clin Chem.* 2007;53:794-795.
12. Pretorius CJ, Dimeski G, O'Rourke PK, et al. Outliers as a cause of false cardiac troponin results: investigating the robustness of 4 contemporary assays. *Clin Chem.* 2011;57:710-718.
13. Brandhorst G, Engelmayer J, Götze S, et al. Pre-analytical effects of different lithium heparin plasma separation tubes in the routine clinical chemistry laboratory. *Clin Chem Lab Med.* 2011;49:1473-1477.