Underfilled tubes revisited: What blood tests can be reported on short draws?

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ABSTRACT

Objective: Underfilled blood tubes (short draws) are often collected from children or those with poor venous access. In a pilot study, we investigated which tests among a large acute care panel could be reported on short draws.

Methods: Blood was drawn in BD vacutainers (short draw: 1 mL [33%-56% fill volume] vs complete draw: 1.8-3 mL [100% fill volume]) from 12 volunteers for 3 coagulation tests, 36 chemistry tests, and the complete blood count (CBC) with differential. Tests that were strong candidates for reporting did not have statistically significant biases between short and complete draws, whereas potential candidates had statistically significant biases that were small (<25% of total allowable error and less than desirable bias from biological variation). Biases that increased or decreased across concentration ranges invalidated reporting candidacy.

Results: Two coagulation tests, 14 chemistry tests, and 15 CBC components were strong candidates for reporting. There were 9 chemistry tests and 2 CBC components that were potential candidates for reporting.

Conclusions: Underfilled blood tubes, or short draws, may be valid collections for several coagulation, chemistry, and hematology tests—which may prevent additional unnecessary phlebotomy. Laboratories should perform their own studies to determine if short draws are acceptable for limited testing using their tube and instrument types.

INTRODUCTION

Underfilling of evacuated blood tubes can be caused by air bubbles present in blood collection set tubing, premature removal of evacuated tubes, and unsuccessful blood transfer from a syringe to evacuated tubes using nonsharps devices.[1] Underfilling is also more likely if veins are small or collapse easily, there is a limited volume of blood to collect, or patients make unexpected movements that interrupt blood flow—all of which are frequently encountered in pediatric phlebotomy.[2] Clinical teams may also deliberately underfill tubes in an effort to protect patients from excessive phlebotomy.

KEY POINTS

- Short draws may cause biases that are unacceptable in terms of analytical performance specifications, or that are undesirable in terms of biological variation. However few, if any, studies have examined whether this is truly the case.
- In our pilot study, short draws appeared to be valid for some coagulation, chemistry, and hematology tests—contradicting the prevailing opinion that tubes should always be filled.
- Laboratories should perform their own studies to determine if limited testing of short draws is appropriate given their collection methods, instrumentation, test menus, and patient populations.

Key words

underfilled tube; short draw; pediatric; coagulation; clinical chemistry; hematology; complete blood count; allowable error; desirable bias; biological variation

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In evacuated tubes containing anticoagulant, underfilled tubes (short draws) contain elevated concentrations of anticoagulant and a higher anticoagulant/blood ratio—which extends clotting time in coagulation tests.[3,4] Furthermore, when an anticoagulant is in liquid form, it dilutes analytes in an effect proportional to blood volume. Excess anticoagulant may also interfere with chemistry tests[5-10] and hematologic components of the complete blood count (CBC).[11] Importantly, short draws are associated with in vitro hemolysis and release of intracellular components into plasma.[10,12,13] Finally, the larger air space in short draws increases loss off of volatile analytes such as CO_2.[14-17] Consequently, the Clinical Laboratory Standards Institute (CLSI) recommends against testing short draws,[18,19] and it is common practice for laboratories to reject these collections.

Nonetheless, few studies have examined the impact of short draws on extensive laboratory test panels, and none, to our knowledge, have expressed biases as a proportion of analytical performance specifications, including allowable error, or desirable bias defined using biological variation. Few have also examined the distribution of biases across concentration ranges, which could impact the validity of reporting results on short draws. These points are important because some tests may be valid for reporting on short draws—which could be helpful in pediatric phlebotomy, where these collections are commonly encountered, and the risk of iatrogenic anemia is higher due to limited blood volume.[20] It could also be helpful in any situation where repeated blood sampling is challenging or impossible (eg, critically ill patients, elderly patients).

We therefore performed a pilot study with the following objectives: (1) estimate test result biases between underfilled evacuated tubes (short draws) and fully filled evacuated tubes (complete draws) for commonly ordered coagulation, chemistry, and hematology tests; (ii) evaluate biases as percentages of total allowable error (TEa) set by the College of American Pathologists (CAP) and as percentages of what is considered desirable biases[21] using biological variation; (3) determine if biases consistently increase or decrease across measured concentration ranges; and (4) develop a list of candidate tests for reporting on short draws.

METHODS

As this study used anonymized laboratory results from staff volunteers in order to answer a question related to routine clinical and laboratory operations, it was classified as a quality improvement activity and was exempt from ethics review. Procedures documented in this article were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Two sets (complete draw and short draw) of Beckton Dickenson (BD) vacutainers (vacutainers) of blood were collected from each of 12 healthy adult volunteers via venipuncture. Best practices in phlebotomy, specimen handling, and processing as established by the CLSI were followed.[19,22] We used a butterfly needle set (BD Set Blood Vacutainer Winged Safety 23 gauge × 0.75 in. with tubing 12 in. & Luer Adapter—REF 152452) and tube holder (BD Holder Vacutainer Venous Access Non-stackable—REF 260582) to collect a fully filled vacutainer (complete draw), after which the tube holder was removed and a 3-mL syringe (BD Syringe Luer Lock Tip 3 mL—REF 246868) was attached to the Luer Lock connection and 3 mL of blood was slowly removed. The syringe was then attached to a blood transfer device (BD Blood Transfer Device, Vacu with Fem Luer Adaptor—REF 354700), which was used to pierce a separate vacutainer. Then, 1 mL of blood was allowed to enter the tube without depressing the syringe plunger (short draw). We chose 1 mL of blood for short draws for two reasons: (1) it was the smallest volume of blood our laboratory staff could reliably aliquot plasma from after centrifugation, and (2) any tests exhibiting minimal bias between complete and 1-mL draws would be valid to report for blood volumes greater than 1 mL.

Tubes were gently inverted the recommended 8 to 10 times immediately following collection. Plasma was isolated by centrifuging appropriate tubes at 1200 × *g* (1500 × *g* for coagulation tests) for 10 minutes.

Complete draw and short draw tubes were collected together in the appropriate order of draw and in sufficient number to satisfy test volume requirements: 1 complete draw and 1 short draw light blue top buffered sodium citrate (9 NC; 3.2%, liquid) tubes—1.8 mL (REF 363080); 1 complete draw and 3 short draw mint green top PST Gel and Lithium Heparin^N (LH) 56 units—3 mL (REF 367960); 2 complete draw and 2 short draw lavender top K_2 (dipotassium) EDTA (K2E) 5.4-mg tubes—3 mL (REF 367856); and 1 complete draw and 1 short draw gray top sodium fluoride 5 mg and potassium oxalate 4 mg (FX)—2-mL tubes (REF 367921). Fill volumes for short draws were 56% for light blue top tubes, 33% for mint green top and lavender top tubes, and 50% for gray top tubes.

Coagulation tests were international normalized ratio (INR), partial thromboplastin time (PTT), and prothrombin time (PT), which were performed on plasma from blood collected in light blue top buffered sodium citrate tubes. Chemistry tests were anion gap (AGAP), alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), ammonia (NH_3), amylase (AMY), aspartate aminotransferase (AST), bilirubin—direct (BILD), bilirubin—total (BILT), C-reactive protein (CRP), calcium—total (CA), carbon dioxide (CO_2), chloride (CL), cortisol (CORT), creatine kinase (CK), creatinine (CREA), ferritin (FERR), gamma glutamyl transferase (GGT), glucose (GLUC), hemoglobin A1c (HbA1c), iron (FE), lactate (LAC), lactate dehydrogenase (LDH), lipase (LIP), magnesium (MG), NT-proBNP (NTPROBNP), phosphate (PHOS), potassium (K), sodium (NA), thyroid-stimulating hormone (TSH), thyroxine—free (FT4), total protein (TP), triglycerides (TRIG), high-sensitivity cardiac troponin T (TNT), urea (UREA), and uric acid (UA). The Roche hemolysis index (H index), icterus index (I index), and lipemia index (L index) were used to assess specimen quality. All chemistry tests were performed on plasma from blood collected in mint green top lithium heparin PST tubes, except HbA1c (whole blood, lavender top K_2 EDTA tubes), LAC (gray top sodium fluoride and potassium oxalate tubes), and NH_3 (lavender top K_2 EDTA tubes). Hematology tests were the CBC and differential: basophil count (BA), basophil percent (BA%), eosinophil count (EO), eosinophil percent (EO%), hematocrit (HCT), hemoglobin (Hb), lymphocyte percent (LY%), lymphocyte count (LY), mean corpuscular hemoglobin (MCH),

mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean platelet volume (MPV), monocyte count (MO), monocyte percent (MO%), neutrophil count (NE), neutrophil percent (NE%), nucleated red blood cell count (NRBC), nucleated red blood cell percent (NRBC%), platelet count (PLT), red blood cell count (RBC), red cell distribution width (RDW), uncorrected white blood cell count (UWBC) and white blood cell count (WBC).. The CBC and differential were performed on whole blood collected in lavender top K_2 EDTA tubes.

Coagulation tests (INR, PT, and PTT) were performed on a Werfen ACLTOP CTS 500. For chemistry tests, specimens were performed on a Roche Diagnostics Cobas Pro c503 analyzer (AGAP, ALT, ALB, ALP, AMY, AST, BILD, BILT, CA, CK, CL, CO_2, CORT, CRP, CREA, FE, GGT, GLUC, LAC, LDH, LIP, MG, NH_3, PHOS, K, NA, TP, TRIG, UREA, UA, and Roche H index, I index, and L index), a Roche Cobas 8000 e801 analyzer (CORT, FERR, FT4, NTPROBNP, TSH, TNT), and Roche Cobas c513 analyzer (HbA1c). Specimens for NH_3 and LAC were collected on ice, separated in a refrigerated centrifuge, aliquoted, and immediately placed on ice. For hematology tests, which included all components of the CBC with automated differential, specimens were tested on a Beckman Coulter UniCel DxH 800.

The Wilcoxon signed-rank test for paired data was used to evaluate the significance of instrument test result biases between short and complete draws for each volunteer. The median of biases (short draw result minus complete draw result), expressed as percentages of complete draw results, was determined, as well as the median of absolute biases, expressed as percentages of TEa from the CAP, and of desirable bias ($0.25 * \sqrt{[CV_I^2 + CV_G^2]}$) derived using biological variation data.[21] We used the 2025 versions of CAP TEa and recent CAP surveys to identify standard deviation values for survey samples that had peer group means close to median results for analytes in our study. Within-subject biological variation (CV_I) and between-subject biological variation (CV_G) were obtained from the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Database,[23] the Westgard Desirable Biological Variation Database,[24] or other sources.[25]

We defined tests as "strong" candidates for reporting on short draws if there was no statistically significant (ie, $P \geq .05$) bias between short and complete draw results. We defined tests as "potential" candidates for reporting if there was a statistically significant bias (ie, $P < .05$) between short and complete draw results, but absolute bias was small (ie, <25% of CAP TEa and less than the corresponding desirable bias threshold). If it was not possible to determine if absolute bias was small (ie, no TEa or biological variation data), then tests were not candidates for reporting. We also determined the percentage of biases that exceeded TEa and desirable bias thresholds, and prepared Bland-Altman–style plots without mean bias lines and limits of agreement to visualize changes in bias distribution across concentration ranges. If biases (percent TEa, percent desirable bias, or raw bias if no TEa or desirable bias was available) were observed to consistently increase or decrease, then tests were not candidates for reporting because at extreme concentrations, these biases could become large enough to cross thresholds indicated above.

Studio release 3.8.1 (SAS Institute) was used for all analyses.

RESULTS

Table 1 shows tests that were strong or potential candidates for reporting on short draws, whereas **Table 2** shows tests that were not candidates for reporting. **Figures 1 and 2** include Bland-Altman plots for tests where biases increased or decreased across concentration ranges. All other Bland-Altman plots can be found in the supplementary figures. Analyte TEa, peer group information, and biological variation data can be found in the supplementary table. Below, we comment mainly on tests that were strong or potential candidates to report.

Among coagulation tests, there was no significant bias in results for INR and PT between short and complete draws (there was a significant and large bias for PTT; median of absolute biases = 121.1% of TEa and 940.4% of desirable bias), and biases did not consistently increase or decrease across concentration ranges. These tests were therefore considered strong candidates to report on short draws.

Among chemistry tests, there was no significant bias in results for 14 chemistry tests (AST, BILT, CK, CORT, FT4, GLUC, HbA1c, K, LAC, NH_3, NTPROBNP, PHOS, TSH, and UREA) between short and complete draws, and biases did not consistently increase or decrease across concentration ranges. These tests were therefore considered strong candidates to report on short draws. Among the remaining 22 chemistry tests that exhibited statistically significant biases, only ALP, AMY, CRE, FE, FERR, LIP, MG, TRIG, and URA had median absolute biases less than 25% of TEa or less than corresponding desirable biases, and biases did not consistently increase or decrease across concentration ranges. These tests were therefore considered potential candidates to report on short draws. Only 2 tests (CRP and TNT) exhibited consistently increasing or decreasing biases across their concentration ranges (**Figures 1** and **2**), and were therefore not considered candidates to report on short draws..

Among hematology tests (CBC), there was no significant bias in results for BA%, BA, EO%, EO, HCT, LY%, MCH, MCHC, MO%, MO, NE%, NRBC%, NRBC, PLT, and RDW between short and complete draws, and biases did not consistently increase or decrease across concentration ranges. These tests were therefore considered strong candidates to report on short draws. Among the remaining hematology tests that exhibited statistically significant biases, only MCV and MPV exhibited median absolute biases less than 25% of TEa or less than corresponding desirable biases, and biases did not consistently increase or decrease across concentration ranges. These tests were therefore considered potential candidates to report on short draws.

While several tests did not exhibit significant biases between short and complete draws, their median absolute biases exceeded 25% of TEa and/or desirable bias thresholds (K, NH_3, GLUC, BA%, RDW, MCHC, and HCT). In these cases, there were roughly equal numbers of high and low biases across concentration ranges in Bland-Altman plots (supplementary figures)—which would cause absolute median biases to exceed bias thresholds—but overall bias would not be statistically significant.

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Table 1 Tests That Were Strong or Potential Candidates to Report on Short Draws[a]

		Complete draw		Short draw						Evaluation using CAP TEa			Evaluation using Biological Variation				
Analyte	N	Median	IQR	Median	IQR	Median bias	IQR	% Median bias	*P* for bias	TEa	Median of absolute biases as % TEa	% of absolute biases ≥25% TEa	Desirable bias, %	% Median of absolute biases as % desirable bias	% of absolute biases ≥ desirable bias	Biases increasing or decreasing in Bland-Altman plots?	Candidate to report on short draws?
Coagulation																	
PT, s	12	11.65	0.85	11.65	1.10	0.05	0.35	0.4	.29	±15%	5.9	16.7	1.4	62.2	41.7	NO	STRONG
INR	12	1.01	0.08	1.01	0.09	0.01	0.03	0.5	.22	±15%	6.6	16.7	1.3	75.6	33.3	NO	STRONG
Chemistry																	
FT4, pmol/L	12	17.60	2.60	17.50	3.20	0.10	0.35	0.6	.90	±15% or 3.86	5.2	0.0	2.3	43.4	16.7	NO	STRONG
K, mmol/L	12	4.08	0.71	4.06	0.44	–0.03	0.31	–0.6	.68	±0.3	35.0	58.3	1.6	176.8	58.3	NO	STRONG
NTPROBNP, ng/L	12	47.45	18.90	47.50	18.00	0.00	0.70	0.0	.63	±30%	1.6	8.3	4.7	10.2	8.3	NO	STRONG
UREA, mmol/L	11	4.82	2.05	4.84	2.13	0.01	0.16	0.2	.62	9% or 0.71	9.9	9.1	6.1	28.8	0.0	NO	STRONG
NH_3, µmol/L	12	19.00	3.55	18.15	8.75	0.70	7.10	6.0	.39	±3 SD	61.8	75.0	5.5	377.4	83.3	NO	STRONG
LAC, mmol/L	12	0.99	0.49	0.96	0.44	–0.03	0.06	–3.3	.35	±0.4 or ±3 SD	8.4	8.3	8.0	49.0	8.3	NO	STRONG
CORT, nmol/L	12	207.00	113.50	201.00	95.50	–5.50	8.55	–2.5	.28	±20%	13.9	25.0	8.9	31.2	25.0	NO	STRONG
GLUC, mmol/L	12	5.03	0.93	5.03	1.05	0.03	0.38	0.6	.26	±8% or 0.3	25.9	50.0	2.3	88.8	50.0	NO	STRONG
CK, U/L	12	109.50	32.60	108.50	28.75	–0.65	3.20	–0.7	.24	±20%	7.0	8.3	8.9	15.7	0.0	NO	STRONG
PHOS, mmol/L	12	1.08	0.24	1.08	0.22	–0.01	0.02	–0.8	.20	±10% or 0.097	8.8	16.7	3.3	28.1	0.0	NO	STRONG
AST, U/L	12	20.30	6.60	20.15	8.40	0.55	1.40	3.2	.18	±15% or 6	17.5	33.3	5.3	94.8	50.0	NO	STRONG
HbA1c, %	11	5.40	0.27	5.30	0.10	0.00	0.10	0.0	.16	±8%	2.3	9.1	1.4	13.4	25.0	NO	STRONG
BILT, µmol/L	11	8.03	2.73	8.27	2.24	–0.22	0.22	–2.9	.10	±20% or 6.84	3.8	0.0	8.0	45.5	0.0	NO	STRONG
TSH, mIU/L	12	1.76	1.12	1.73	0.98	–0.04	0.10	–1.7	.07	±20% or ±0.2	12.3	16.7	10.1	26.8	0.0	NO	STRONG
TRIG, mmol/L	12	0.94	0.71	0.90	0.65	–0.02	0.06	–2.4	.04	±15%	22.6	33.3	9.6	35.4	0.0	NO	POTENTIAL
UA, µmol/L	12	288.00	89.50	285.00	88.50	–2.50	3.00	–0.9	.02	±10%	10.6	0.0	6.0	17.8	0.0	NO	POTENTIAL
CREA, µmol/L	10	72.40	12.60	68.10	11.80	–1.25	2.10	–2.0	.01	±10% or 17.7	7.1	10.0	4.2	47.3	16.7	NO	POTENTIAL
FE, µmol/L	12	16.70	5.05	16.35	4.95	–0.29	0.40	–1.6	.01	±15%	10.5	16.7	9.6	16.5	0.0	NO	POTENTIAL
MG, mmol/L	12	0.82	0.06	0.82	0.06	–0.01	0.01	–0.8	.01	±15%	5.3	0.0	1.6	51.2	25.0	NO	POTENTIAL
LIP, U/L	12	31.60	18.70	31.10	17.30	–1.05	1.55	–3.2	.01	±30%	11.3	0.0	6.6	51.5	8.3	NO	POTENTIAL

Table 1. Continued

Analyte	N	Complete draw		Short draw		Median bias	IQR	% Median bias	*P* for bias	Evaluation using CAP TEa			Evaluation using Biological Variation			Biases increasing or decreasing in Bland-Altman plots?	Candidate to report on short draws?
		Median	IQR	Median	IQR					TEa	Median of absolute biases as % TEa	% of absolute biases ≥25% TEa	Desirable bias, %	% Median of absolute biases as % desirable bias	% of absolute biases ≥ desirable bias		
FERR, µg/L	12	60.95	44.75	58.85	41.05	−2.25	8.70	−3.6	<.01	±20%	21.2	41.7	5.2	82.2	41.7	NO	POTENTIAL
ALP, U/L	12	69.70	22.25	66.00	21.80	−2.35	1.05	−3.2	<.01	±20%	16.1	25.0	6.7	48.0	8.3	NO	POTENTIAL
AMY, U/L	12	68.20	50.05	65.80	48.00	−2.40	1.60	−3.6	<.01	±20%	17.8	16.7	6.3	56.7	0.0	NO	POTENTIAL
Roche L index	12	19.00	20.00	8.50	6.00	−8.50	12.00	−34.2	.01								
Roche H index	12	9.00	5.50	29.50	15.00	17.00	18.00	182.5	.01								
Roche I index	12	1.00	0.00	1.00	0.00	0.00	0.00	0.0	.99								
Hematology																	
BA, 10^9/L	12	0.10	0.10	0.05	0.10	0.00	0.00	0.0	≥.99	±3 SD	[b]	[b]	7.7	0.0	16.7	NO	STRONG
NRBC%	12	0.00	0.10	0.05	0.10	0.00	0.10	−12.5	≥.99							NO	STRONG
BA%	12	0.90	0.35	0.80	0.35	0.00	0.15	0.0	.77	±3 SD	166.7	66.7				NO	STRONG
NE%	12	59.75	21.85	59.95	22.15	0.00	0.70	0.0	.67	±3 SD	10.2	33.3				NO	STRONG
LY%	12	32.00	19.00	32.00	18.80	0.05	1.00	0.2	.65	±3 SD	17.2	33.3				NO	STRONG
MO, 10^9/L	12	0.40	0.30	0.40	0.20	0.00	0.05	0.0	.63	±3 SD	0.0	33.3	6.5	0.0	33.3	NO	STRONG
NRBC, /100 WBC	12	0.00	0.01	0.00	0.01	0.00	0.01	0.0	.63							NO	STRONG
RDW, %	12	13.25	0.65	13.30	1.20	−0.05	0.25	−0.4	.61	±3 SD	27.8	50.0	1.7	69.9	33.3	NO	STRONG
EO, 10^9/L	12	0.15	0.15	0.15	0.10	0.00	0.00	0.0	.50	±3 SD	0.0	16.7	16.0	0.0	16.7	NO	STRONG
MO%	12	7.05	2.50	7.20	2.05	−0.25	0.50	−3.4	.39	±3 SD	26.5	50.0				NO	STRONG
MCH, pg	12	30.15	2.90	30.50	3.00	0.10	0.15	0.3	.38	±3 SD	7.4	16.7	1.2	29.8	16.7	NO	STRONG
MCHC, g/L	12	338.50	16.50	337.50	16.50	−1.00	2.50	−0.3	.13	±3 SD	10.6	25.0	0.4	100.4	50.0	NO	STRONG
HCT, %	12	0.41	0.04	0.41	0.03	−0.01	0.01	−1.2	.12	±4%	40.1	58.3	1.6	102.4	58.3	NO	STRONG
EO%	12	2.10	3.05	2.00	2.45	−0.10	0.40	−6.1	.11	±3 SD	13.9	33.3				NO	STRONG
PLT, 10^9/L	12	261.00	57.50	240.50	59.50	−8.50	17.50	−4.1	.11	±25%	23.4	33.3	3.7	156.3	58.3	NO	STRONG
MCV, fL	12	88.55	7.30	89.20	7.70	0.45	0.60	0.5	.01	±3 SD	23.4	50.0	1.0	49.2	0.0	NO	POTENTIAL
MPV, fl	12	8.15	0.75	7.95	0.80	−0.10	0.30	−1.4	.01	±3 SD	22.2	41.7	1.9	74.5	41.7	NO	POTENTIAL

Abbreviations: AGAP, anion gap; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; AST, aspartate aminotransferase; BA, basophil count; BILD, bilirubin—direct; BILT, bilirubin—total; CA, calcium—total; CAP, College of American Pathologists; CBC, complete blood count; CK, creatine kinase; CL, chloride; CO_2, carbon dioxide; CORT, cortisol; CREA, creatinine; CRP, C-reactive protein; EO, eosinophil count; FE, iron; FERR, ferritin; FT4, thyroxine—free; GGT, gamma glutamyl transferase; GLUC, glucose; H index, hemolysis index; Hb, hemoglobin; HbA1c, hemoglobin A1c; HCT, hematocrit; I index, icterus index; INR, international normalized ratio; K, potassium; L index, lipemia index; LAC, lactate; LDH, lactate dehydrogenase; LIP, lipase; LY, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MG, magnesium; MO, monocyte; MPV, mean platelet volume; NA, sodium; NE, neutrophil; NH_3, ammonia; NRBC, nucleated red blood cell count; NTPROBNP, NT-proBNP; PHOS, phosphate; PLT, platelet; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cell count; RDW, red cell distribution width; TEa, total allowable error (when 2 thresholds were provided, the higher threshold was used); TNT, high-sensitivity cardiac troponin; TP, total protein; TRIG, triglycerides; TSH, thyroid-stimulating hormone; UA, uric acid; UWBC, uncorrected white blood cell count, UREA, urea; WBC, white blood cell count.

[a]Table is sorted by *P* values, within testing division. Blank cells indicate data not available. Cells are highlighted if they contain *P* values less than .05, if biases exceed 25% of TEa or 100% of desirable bias derived from biological variation ($0.25 \times \sqrt{[CV_I^2 + CV_G^2]}$),[21] or if biases were noted to continually increase or decrease in Bland-Altman plots. Median bias is defined as the median of test result differences within volunteers between short and complete draws (short draw result minus complete draw result). % Median bias is defined as the median of test result differences within volunteers between short and complete draws (short draw result minus complete draw result) each divided by test result for the complete draw × 100.

[b]Cannot calculate as peer group SD = 0.00. Short draw fill volumes were 1 mL, yielding a fill volume of 33% for mint green (AGAP, ALT, ALB, ALP, AMY, AST, BILD, BILT, CRP, CA, CO_2, CL, CORT, CK, CREA, FE, FERR, GGT, GLUC, LAC, LDH, LIP, MG, NH_3, PHOS, K, NA, TP, TRIG, UREA, UA) and lavender top tubes (CBC, HbA1C, NH_3), 56% for light blue top tubes (INR, PT, PTT), and 50% for gray top tubes (LAC).

Table 2 Tests That Were Not Candidates to Report on Short Draws[a]

Analyte	N	Complete draw		Short draw		Median bias	IQR	% Median bias	*P* for bias	Evaluation using CAP TEa			Evaluation using biological variation			Biases increasing or decreasing in Bland-Altman plots?	Candidate to report on short draws?
		Median	IQR	Median	IQR					TEa	Median of absolute biases as % TEa	% of absolute biases ≥25% TEa	Desirable bias, %	% Median of absolute biases as % desirable bias	% of absolute biases ≥ desirable bias		
Coagulation																	
PTT, s	12	34.30	4.15	41.50	5.70	6.25	2.15	18.2	<.01	±15%	121.1	91.7	1.9	940.4	91.7	NO	NO
Chemistry																	
CRP, mg/L	12	0.88	2.10	0.85	2.03	−0.03	0.07	−2.5	.02	±3 SD	0.8	0.0	23.2	10.7	0.0	YES	NO
GGT, U/L	12	17.45	11.15	15.85	10.95	−1.95	1.72	−6.6	<.01	±15% or 5	29.0	50.0	11.5	57.2	33.3	NO	NO
ALT, U/L	12	11.30	12.00	5.83	9.00	−5.30	4.29	−29.9	<.01	±15% or 6	88.3	75.0	9.3	322.9	75.0	NO	NO
TNT, ng/L	12	5.15	7.29	3.62	1.18	−0.47	6.86	−10.8	<.01	±30% or 3 SD	2.5	33.3	8.2	131.3	58.3	YES	NO
AGAP	12	7.40	1.70	8.10	1.65	0.90	0.95	12.8	<.01							NO	NO
CL, mmol/L	12	107.05	2.40	107.75	2.15	0.95	0.70	0.9	<.01	±5%	17.8	16.7	0.4	217.2	66.7	NO	NO
LDH, U/L	12	155.00	27.50	173.00	32.00	18.00	14.00	11.2	<.01	±15%	74.8	75.0	3.1	356.4	75.0	NO	NO
BILD, µmol/L	11	3.20	1.24	2.82	0.71	−0.54	0.66	−14.8	<.01	±20% or 6.84	7.9	0.0	14.2	104.5	50.0	NO	NO
CA, mmol/L	12	2.33	0.06	2.28	0.05	−0.05	0.02	−1.9	<.01	±0.025	180.0	100.0	0.8	228.0	91.7	NO	NO
ALB, g/L	12	39.30	4.80	37.15	5.15	−1.45	0.60	−3.5	<.01	±8%	43.7	83.3	1.2	291.3	83.3	NO	NO
CO_2, mmol/L	12	25.25	2.65	21.55	1.95	−3.25	0.90	−13.1	<.01	±20%	65.3	100.0	1.6	835.6	100.0	NO	NO
NA, mmol/L	12	138.75	1.20	137.05	1.75	−1.60	1.10	−1.2	<.01	±4	40.0	83.3	0.2	538.9	91.7	NO	NO
TP, g/L	12	70.15	5.05	69.35	4.75	−1.50	1.70	−2.2	<.01	±8%	27.7	58.3	1.1	203.2	75.0	NO	NO
Hematology																	
RBC, 10^{12}/L	12	4.68	0.46	4.62	0.36	−0.09	0.14	−1.8	.04	±4%	50.6	66.7	1.9	107.5	58.3	NO	NO
Hb, g/L	12	139.50	14.50	139.50	16.00	−2.00	2.50	−1.3	.02	±4%	32.7	58.3	1.8	71.2	25.0	NO	NO
LY, 10^9/L	12	2.00	1.00	1.90	0.90	−0.10	0.10	−5.1	<.01	±3 SD	45.7	75.0	6.2	81.8	33.3	NO	NO
NE, 10^9/L	12	3.40	1.45	3.15	1.50	−0.10	0.10	−3.7	<.01	±3 SD	41.7	83.3	7.0	52.7	16.7	NO	NO
UWBC, 10^9/L	12	6.10	2.20	5.80	2.10	−0.25	0.20	−4.7	<.01							NO	NO
WBC, 10^9/L	12	6.10	2.20	5.80	2.10	−0.25	0.20	−4.7	<.01	±10%	46.5	83.3	5.1	90.9	50.0	NO	NO

[a]Table is sorted by *P* values, within testing division. Blank cells indicate data not available. Cells are highlighted if they contain *P* values less than .05, if biases exceed 25% of TEa or 100% of desirable bias derived from biological variation ($0.25 \times \sqrt{[CV_I^2 + CV_G^2]}$),[21] or if biases were noted to continually increase or decrease in Bland-Altman plots. Median bias is defined as the median of test result differences within volunteers between short and complete draws (short draw result minus complete draw result). % Median bias is defined as the median of test result differences within volunteers between short and complete draws (short draw result minus complete draw result) each divided by test result for the complete draw ×100. Short draw fill volumes were 1 mL, yielding a fill volume of 33% for mint green (AGAP, ALT, ALB, ALP, AMY, AST, BILD, BILT, CRP, CA, CO2, CL, CORT, CK, CREA, FERR, GGT, GLUC, IRON, LAC, LDH, LIP, MG, NH_3, PHOS, K, NA, TP, TRIG, UREA, UA) and lavender top tubes (CBC, HbA1C, NH_3), 56% for light blue top tubes (INR, PT, PTT), and 50% for gray top tubes (LAC). For definitions of abbreviations, see Table 1.

DISCUSSION

In our pilot study, we identified a list of tests that may be reported on 1-mL (33% to 55% fill volume, depending on tube type) blood draws in BD vacutainers using specific instruments. Our results may be of interest to laboratories that wish to perform limited testing on short draws—especially those from pediatric patients.

While the CLSI recommends against testing short draws,[19] this position is supported by a relatively small body of literature, some of which is quite old. As such, we were curious whether short draws always result in unacceptable biases as a proportion of TEa and desirable bias calculated from biological variation, as well as whether bias distributions change across concentration ranges. Our interest in this subject arose from requests to perform testing in cases of difficult pediatric phlebotomy, leading to the present study.

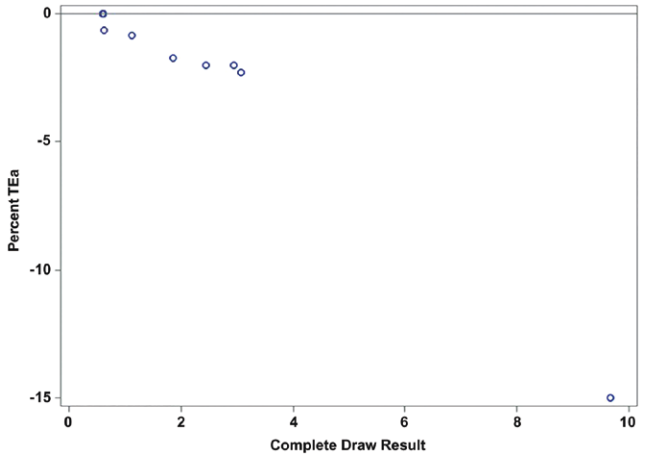

Figure 1 Bland-Altman plot showing increasing negative bias as percent TEa for CRP. Percent TEa is calculated as 100 × (short draw result – complete draw result)/TEa. CRP, C-reactive protein; TEa, total allowable error.

An important characteristic of short draws is their elevated anticoagulant concentration and anticoagulant/blood ratio.[19] In coagulation testing, citrate reversibly chelates calcium—which is required for both intrinsic and extrinsic coagulation pathways.[26] Excess citrate in short draws diminishes calcium availability, thereby reducing activity of both pathways and potentially elevating PT, INR, and PTT. This phenomenon is further influenced by dilution from liquid anticoagulants. Consequently, the CLSI recommends that tubes used for coagulation testing be at least 90% full.[18] In our study, however, only PTT significantly increased in short draws (56% fill volume; median bias +18.2%) vs complete draws—which was over 100% of TEa and nearly 10-fold higher than what is considered a desirable bias derived from biological variation. Our findings align with those of another study, which also used 3.2% sodium citrate tubes and reported a significant bias in PTT only when fill volume fell below 70%, and in PT only when fill volume fell below 60%.[3] Importantly, tubes containing higher concentrations of sodium citrate (3.8%) are more susceptible to fill volume–related bias, with significant deviations in PTT and PT observed at much higher fill volume thresholds (eg, <90% for PTT, <80% for PT).[3] Excess anticoagulant may also affect some chemistry tests. For example, high heparin concentration negatively biases sodium measurement using ion-selective electrodes on QuidelOrtho Vitros 950 analyzers.[8] Consistent with this effect, we observed significantly decreased sodium in short draws (median bias –1.2%, median 40% of TEa and 538.9% of desirable bias) vs complete draws in our study. Whether elevated heparin concentration affects other chemistry tests is less clear, however, as reagent formulations and assay principles likely respond differently to anticoagulant concentration. Response may also differ according to tube type, manufacturer, and fill volume. Older reports attribute elevated heparin concentration in short draws (33% fill volume) using Greiner Bio-One Vacuettes to increased CK on Beckman UniCell DxC analyzers and GGT on Roche analyzers vs complete draws.[6] However, in our study, which used BD vacutainers (33% fill volume) and Roche Cobas analyzers, we noted a small but significant negative bias only for GGT in short

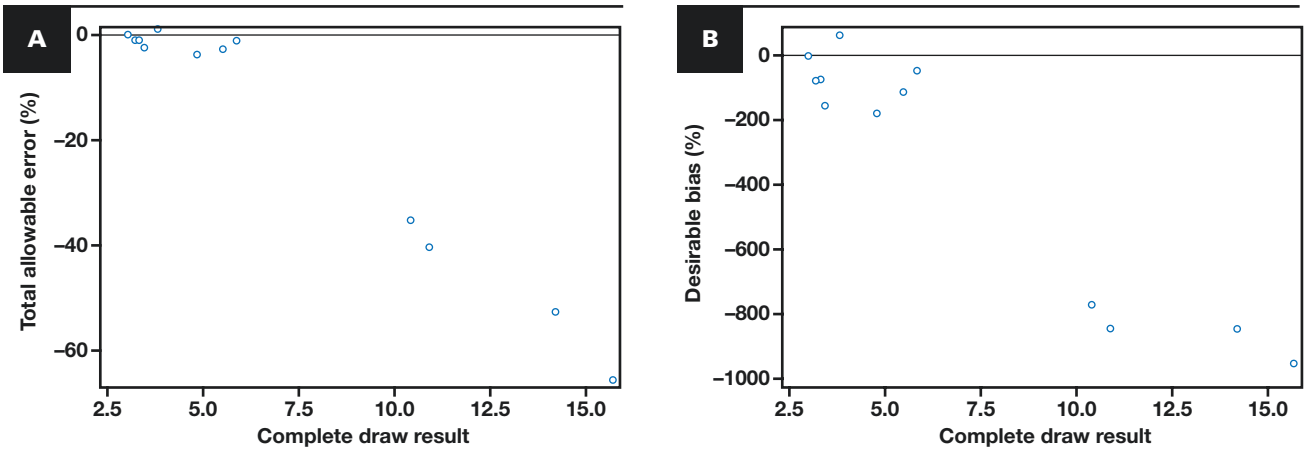

Figure 2 Bland-Altman plots showing increasing negative bias as percent TEa and percent desirable bias for high-sensitivity cardiac troponin T. Percent TEa is calculated as 100 × (short draw result – complete draw result)/TEa. Percent desirable bias is calculated as 100 × ((100 × [short draw result – complete draw result]/complete draw result)/desirable bias). CRP, C-reactive protein; TEa, total allowable error.

HTTPS://DOI.ORG/10.1093/AJCP/AQAF109

draws (33% fill volume; median bias –6.6%, median 29.0% of TEa, and 57.2% of desirable bias)—the reason for which is unclear. While there are other reports of assay- and tube-specific biases attributed to elevated heparin concentration in short draws,[5,27] these studies evaluated only the impact of anticoagulant concentration on a small number of tests. In hematology testing, elevated EDTA concentration is known to affect CBC and differential by accelerating platelet degradation, enlarging platelets, and causing RBCs and WBCs to shrink.[19] However, many of the studies used to justify volume requirements for CBC testing were performed in the 1960s and 1970s using liquid K_3-EDTA–containing glass tubes rather than spray-dried K_2-EDTA–containing plastic tubes.[28] This has been suggested as to why, in more recent studies, short draws in BD vacutainers (25% to 50% fill volume) containing dried K_2-EDTA result in only minor changes in CBC components.[28,29] In our study, among CBC components that were significantly different between short (33% fill volume) and complete draws, we found similarly small changes. For example, while RBC, Hb, MCV, MPV, LY, NE, and WBC were significantly different in short vs complete draws, median absolute biases were all less than 6%. However, when these biases were expressed as proportions of TEa and desirable bias, they were substantially larger (median percent TEa: 22.2% to 50.6% and percent desirable bias: 52.7% to 107.5%). This highlights the importance of evaluating bias observed in short draws not just in terms of statistical significance but also clinically and biologically significant degrees of change. Interestingly, all significant biases among CBC components except for MCV were negative, which is consistent with in vitro hemolysis.

Hemolysis is a major concern in short draws and most likely results from a 3× higher initial fill rate, which imparts greater shear stress on the small volume of blood collected.[10] Consequently, hemolysis has been observed in short draws of both heparinized and serum tubes.[10] In a study using Greiner Bio-One Vacuettes lithium heparin tubes, a fill volume of 50% was attributed to increased hemolysis (+210%), K (+4.2%), and LDH (+21.6%) vs complete draws.[10] In our study, we observed a smaller increase in hemolysis, as measured by the Roche H index (+182.5%), but a smaller increase in LDH (+11.2%), despite using a lower fill volume of 33%. The reason for this is unclear. However, we note that studies retrospectively examining blood samples—which may include short draws—already collected in the course of patient care may inadvertently include those collected using intravenous catheters, which subject blood to much greater shear stress than standard venipuncture[30] and produce twice the number of hemolyzed samples in short draws (50% fill volume) vs complete draws when using butterfly needles.[31] In a larger study (n = 156 participants), significant hemolysis (+407.8%) was observed in short draws (50% fill volume) vs complete draws, as well as a much larger increase in K (+11.8%), LDH (+50%), and AST (+19.4%).[9] Interestingly, a significant increase in lipemia (+46.3%) was observed in this study, whereas in our study, we observed a significant decrease in lipemia (–34.2%). While the reason for this is unclear, as is the actual degree of hemolysis expected when performing short draws, implementing spectrophotometric index thresholds for appending interpretive comments or canceling tests remains extremely important in guiding clinical interpretation.

A greater air gap is another consequence of short draws. This increases specimen surface area-to-volume ratio in evacuated tubes, increasing the rate of gas exchange. Most notable is an effect on CO_2, which rapidly escapes from venous blood (21%; 160 mm Hg) to the atmosphere, where it is much lower (0.02%; 0.25 mm Hg). Not surprisingly, we found a significant and large negative bias in CO_2 in short draws vs complete draws (median bias –13.1%, median 65.3% of TEa and 835.6% of desirable bias), which was consistent with multiple other studies.[14-17]

Our study has several strengths. The first is that it examined the impact of short draws on the most extensive list of acute care tests so far. Second, it evaluated biases in terms of not only statistical significance but also clinical and biological significance. It also looked for changes in bias distribution, which could alter conclusions regarding the appropriateness of short draws depending on analyte concentration. Third, rather than focusing on identifying tests that *could not* be reported in short draws, its goal was to identify tests that *could* be reported, which would be of practical interest to laboratories.

Our study also has some limitations. First, only healthy volunteers were used, which means that the full range of analyte concentrations—including those of therapeutic drugs—in acute care patients was not represented. We acknowledge that using healthy volunteers could miss important changes in bias distributions at much higher or lower analyte concentrations. However, our approach was both convenient and practical, and it is commonly used in our laboratories to evaluate new container types in pilot studies. Second, we drew blood into syringes and transferred 1 mL into vacutainers to simulate short draws occurring in routine practice. This extra step may have increased hemolysis compared to short draws of blood directly into vacutainers. However, an accurate 1-mL blood draw directly into vacutainers was not possible without using a syringe. Third, we evaluated test biases only between a fixed short draw blood volume of 1 mL and complete draw fill volumes. This design, while practical, reduced the generalizability of our findings because laboratories receive short draws containing a variety of blood volumes. As previously mentioned, we chose 1 mL for our short draw blood volume because it was the smallest blood volume that our staff could reliably aliquot plasma from after centrifugation, but this prevented us from examining the impact of smaller volumes. In addition, while tests that were valid to report in 1-mL short draws were likely valid to report in larger (eg, 2 mL) short draws, our design prevented us from determining whether tests that were not valid to report in 1-mL short draws were valid to report in larger short draws. Fourth, we recruited only 12 volunteers, which means that our study was likely underpowered to detect significant biases compared to larger studies. However, this reduction in statistical power primarily concerns our ability to detect small biases, which may be inconsequential in the reporting of test results on short draws. Fifth, our study results may be applicable only to the tube types, tests, and instrumentation used. Sixth, the thresholds we used could be considered arbitrary. For example, while one

laboratory may view 25% of TEa to be an unacceptable threshold to cross when dealing with short draws, other laboratories may not. This also applies to desirable bias criteria, which assume imprecision is near zero, which is not the case.[32] Finally, due the large number of statistical tests performed, we may have observed significant differences purely due to chance. Despite these limitations, however, we feel our study has intrinsic value due to its broad assessment of multiple tests and its practical approach. However, since some tests should not be reported on short draws, complete draws should remain the default specimen type, with short draws remaining alternative specimen types for specific clinical scenarios and tests. We hope our pilot study will encourage laboratories to perform their own investigations regarding the validity of short draws.

CONCLUSIONS

Underfilled blood tubes, or short draws, may be valid collections for several coagulation, chemistry, and hematology tests, which may prevent additional unnecessary phlebotomy. Laboratories should perform their own investigations to determine if short draws are acceptable for limited testing using their own tube and instrument types.

Supplementary material

Supplementary material is available at *American Journal of Clinical Pathology* online.

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Conflicts of Interest

None declared.

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