INFLUENCE OF BLOOD SPECIMEN COLLECTION METHOD ON VARIOUS PREANALYTICAL SAMPLE QUALITY INDICATORS

Tester F Ashavaid , Sucheta P Dandekar*, Bhamini Keny and Vishaal R Bhambhwani*

Department of Lab.Medicine,Jt.Dir-Research, P.D.Hinduja National Hospital & Medical Research Center,Mahim, Mumbai

*Department of Biochemistry, Seth G.S.Medical College & KEM Hospital, Parel, Mumbai-400012

ABSTRACT

Preanalytical errors contribute to a large proportion of total laboratory errors. In order to achieve continuous laboratory improvement, it is important to focus on all phases of patient specimen testing i.e. preanalytical, analytical and post-analytical. With large variations in the way venous blood specimens are collected using diverse devices in the country, the effect of such practices on specimen quality is not known. The purpose of this study was to monitor fourteen specimen preanalytical quality indicators in order to compare the usage of evacuated blood collection devices with needle and syringe open collection using either disposable tubes or re-washed glass vials. The study involved 26638 patient specimens assessed over a period of 6 months. The results demonstrated that evacuated closed blood collection resulted in an approximate 100-fold reduction in the incidence of hemolysis in samples. Similarly, there was a 200-fold reduction in incidence of insufficient specimen quantity while using evacuated collection system. It was also found that incidence of specimen contamination, improper volume of sample collected, and specimen spillage was also lower when the evacuated collection system was used. Further, it was also observed that the facility with a laboratory information system demonstrated much lower specimen identification and related errors. The observed results clearly demonstrate that the usage of the evacuated blood collection system resulted in improvement of preanalytical specimen quality as compared to needle and syringe usage.

KEY WORDS

Preanalytical, Vacutainer, Hemolysis, Total quality, Needle and Syringe, Open collection.

INTRODUCTION

Importance of clinical chemistry results for effective patient care has been continually increasing. With the increasing focus on achieving better analytical quality through usage of improved laboratory analyzers, internal and external quality control - steady improvement in analytical quality is almost certain. However, it has been long realized that in order to achieve total quality, laboratories would need to focus on all steps in laboratory testing i.e. preanalytical, analytical and post-analytical. Once we are aware of the causes of mistakes, corrective action could be taken to reduce them which could

further improve total quality of laboratory services.

One of the earlier studies analyzing the causes of laboratory errors found that out of total studied errors, 46% were contributed by pre-analytical and 47% by post-analytical phase, whereas, analytical phase contributed only to 7% of errors (13). Later, Plebani and Carraro (14) attributed 68% of laboratory errors to preanalytical phase. Similar observations were made through several other publications (15, 16). These findings clearly indicate that in order to further improve upon the quality of laboratory services, a complete focus on all aspects of preanalytical, analytical and post-analytical phases is required.

Although, there are guidelines by CLSI (2) suggesting procedure for diagnostic specimen collection, these are not mandatory in India. Therefore there is large diversity in sample collection procedures. Various modes of venous blood specimen collection methodology and devices are used in the

Address for Correspondence :

Dr T. F. Ashavaid

Department of Lab Medicine, Jt. Dir. Research,
PD Hinduja National Hospital & Medical Research Center
Mahim, Mumbai-400016
E-mail : dr_tashavaid@hindujahospital.com

country. Closed evacuated blood collection system is being used by only small number of users, while majority use needles and syringes and transfer blood to re-used glass vials or very few use non vacuum tubes. The influence of such practices on quality of specimens has not been established in the past.

In view of the above, following study was designed in order to find out the impact of modes of venous blood collection on various preanalytical specimen quality indicators.

MATERIAL AND METHODS

Subjects: All samples examined were collected from patients that had been referred to the laboratories for various clinical chemistry assays and therefore the study did not involve collection of any additional blood specimens. In total, 26,638 patient specimens were monitored during 6 months of study period at two institutions for various preanalytical specimen quality checks. Both participating facilities used their existing mode of specimen collection without any modifications.

Mode of Specimen Collection: Venous blood specimens were collected using two different modes:

1. **Closed Collection Mode:** Patient blood was collected by employing closed blood collection method using the BD Vacutainer® blood collection system. This involved usage of BD Vacutainer® multi sample needles, BD Vacutainer® holder and BD Vacutainer® serum tubes (red top, 5ml tube). Blood was allowed to fill the tube until the vacuum was exhausted and blood flow ceased. The specimen was mixed by complete inversions for 8 -10 times immediately after the draw. The order of specimen draw was maintained as recommended by CLSI (2). The needle was discarded into sharps container, while the (re-usable) BD Vacutainer® holder was re-used. Specimens thus collected were kept at room temperature for 60min before centrifugation. The un-opened tubes were centrifuged after ensuring balancing, using Beckman Coulter SPINCHRON DLX centrifuge at 1300g for 10 minutes.
2. **Open Collection:** All blood specimens under this mode were collected using normal hypodermic needle (22G) and disposables syringes (5ml, 10ml) procured from normal hospital purchase process. Open collection was carried out using either of the following methods using different specimen containers.
 - a. **Open Collection with BD Vacutainer® tube:** Venous

blood was collected using needle and syringe (as mentioned above). After sufficient amount of blood was withdrawn from patient in syringe, needle was mutilated using needle burner. The mutilated needles was then re-capped and removed from the syringe. The venous blood was transferred to the BD Vacutainer® serum tube (red top, 5ml) after the Hemogard™ cap of the tube was removed. Precautions were taken to minimize froth formation while transferring the specimen. The syringe and the recapped, mutilated needle were discarded in hypochlorite solution. The specimens were left at room temperature for 60min before centrifugation at 1500g for 10 minutes using REMI R8C centrifuge.

- b. **Open Collection using glass, reused vials:** Specimens collected using needle and syringe (as mentioned above) were transferred to glass, re-used, washed, dry, injection vials. All care was taken to reduce any froth formation during the transfer of specimens. Samples were transferred at room temperature to the laboratory in a sample tray in upright position. After minimum one hour of collection, samples were centrifuged at 1500g for 10 min using REMI R8C centrifuge.

Washing of Vials: The vials were washed in the hospital CSSD unit following regular procedure. Washing process involved - disinfection, autoclaving and removal of residual blood by sequential thorough washing in tap water and detergent. The vials were finally rinsed in running tap water and dried in hot-air oven. The rubber caps used were washed in the same way as vials except that they were air dried.

Laboratory / Hospital Information System: All BD Vacutainer® closed collections were carried out at the facility having Hospital / Laboratory Information system for requisition and report management. However, 'Open collections' were carried out at a facility with manual data management.

Data Handling: At the time of collection of all specimens, the phlebotomists were required to fill up a form (Figure 1). This form traveled along with the specimen to the laboratory. The form was divided into two sections, one filled at the time of collection, while the other was filled once the specimen entered the laboratory (site of analysis). All preanalytical specimen quality checks were carried out in the laboratory.

Preanalytical Quality Checks: All specimens were checked for preanalytical quality using the method / criteria given in the Table 1 (also refer Figure 1).

Must be filled at the time of Collection

☐ Routine

Accession Number _____

Date of Collection _____

Time of Collection _____

Department _____

Sample Type

☐ Serum

☐ EDTA

☐ Sugar

☐ Heparinized

☐ Urgent

Sample Collected by

☐ Phlebotomist

☐ Lab Personnel

☐ Nursing Staff

☐ Doctor

Collection Mode

☐ Closed Vacutainer® Collection

☐ Needle Syringe - Vacutainer®

☐ Needle Syringe - Vial

Quality Assessment of Specimen:

Time of Sample Receipt _____

☐ Specimen Lost / Not received

☐ Specimen ID and Order form Mismatch

☐ One or more mandatory ID Missing e.g. Name, Hospital ID etc.

☐ QNS (Insufficient Volume to perform test)

☐ Inappropriate Transport Temperature

☐ Incorrect specimen Collection time

☐ Inadequate separation of serum from clot / cells, red cell contamination

☐ Received Leaking Specimen

☐ Tube Broken / Spillage in Lab

☐ Post-centrifugation Fibrin Clot in Serum

☐ Mild Hemolysis

☐ Moderate Hemolysis

☐ Gross Hemolysis

☐ Tube / Vial Under filled

☐ Tube / Vial Over filled

☐ Test Order Form Missing

☐ Incorrect tube Collected

☐ Contaminated, IV fluids, Order of Draw

☐ Inappropriate Patient preparation viz. Icteric serum in fasting patient etc.

☐ Tube / Vial Exterior Soiled with Blood

☐ Any Other (please specify) _____

☐ Sample Clotted (when Anticoagulated)

☐ Platelet Clumps (flagged by instrument / Microscopy)

☐ Review Differential Flag on Instrument

Test Profile

☐ LFT

☐ RFT

☐ Electrolytes

☐ Health Check

☐ Cardiac Markers

☐ Emergency Panel

☐ CBC

☐ ESR

Action Taken

☐ Specimen Rejected

☐ Sub-optimal (Redraw)

☐ Sub-optimal (No Redraw)

Analysis / Analyzer Flags

☐ Primary Tube Sampling

☐ Hemolysis

☐ Lipemia

☐ Delta Check

☐ Platelet Clumps

☐ Review Differential Flag

☐ Clot Detected

Time of Report (From Analyzer Report) _____

Figure 1 : Form used for specimen quality assessment

Data Entry: The forms from all study groups (BD Vacutainer® closed collection, syringe and needle collection transferred to BD Vacutainer® tube and syringe and needle collection transferred to glass vials), were entered into Microsoft Excel® format. The data were analyzed using Pivot table for incidence of various specimen quality indicators under each of collection modes.

Data Analysis: Total incidence of specimen quality indicators in each of specimen collection modes was derived. Since the number of specimens studied under each of the blood sample collection groups varied from 3787 to 18512, the incidence of quality check for each mode was plotted to a common base. This was done through calculation of incidence per 10,000 specimens.

RESULTS AND DISCUSSION

Of the total number 26,638 specimens collected, 18,512 were BD Vacutainer® closed collections and 8,126 were open (which included 4,339 using vials and 3,787 transferred to open BD Vacutainer® tubes). (Figure 2)

Table 1: Specimen quality variables evaluated in the study

Preanalytical Check	Observed for	Method
Specimen lost or not received	Form and requisition received but no specimen	Manual verification
Specimen ID and order form mismatch	Match ID number on specimen and order form	Manual verification
One or more mandatory ID missing	Patient's age, sex, name	Manual check
Test order form missing	Specimen was received but no requisition form reached the laboratory	Manual observation
Insufficient volume to perform test	When laboratory was not able to report one or more tests due to insufficient volume	Check reported tests vs. requisition
Tube / vial under filled / overfilled	Checked against the standard recommended volume for the device	Visual observation
Specimen contaminated	Test results not correlating with patient follow-up	Manual verification from comments on final report
Post-centrifugation fibrin clot	Presence of fibrin in the serum	Visual observation before analysis
Mild hemolysis	Serum color	Visual verification
Moderate hemolysis	Serum color	Visual verification
Gross hemolysis	Serum color	Visual verification

The observations made for quality check of studied specimens belonging to each of venous blood collection method have been presented in the Table 2. All incidences of specimen quality errors in this table have been calculated to per 10,000 samples for each collection mode.

Specimen Identification and Tracking Related Observations (refer column 'a, b & c and rows i - iv' in Table 2): It is observed that the facility which had laboratory information system (BD Vacutainer® closed collection user), does not show any major identification related errors. There has been incidence of just 4 specimens in 10,000 where one or more mandatory ID is found to be missing. As compared to this, the facility which has manual tracking system for samples (where all open collections were done) has 6 specimens with sample ID and form mismatches (serious error) and incidence of 10 samples getting lost in transit per 10,000 specimens. Besides this, the facility also showed 199 specimens that have one or more mandatory ID missing posing potential risk of error.

Figure 2 : Venous specimen collection mode
(Numbers indicate number of specimen studied for each group)

The above demonstrates that Laboratory / Hospital information system results in reducing identification and tracking related errors. This observation is in line with the reported role of bar coding / information system in ensuring accuracy in specimen identifications (1)

Specimen Volume Related Errors (refer column d, e, f and

g, Table 2): Only one specimen per 10,000 collected using BD Vacutainer® closed collection system had insufficient volume to perform the analysis. As compared to this, there are 222 incidences of inability to perform one or more analysis in case of open collection method – this required redraw of specimen from the patients. Besides this, the incidence of under-filling specimen containers was also observed to be more than 10 times higher in case of open collections as compared to closed collections.

Since BD Vacutainer® tubes have been previously evacuated to a pre-determined level (2), the vacuum ensures that the correct volume fills the tube and is responsible for the very low incidence of insufficient specimen volume. As compared to this, volume of blood delivered to the containers in case of open collection is subject to human error. With the fixed volume syringes are used, there may be a possibility of variation in the volume delivered into the open containers when smaller than needed volumes of collected specimen were coupled with the inclination of the phlebotomists to avoid second venipuncture.

However, we also observe incidence of low specimen volumes

Table 2: Observations made in venous specimens using preanalytical quality criteria

	Sample ID/Tracking Related Errors				Sample Volume Related Errors			Sample Leakage / Spillage		Sample Hemolysis and Contamination				Misc
	Sample Lost/Not received	Sample ID/order form mismatch	One or more mandatory ID Missing	Test Order form Missing	Insufficient Volume to perform test	Tube/Vial Underfilled	Tube/Vial Overfilled	Received Leaking Specimen	Tube broken/spillage in Lab	Specimen Contaminated	Mild Hemolysis	Moderate Hemolysis	Gross Hemolysis	Rest Centrifugation fluid not
	a	b	c	d	e	f	g	h	i	j	k	l	m	n
i) BD Vacutainer® Closed Collections	0	0	4	1	1	18	3	0	2	3	1	2	3	0
ii) Syringe Needle collection into BD Vacutainer® Tube	8	3	195	32	214	296	0	0	16	26	209	124	82	3
iii) Syringe Needle collection into Glass Vial	12	9	203	53	228	203	0	2	51	233	260	224	210	21
iv) Total Open Collection	10	6	199	43	222	246	0	1	34	137	236	177	150	12

1. All numbers given above indicate number of incidences under each criterion extrapolated to per 10,000 specimens

2. Entries under 'Total open collections' is calculated from total incidences (sum of actual incidence in Syringe needle collection transferred to BD Vacutainer® tube and Vials) and then extrapolated to per 10,000 specimens. Therefore, the figure is not mathematically total of each in row b and c.

(18 per 10,000) in case of closed collection system. This could happen if the phlebotomists withdraw the tube before the vacuum has exhausted. Such a practice, although may not have much impact on the serum sample quality, could result in introduction of preanalytical errors due to changed sample to additive ratios and therefore potentially impact quality of results for certain parameters (3, 4).

Specimen Leakage and Spillage (column h and i in Table 2) : It is observed that 51 specimens collected in glass vials (per 10,000) had been either broken or spilled in the laboratory. This could be primarily due to the containers being small and prone to breakage (glass) or could also be due to loosely attached rubber caps. When open collection was done using BD Vacutainer® tubes, spillage was not completely eliminated – although it was one third of that observed using vials as specimen containers. This higher spillage in case of open collection could also be attributed to the fact that in case of all open collections, serum was transferred to sample cups before loading into analyzer. This additional step could have posed another increased risk of specimen spillage. As compared to this it is worth noting that in case of closed collection, spillage contributed to just 2 occurrences per 10,000 specimens – which may have been primarily due to accidental dropping of specimens.

Contaminated Specimen: (Refer column j in Table 2) : High incidence of contaminated specimens (233 per 10,000) can be seen when vials had been used for collection. Given that re-washed vials were used, contamination could be attributed to the possibility of residual detergent, moisture or remnant of anticoagulant in the vial due to inadequate washing. When open collections were carried out in BD Vacutainer® tubes, there is approximately 10-fold lower incidence of contamination. The total incidence of contamination in case of open collections can also be attributed to the fact that re-washed sample cups were used in case of both vials as well as open BD Vacutainer® tubes. As compared to open collections, closed collections had very low (3 in 10,000) incidence of contaminated samples. The analysis was carried out in primary tubes for all closed collection specimens. Although the incidence of contaminated specimen in case of closed collection is miniscule, these could be arising from factors such as not following order of draw (2, 5). Besides the above, there have been several reports indicating the impact of inappropriate phlebotomy procedure on specimen quality such as prolonged tourniquet application (6), fist clenching (7, 8) which may affect specimen quality.

Hemolysis (refer column k, l, and m in Table 2) : The incidence

of hemolysis in samples collected with needle and syringe (open collection) is up to 200 times higher than that of BD Vacutainer® closed collections. More than 2% of specimens were mildly hemolyzed in all open collections (vials and open BD Vacutainer® tubes). Whereas, vial usage continued to demonstrate more than 2% (>200 per 10,000) of moderate and gross hemolysis each, the incidence was lower (124 moderate and 82 gross per 10,000) in case of collection into open BD Vacutainer® tubes. As compared to open collections, use of BD Vacutainer® closed collection system demonstrated very low - 1, 2 and 3 in 10,000 incidence of mild, moderate and gross hemolysis respectively. When BD Vacutainer® closed collection was compared with total open collection for incidence of hemolysis (cumulative of mild, moderate and gross) we observe about 100-fold increased hemolysis (6% hemolysis) in syringe needle (open) collections (Figure 3)

Figure 3 : Incidence of hemolysis (Open vs. closed collections)
Numbers indicate incidence of total hemolysis per 10,000 samples for each mode of specimen collection

Hemolysis, caused by rupture of erythrocytes, could arise due to various reasons – some of these are related to the devices used and others to the procedure of phlebotomy. Hemolysis occurs whenever there is trauma to relatively fragile red blood cells, either during collection or after phlebotomy is completed. If blood is drawn with a syringe, drawing the plunger back forcefully or injecting blood into container using pressure could cause hemolysis (9). Very small amount of hemolysis observed in closed collection system could be even due to various factors such as some metabolic disorders, infectious agents or mechanical issues (12). Besides the above factors, as discussed in case of contamination, remnants of detergent / moisture in the re-washed vials could have been responsible for consistently higher degree of hemolysis in this category.

Hemolysis affects various tests due to different mechanisms. There have been reports of several parameters including Potassium, LDH, AST, ALT, CK, GGT, and ALP being affected

by hemolysis due to efflux of intracellular constituents (10). Further, hemolysis could cause chemical, biological, immunological interference with reaction mechanism of several assays (11). Therefore, higher incidence of hemolysis in case of open collections could potentially pose one of the largest risk for specimen result not correlating with patient condition.

Fibrin Clot: (refer column n in Table 2) : We observed incidence of post-centrifugation fibrin in 21 (per 10,000) specimens collected in vials. Presence of latent fibrin in serum poses a risk of blocking analyzer probe resulting in no (or reduced) sample aspiration / system breakdown. Post-centrifugation fibrin clot can be formed due to insufficient time given for specimen to clot before centrifugation. This can also happen if the patient is on dialysis or on anticoagulant therapy (9)

In view of the above results and discussions, it is evident that BD Vacutainer® closed system for venous blood specimen collection resulted in lower number of preanalytical errors. The usage of needle and syringe collection with re-washed vials and disposable tubes (in this study open BD Vacutainer® tubes) resulted in much higher incidence of specimen quality issues. These additional errors would have a clear impact on avoidable repeats in terms of specimen redraw, retesting, or even potentially producing wrong laboratory results.

ACKNOWLEDGEMENT

We thank Becton Dickinson (India) Pvt. Ltd. for providing us with BD Vacutainer® tubes for performing open collections. All closed collections were carried out using BD Vacutainer® procured by the institute as part of routine testing.

REFERENCES

- Weilert M, Tilzer LL. Putting bar codes to work for improved patient care. *Clin Lab Med* 1991; 11:227-30.
- CLSI Document. Procedures for collection of diagnostic blood specimens by venipuncture; Approved standard 2006;H03-A5
- Peterson P, Gottfried EL. The effects of inaccurate blood sample volume on prothrombin time (PT) and activated partial thromboplastin time (aPTT). *Thrombosis and Haemostasis* 1982; 47:101-3.
- Reneke J, Etzell J, Leslie S, Ng VL, Gottfried EL. Prolonged prothrombin time and activated partial thromboplastin time due to filled specimen tubes with 109 mmol/L (3.2%) citrate anticoagulant. *Am J Clin Pathol* 1998;109:754-7
- Calam RR, Cooper MH. Recommended "order of draw" for collecting blood specimens into additive-containing tubes. *Clin Chem* 1982; 28:1399-401.
- Junge B, Hoffmeister H, Heddersen HM, Rocker L. Standardisierung der Blutentnahme. *Dtsch Med Wschr* 1978; 103:260-5.
- Don BR, Sebastian A, Cheitlin M, Christiansen M, Schambelan M. Pseudohyperkalemia caused by fist clenching during phlebotomy. *N Engl J Med* 1990; 322: 1290-2.
- Skinner SL, Adelaide MB. A cause of erroneous potassium levels. *Lancet* 1961; 1: 478-80.
- Lawrence AK, Amadeo JP, Steven CK. *Clinical Chemistry 2003: Theory, analysis, correlation: Fourth Edition.*
- Guder WG, Narayanan S, Wisser H, Zawta B. *Samples: from the Patient to the laboratory – Impact of preanalytical variables on the quality of laboratory results 2003; Willey – VCH, 3rd Edition.*
- Guder WG, da Fonseca-Wolheim F, Heil W, Schmitt YM, Topfer G, Wisser H et al. *The haemolytic, icteric and lipemic sample – Recommendations 2000.*
- Henry JB, ed. *Todd-Sanford-Davidsohn Clinical Diagnosis and Management by Laboratory Methods; Vol 1 16th ed.* Philadelphia, PA: WB Saunders Co. 1979;1005-7
- Ross JW, Boone DJ. Assessing the effect of mistakes in the total testing process on the quality of patient care [Abstract 102]. In: Martin L, Wagner W, Essien JDK, *Institute of Critical Issues in Health Laboratory Practice.* Minneapolis, MN: DuPontPress, 1991.
- Plebani M, Carraro P. Mistakes and stat laboratory: types and frequency. *Clin Chem* 1997; 43: 1348-51.
- Bachner P, Boone DJ, Herron R, Howanitz PJ, Meier F, Schiffman R, Zarbo RJ. *College of American Pathologists Outcomes Working Group, blood bank quality assurance questionnaire.* Northfield, IL: CAP, 1991.
- Boone J, Steindel SD, Herron R, Howanitz PJ, Bachner P, Meier F, Schiffman RB, Zarbo RB. *Transfusion medicine monitoring practices.* *Arch Pathol Lab Med* 1995; 119:999-1006.