ORIGINAL ARTICLE

INFLUENCE OF METHOD OF SPECIMEN COLLECTION ON VARIOUS PREANALYTICAL SAMPLE QUALITY INDICATORS IN EDTA BLOOD COLLECTED FOR CELL COUNTING

Tester F Ashavaid, Sucheta P Dandekar**, S Khodaiji*, M H Ansari*** and Adarsh Pal Singh****

Departments of Laboratory Medicine and *Hematology, P.D. Hinduja National Hospital & Medical Research Center, Mahim, Mumbai – 400016, Departments of **Biochemistry and ***Pathology, Seth G.S. Medical College & KEM Hospital, Parel, Mumbai-400012, ****BD Diagnostics – Preanalytical Systems, BD (India).

ABSTRACT

Improving specimen quality as well as healthcare worker (HCW) safety poses significant concerns for today's laboratories. With an increasing number of diagnostic tests requested, laboratory professionals are faced with challenges to reduce laboratory errors, improve the quality of laboratory results to assure accurate diagnosis and implement initiatives to ensure healthcare worker safety and minimize risk of exposure to bloodborne pathogens. A prior study conducted in 2008 reported that variations in blood collection methods for clinical chemistry assays may affect overall specimen quality. As a follow up, the current study assessed the quality of 22563 patient specimens for cell counting in EDTA blood collection tubes that were obtained with needle and syringe collection (open) using either disposable tubes or re-washed glass vials or with an evacuated blood collection system (closed). Based on the observations, the use of the evacuated blood collection system resulted in better preanalytical specimen quality as compared with needle and syringe collection. The findings also showed an approximately 70-fold reduction in the incidence of clotting as well as fewer instrument-generated flags using the evacuated collection system. In addition, the use of an evacuated collection system for venous blood collection demonstrated lesser chance of blood exposure to healthcare workers.

KEY WORDS

Preanalytical, Sample quality, EDTA blood collection, Evacuated collection.

INTRODUCTION

Managing preanalytical variability in laboratory testing is a critical factor for ensuring accurate results. These preanalytical errors are influenced by specimen collection, specimen handling, interfering substances, and patient factors and have been well documented in the medical literature (1-4). While the majority of these did not influence patients' outcomes, an evaluation conducted to review the incidence of laboratory errors at the University-Hospital of Padova showed that 19% of errors were correlated with further inappropriate

investigations and subsequently, an unjustifiable increase in laboratory costs (5).

One study tracked preanalytical errors in a laboratory medicine department over a one-year period; the data obtained showed that hemolysis and clotting were most frequently related to specimen collection (6). In a recent hospital study, clotted specimens were a recurrent cause of specimen rejection for inpatients (7). In addition, a retrospective data analysis (2001-2005) from 105 laboratories that summarized the main factors affecting preanalytical phase quality determined that 14.4% of rejected specimens were due to clotting (8).

Although a reduction in preanalytical errors has been documented, these errors remain a concern for laboratory professionals (5, 9). As such, studies have been conducted to ascertain whether the mode of specimen collection could play a role in reducing laboratory errors. A prior assessment

Address for Correspondence :

Dr. T. F. Ashavaid,
Department of Laboratory Medicine,
P. D. Hinduja National Hospital & Medical Research Centre,
Veer Savarkar Marg, Mahim, Mumbai-400 016
E-mail: dr_tashavaid@hindujahospital.com

of the effect of blood collection methods (needle/syringe [open] versus an evacuated system [closed]) on specimen quality had been reported by the authors for clinical chemistry testing; the closed method for blood collection resulted in a fewer number of preanalytical errors (e.g. errors related to specimen volume, specimen leakage, contamination, hemolysis) than the open system (10).

As a follow-up to this study, a multi-center evaluation was performed to monitor specimens anticoagulated with EDTA obtained with open and closed collection modes and utilized for cell counting. Specifically, various preanalytical specimen quality indicators were assessed in these specimens and their impact on test results was evaluated.

MATERIALS AND METHODS

Subjects: All samples examined were collected from patients that had been referred to the laboratories for hematology tests requiring collection of EDTA anticoagulated whole blood specimens. The study, therefore, did not involve collection of any additional blood specimens. In total, 22563 patient specimens were monitored in two institutions for various preanalytical specimen quality indicators and instrument-generated flags. 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 a closed blood collection method using the BD Vacutainer® blood collection system in one institution. This involved utilization of BD Vacutainer® multi-sample needles, BD Vacutainer® reusable standard size holder for 13 mm and 16 mm diameter tubes and BD Vacutainer® Plus plastic whole blood tube with lavender BD Hemogard™ closure, paper label, with 5.4mg (spray dried) K₂EDTA (BD Vacutainer® EDTA 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 collection. The order of specimen collection was maintained as recommended by the Clinical Laboratory Standards Institute (CLSI).⁽¹¹⁾ The needle was discarded into a sharps container, while the (reusable) BD Vacutainer® holder was re-used. Blood samples were analyzed using Beckman Coulter® HmX or Sysmex SE2100 cell counters with autoloader. The sample load was divided randomly between the two analyzers. All BD Vacutainer® closed collections were performed at a facility that had a Hospital/Laboratory Information System (HIS/LIS)

for requisition and report management.

2. Open Collection: All blood specimens were collected in one institution using a hypodermic needle (22G, Unolok, Dispovan) and disposable syringes (5ml, 10ml, Unolok, Dispovan) procured from the standard hospital purchasing process. Open collection was carried out in two different ways.

a. Open Collection with BD Vacutainer® EDTA tubes: Venous blood was collected using needle and syringe (as mentioned above). After a sufficient amount of blood was withdrawn from the patient in the syringe, the needle was mutilated using a needle burner. The mutilated needle was then re-capped and removed from the syringe. The venous blood was transferred to the BD Vacutainer® EDTA tube (lavender top, 3ml) after the Hemogard™ cap of the tube was opened. Precautions were taken to minimize froth formation while transferring the specimen. The syringe and the recapped, mutilated needle were discarded in hypochlorite solution. The tube was re-capped and the specimens were mixed by inversion per manufacturer's instructions.

b. Open Collection using glass, reused vials: Specimens collected using needle and syringe (as mentioned above) were transferred to re-used, washed (see discussion below) and dried, injection glass vials containing 4 mg of EDTA. Care was taken to reduce any froth formation during the transfer of specimens. Samples were transported to the laboratory at room temperature in a sample tray in an upright position.

Washing of Vials: The vials were washed in the Central Sterile Supply Department of the hospital following established procedures. The 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 a hot-air oven. EDTA was delivered into the vials in liquid form such that each vial contained 4 mg of EDTA (sufficient for 2 ml of blood). The rubber caps used were washed in the same way as the vials except that they were air dried.

All open blood samples were analyzed using an ERMA three-part differential analyzer (without autoloader)(P170). Both modes for open collections were performed at the same facility with manual patient data management.

Data Handling: At the time of collection of all specimens, the phlebotomists were required to complete a specimen quality assessment form. This form had been validated in the previous study (10). This form accompanied the specimen to the laboratory and was divided into two sections: one filled at the

Table 1: Specimen Quality Variables Evaluated in the Study

Preanalytical Variable	Observation	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	Laboratory was not able to report one or more tests due to insufficient volume	Check reported tests vs. requisition
Tube / vial underfilled/ overfilled	Checked against the standard recommended volume for the device	Visual observation
Specimen clotted	Presence of visibly clotted specimen	Visual observation
Review differential flags	Flagging of differential count by the cell counter	Analyzer generated flag
Platelet clumps	Analyzer flag for platelet count	Analyzer generated flag
Tube / vial exterior soiled	Blood-stained exterior of the container	Visual observation
Tube / vial spillage	Spillage of specimen	Visual observation / record

time of collection and the other filled once the specimen was received in the laboratory (site of analysis). All preanalytical specimen quality indicators were assessed in the laboratory.

Preanalytical Quality Checks: All specimens were checked for preanalytical quality using the criteria in Table 1.

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

Data Analysis: Total incidence of specimen quality indicators in each specimen collection mode was derived. Since the number of specimens studied under each of the blood sample collection groups varied from 4441 to 11265, the incidence of quality indicator 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 of 22563 specimens, 11265 were collected using the BD Vacutainer® closed collection system, and 11298 were from open collection (which included 6857 specimens collected in vials and 4441 transferred to open BD Vacutainer® tubes).

The results obtained for various preanalytical quality indicators on the studied specimens from each venous blood collection method are presented in Table 2.

Table 2: Observations Using Preanalytical Quality Criteria^{1,2}

	Tube/vial exterior soiled with blood	Sample clotted	Review differential flag ³	Sample lost/not received	One or more mandatory ID missing	Test order form missing	Insufficient volume to perform test	Tube broken/ spillage in lab	Platelet clump flagged by analyzer
BD Vacutainer® closed collection	—	1	2	—	1	1	7	1	11
Needle/Syringe collection into BD Vacutainer® tube	—	101	—	—	—	—	—	—	—
Needle/Syringe collection into glass vial	19	51	70	7	—	—	—	—	—

1. No instances of specimen contamination, tube/vial overfilling, or receipt of leaking specimens were observed. 2. All numbers given above indicate the number of incidences under each criterion extrapolated to per 10,000 specimens. 3. Results depict a worst case scenario and may be more favorable if a 5-part differential count analyzer is used for analyzing specimens collected using the open system.

Tube / Vial Exterior Soiled with Blood: It was observed that 19 of the 10,000 samples collected using the open collection method and re-used glass vials demonstrated soiling of the exterior of the container with patient blood. In the specimens collected using BD Vacutainer® closed collection and open collection using syringe and needle and BD Vacutainer® tubes, there was no blood on the outside of the tubes. Since the specimen containers pass through several laboratory and non-laboratory personnel before analysis and final disposal, soiled exteriors could pose potential blood exposure to these healthcare workers. Several bloodborne pathogens (i.e. hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV) are transmitted through blood exposure. In addition, reports indicate that the HBV virus can survive for at least one week in dried blood on environmental surfaces or contaminated needles and instruments (12).

Samples Clotted: Fifty-one specimens per 10,000 were clotted in the cases of open collection using re-used vials, while the incidence of clotted samples was observed to be higher for open collection in BD Vacutainer® tubes (101 per 10,000 samples). The BD Vacutainer® closed collection method resulted in much lower (approximately 70-fold) incidence of clotting. Since increasing number of cell counting occurs using automated hematology analyzers, micro-clots in whole blood could pose a major risk of erroneous results and analyzer breakdown. While it is easier to detect a micro-fibrin clot in serum samples, identification in whole blood may be difficult. The presence of clots in EDTA samples can be explained primarily due to increased blood to additive ratio (could be due to higher than optimal volume transferred to tubes in open collection) or improper mixing of the sample after collection. The phlebotomists who performed open collection routinely mixed samples without inverting them; in contrast, the samples collected in tubes had to be inverted for proper mixing. It is possible, however, that the phlebotomists continued to mix the tubes in the same manner as the vials, which could have resulted in a higher rate of clot formation in open collections using BD Vacutainer® tubes.

Review Differential Flags: Five-part differential cell counters use two parameters (e.g. size and cytochemical properties) to differentiate cell types, while three-part differential counters use only one parameter (i.e. size). Morphological changes in the cell populations could impact the ability of the cell counter to report/separate different populations of cells and hence raise a flag that indicates the same. This requires the laboratory to either report differential count using microscopy or to request

a repeat specimen, both of which necessitate additional time and re-work.

We observed that 70 samples per 10,000 of those collected using an open method in re-used vials raised a differential flag, whereas, only 2 samples per 10,000 raised a differential flag in closed collections. There was no report of differential flag generation in cases of open collection using BD Vacutainer® tubes. The latter was an unexpected finding; however, it may be explained by the fact that the analyzer used for open collections was a basic cell counter, while the one used for closed collections was a high-end, five-part differential cell counter with advanced flagging capabilities. The cell counter for open collections may have missed sample compromises in open collection in vials and BD Vacutainer® tubes due to its inherent technological limitations. Although the data clearly demonstrated that closed collection resulted in an almost 35-fold lesser differential flagging, more prominent findings may be expected when analyzers with comparable flagging capabilities are used.

Samples Lost or Not Received: The facilities used for open collections did not have positive specimen identification (bar code) and Laboratory Information System (LIS) capabilities; whereas, the closed collections were carried out at a hospital that was linked to an HIS/LIS. In open collections, 7 samples per 10,000 were either lost in transit or were not received; patient specimens then needed to be re-collected. This was noteworthy since in open collections; the specimen collection and analysis area was connected, offering little chance of specimen misplacement. Therefore, the findings of lost/not received specimens in the instance of open specimen collection into glass vials can be most likely attributed to the presence of an HIS/LIS in the facility rather than the mode of specimen collection.

Specimen Identification-Related Errors: Specimens collected with closed collection systems demonstrated 1 specimen per 10,000 each with 'one or more specimen ID' and 'test order form missing'. No such error was detected in samples collected using an open collection system in a facility without an HIS/LIS.

Volume-Related Issues: For those samples collected using the closed method, 7 samples per 10,000 contained insufficient volume of blood (QNS) to perform one or more tests. Further, 12 tubes per 10,000 of closed collection had been underfilled. This could be attributable to the early withdrawal of the tube before the vacuum was exhausted. These errors can be significantly reduced by proper education and training of the

phlebotomists on the use of evacuated blood collection systems. No QNS/underfilling was observed in cases of open system usage. One factor for this could be that the cell counter used in open collections required only 50µl of blood, while the one in the facility using closed collection required 300ul per aspiration. In combination with low draw volume during venipuncture, this could be responsible for a high incidence of QNS in closed collections. Acquisition of lower than the recommended volume of blood in tubes could result in higher concentration of EDTA per ml of blood than that recommended by CLSI. This, in turn, may lead to cell shrinkage and lower mean cell volume (MCV).

Tubes Broken / Spillage in Laboratory: For samples obtained using a closed blood collection, one tube out of 10,000 samples had been broken/spilled in the laboratory. No such incident was reported in open collections.

Platelet Flags: Specimen flagging is directly related to the capabilities of the analyzers used. We observed that the analyzers used for studying specimen compromises in blood collected with open systems were unable to detect platelet clumps/morphological changes. The analyzer used in closed collections employed an advanced platelet clump detection algorithm. Using the closed collection method, 11 platelet flags per 10,000 specimens were noted in which the analyzer was unable to report the platelet counts. However, no similar flag was detected in open systems, which was attributed to equipment limitation as explained previously.

Based on the observations in this study, it is evident that the use of an evacuated closed collection system for venous blood collection decreased the potential exposure of healthcare workers to blood and bloodborne pathogens. In addition to safety considerations, blood collection by a closed system also demonstrated fewer incidences of clotted samples, which if present, may result in an inadequate specimen for any hematological estimation, and require re-collection of the specimens. This, in turn, could delay patient diagnosis, cause inconvenience for the patient and unnecessarily increase the cost of specimen collection. The closed blood collection also showed decreased frequency of differential flags by the analyzers used. We were not able to compare other instrument-generated flags in samples collected by open and closed systems due to differences in the capability of the analyzers used, which may be of interest for future studies.

ACKNOWLEDGEMENT

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

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