Stability of haematology parameters on the LH750: comparison of Sarstedt Monovettes and BD Vacutainers

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ABSTRACT

Haematological analysis of white blood cells, red blood cells and platelets is used to aid diagnosis and treatment. Although most laboratories aim to analyse haematology samples on the day of collection, this is not always possible, particularly when the laboratory is remote from the patient. The integrity of a haematological sample is known to depend on time and temperature: measurement technique has already been found to have an impact on stability. This study aims to evaluate whether or not the type of EDTA specimen tube affects the stability, and the effect on stability using two commonly used blood collection systems (Becton Dickinson Vacutainers and Sarstedt Monovettes). Blood was drawn from 20 volunteers and stored refrigerated. Haematological analysis was conducted on a Beckman Coulter LH750 haematology analyser at multiple time points up to 72 h. The results were examined using analysis of variance (ANOVA), to look for imprecision both within-run and between run. Stability assessment was performed using an in-house method based on the manufacturer's stated precision limits. An analyte was classed as unstable when the cumulative SD/CV exceeded the precision limits of that assay. The method used to assess stability was found to provide robust stability information that matched data provided by the manufacturer and other researchers. Accurate full blood count results can be obtained on samples up to 48 h, provided that the samples are stored in a refrigerator. The tube type was found to have minimal impact on the stability of haematological samples.

KEY WORDS: Hematologic tests/instrumentation.
Reproducibility of results.
Time factors.

Introduction

Diagnosis, treatment and follow-up of a broad range of diseases depends on the provision of accurate and precise clinical pathology data. One key part of these data is the full blood count (FBC), which consists of the quantification and characterisation of the three major cellular components of blood: white blood cells, red blood cells and platelets.

Standard haematology textbooks such as that by Dacie and Lewis[1] discuss the effects of storage on both the numerical and morphological aspects of blood. Refrigeration slows these storage effects, but the recommended practice is to analyse samples within 24 h for maximum accuracy.[1–3] The duration of storage before the quality of the FBC is adversely affected is known to depend on the temperature of storage, the anticoagulant in the sample tube, and the analyser used. The integrity of a haematological sample is therefore critically dependent on the time (and temperature) between collection and analysis.

Most laboratories aim to analyse haematology samples on the day of collection. However, where laboratories are remote from the patient, and receive samples by courier and/or post, analysis can be delayed.

Where the stability of haematological parameters has been studied, there has been little consistency of methodology, leading to inconsistent results and conclusions. Imeri *et al*.[4] recently recommended that the International Council for Standardization in Haematology (ICSH) adopts a standard reference method to address this. The main focus of haematological stability studies so far has been the impact of measurement technique. A number of researchers[4–6] have found evidence in support of Lewis and Tatsumi's[1] claims that the stability of haematological samples depends on the analyser used.

However, to date, no studies appear to have compared the impact of the different sample collection tubes. Becton Dickinson (BD) Vacutainers and Sarstedt Monovettes are commonly used for the collection of EDTA-anticoagulated whole blood specimens for FBC analysis. The present study examines the hypothesis that the stability of haematological samples also depends on the type of EDTA specimen tube used to collect the sample.

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Materials and methods

Twenty healthy volunteers (10 males, 10 females) were recruited. No restrictions were placed on these volunteers. A total of 36.9 mL blood was taken from each volunteer, collected using a cannula into seven 2.7 mL EDTA Sarstedt Monovettes and four 4.5 mL EDTA BD Vacutainers. Both types of tube are plastic and contain potassium K3 EDTA as

Table 1. Full blood count parameters measured by the Beckman Coulter LH750 haematology analyser.

	Directly measured	Derived from size distributions	Derived from AccuGate analysis	Computed
CBC parameters (measured by Coulter Principle)	WBC, RBC, Hb	MCV, RDW, PLT, MPV	NA	MCH, MCHC, HCT
WBC differential (measured by VCS technology)	NA	NA	LY%, MO%, NE%, EO%, BA%	LY, MO,NE, EO, BA
Reticulocyte count (measured by VCS technology)	NA	NA	RET%, IRF, MRV	RET

CBC: complete blood count; VCS: volume, conductivity, scatter; WBC: white blood cells; RBC: red blood cells; Hb: haemoglobin; MCV: mean cellular volume; RDW: red blood cell distribution width; PLT: platelets; MPV: mean platelet volume; LY: lymphocytes; MO: monocytes; NE: neutrophils; EO: eosinophils; BA: basophils; RET: reticulocytes; IRF: immature reticulocyte fraction; MRV: mean reticulocyte volume; MCH: mean cellular haemoglobin; MCHC: mean cellular haemoglobin concentration; HCT: haematocrit; NA: not applicable/not available.

Taken from Beckman Coulter LH 700 series operator manual; operation principles.[7]

an anticoagulant. Becton Dickinson tubes contain 7.2 mg K3 EDTA in a 4 mL tube; Sarstedt tubes contain sufficient K3 EDTA to achieve a concentration of 1.2–2 mg EDTA/mL blood, with a maximum dilution effect of 1%.

Samples were taken within a 1-h timeframe and then mixed for 10 min on a roller mixer. In order to eliminate potential bias between collections in the different bottles, two separate pools were created for each volunteer: one pool containing all the Monovette samples, the other pool containing all the Vacutainer samples. These pooled samples were mixed for a further 5 min, and then an approximate 1.5 mL sample was transferred to each of 22 neutral blood tubes (3.0 mL, no anticoagulant or additive). Each sample aliquot represented a single time point. Baseline samples were analysed immediately following completion of processing the primary samples (within 30 min of completion of collection). All other tubes were stored refrigerated at 2–10°C for up to 72 h. Designated tubes were removed from the refrigerator after 4, 8, 12, 24, 30, 36, 42, 48, 60 and 72 h, and mixed for 5–10 min on a roller mixer before being analysed.

Table 2. Beckman Coulter specifications for precision.

	Approximate level	LH750 precision specification		
		1 SD	2 SD	CV
WBC	9–11 x 10^9/L	NA	NA	1.7
RBC	4.5–5.5 x 10^{12}/L	NA	NA	0.8
Hb	14–16 g/dL	NA	NA	0.8
MCV	80–90 fL	NA	NA	0.8
RDW	12–14%	NA	NA	2.2
PLT	280–320 x 10^9/L	NA	NA	3.3
MPV	8–10 fL	NA	NA	2.2
NE	50–60%	NA	3.0	NA
LY	25–35%	NA	3.0	NA
MO	5–10%	NA	2.0	NA
EO	2–5%	NA	1.0	NA
BA	0.5–1.5%	NA	1.0	NA
RET	1.5–4%	0.68	NA	11.0

SD: standard deviation; CV: coefficient of variation; NA: no specifications available. See Table 1 for abbreviations.

Taken from Beckman Coulter LH 700 series operator manual: performance specifications.[7]

The EDTA samples were analysed for FBC and reticulocytes using an LH750 haematology analyser (Beckman Coulter). This analyser provides a comprehensive haematological profile (i.e., complete blood cell analysis [CBC], differential leucocyte cell count and reticulocyte analysis[7]). The FBC parameters are given in Table 1.

Statistical analysis and stability assessment

Analysis is based on the assumption that the stability of calculated parameters depends on the stabilities of the parameters used in their calculation. Hence, for the purposes of this study, only directly measured or derived parameters were used for analysis of sample stability (Table 1). All data analyses were performed using Microsoft Excel 2003. Any results flagged by the LH750 as unreliable were excluded from statistical analysis.

Analysis of variance (ANOVA) was used to calculate within-run and between-run imprecision. Using the principles outlined by Thiers *et al.*,[8] an in-house technique for assessing sample stability was developed, based on the manufacturer's stated precision limits for each assay (Table 2). The method involves calculating the cumulative standard deviation (SD) and/or cumulative coefficient of variation (CV) using the mean results of each time point. This parameter is then plotted against the manufacturer's analytical precision limits. An analyte is classed as unstable when the SD/CV exceeds these stated precision limits of the analyser for that parameter.

Results

Samples in this study were tested for FBC at 11 different time points using both BD Vacutainer tubes and Sarstedt Monovette tubes. The mean, standard deviation and *P* value for each parameter at all storage times with both tube types are given in Tables 3–6. The stability of selected parameters is shown in Figures 1 and 2. A summary of the stability times (i.e., the time beyond which the manufacturers' recommended precision limits are exceeded) achieved in BD and Sarstedt tubes, together with comparative data from the literature, is given in Table 7. Each run contained internal

Table 3. Mean (standard deviation) and appropriate *P* values for CBC and reticulocyte parameters using Becton Dickinson tubes.

		0 hours	4 hours	8 hours	12 hours	18 hours	24 hours	30 hours	36 hours	42 hours	48 hours	60 hours	72 hours	*P* value
WBC	(x10^9/L)	5.7 (1.2)	5.7 (1.1)	5.7 (1.1)	5.6 (1.1)	5.6 (1.1)	5.7 (1.1)	5.6 (1.0)	5.5 (1.2)	5.5 (1.1)	5.4 (1.3)	5.3 (1.1)	5.2 (1.2)	0.9571
RBC	(x10^{12}/L)	4.31 (0.51)	4.30 (0.50)	4.30 (0.49)	4.28 (0.51)	4.30 (0.51)	4.30 (0.51)	4.30 (0.50)	4.31 (0.49)	4.33 (0.50)	4.31 (0.50)	4.31 (0.50)	4.32 (0.50)	1.0000
Hb	(g/dL)	12.9 (1.4)	12.9 (1.4)	12.9 (1.4)	12.9 (1.4)	12.9 (1.4)	12.9 (1.4)	12.9 (1.4)	12.9 (1.4)	12.9 (1.4)	12.9 (1.4)	12.9 (1.4)	13.0 (1.4)	1.0000
MCV	(fL)	88.2 (3.2)	88.2 (3.3)	88.6 (3.3)	88.4 (3.3)	88.4 (3.3)	88.8 (3.6)	88.8 (3.6)	88.7 (3.4)	88.6 (3.5)	88.6 (3.5)	89.0 (3.5)	89.3 (3.6)	0.9992
RDW	(%)	13.6 (1.2)	13.6 (1.2)	13.5 (1.3)	13.5 (1.2)	13.5 (1.3)	13.6 (1.2)	13.6 (1.2)	13.6 (1.3)	13.6 (1.2)	13.5 (1.2)	13.5 (1.3)	13.5 (1.3)	1.0000
PLT	(x10^9/L)	221 (47)	218 (46)	217 (46)	217 (47)	220 (48)	219 (49)	216 (48)	216 (49)	213 (45)	211 (47)	210 (42)	204 (45)	0.9945
MPV	(fL)	8.9 (1.1)	9.1 (1.1)	9.2 (1.2)	9.2 (1.2)	9.4 (1.2)	9.5 (1.2)	9.6 (1.2)	9.7 (1.2)	9.8 (1.2)	9.8 (1.2)	9.9 (1.2)	10.1 (1.3)	0.0433
RET	(%)	1.3 (0.6)	1.1 (0.6)	1.0 (0.6)	1.0 (0.6)	1.0 (0.6)	1.1 (0.6)	1.0 (0.6)	1.0 (0.6)	1.0 (0.7)	1.0 (0.6)	1.2 (0.6)	1.0 (0.6)	0.9691

See Table 1 for abbreviations.

quality control samples used as part of the laboratory protocol, and all results were within specified limits of acceptance.

Discussion

Literature review

Beckman Coulter states in its LH700 reference information that the FBC is stable for 48 h when the sample is stored at 4°C.[7] However, these data are based on the results of five normal samples only. There is a growing realisation that the technology used to analyse FBC and reticulocytes has a major impact on the stability of the results. Three groups[4–6] recently evaluated various haematology analysers and concluded that the LH750 gave better stability than the other analysers under evaluation.

Bourner *et al*.[5] claim 72-h stability for all FBC parameters using a Beckman Coulter LH750; however, the results quoted in their paper do not fully back this up. They achieved CVs of 1.75 and 11.6 for WBC and reticulocytes, respectively, but these figures exceed the precision specifications quoted by Beckman Coulter.

Jackson *et al*.[9] tested stability only up to 36 h at 4°C. They claim that any deterioration in results caused by a delay in analysis of 36 h is likely to be minimal, and will still be representative for that patient. Their conclusions were drawn using expected percentage change and probability of negative trend, rather than defined precision limits of the individual assays.

Various researchers have evaluated and validated the LH750 without performing stability assessment.[10,11] Other researchers make claims about the stability of haematology samples without reference to the technology used. Thus, Buttarello[2] suggests that all haematology samples be analysed promptly, and recommends that the maximum allowable storage time should be 6 h at room temperature and 24 h in the refrigerator.

Table 4. Mean (standard deviation) and appropriate *P* values for differential parameters using Becton Dickinson tubes.

		0 hours	4 hours	8 hours	12 hours	18 hours	24 hours	30 hours	36 hours	42 hours	48 hours	60 hours	72 hours	*P* value
NE	(%)	55.20 (8.11)	54.87 (7.83)	54.72 (8.47)	54.94 (8.20)	56.40 (7.87)	57.33 (8.45)	57.16 (8.83)	59.10 (7.73)	60.11 (10.34)	60.03 (11.03)	58.99 (11.40)	50.16 (16.06)	0.0871
LY	(%)	31.70 (8.96)	31.55 (8.59)	31.52 (8.99)	30.95 (8.81)	30.18 (8.61)	29.93 (8.91)	29.74 (9.35)	28.56 (7.86)	27.78 (12.10)	30.06 (8.68)	30.72 (10.41)	36.40 (13.28)	0.5024
MO	(%)	8.57 (1.58)	8.87 (1.71)	8.75 (1.67)	8.58 (1.76)	8.47 (1.89)	8.10 (1.98)	7.93 (1.57)	8.32 (1.95)	7.51 (3.93)	6.98 (3.41)	6.75 (3.10)	9.82 (3.72)	0.0136
EO	(%)	3.91 (3.28)	3.99 (3.26)	4.06 (3.31)	4.10 (3.34)	4.04 (3.26)	3.66 (2.49)	3.62 (2.95)	3.15 (2.61)	2.99 (2.79)	2.37 (1.52)	2.80 (2.35)	3.11 (2.32)	0.6251
BA	(%)	0.62 (0.23)	0.96 (0.55)	0.96 (0.55)	1.44 (1.16)	0.93 (0.52)	0.99 (0.97)	1.55 (2.53)	0.87 (1.13)	0.45 (0.31)	0.57 (0.83)	0.47 (0.42)	0.39 (0.41)	0.0025

See Table 1 for abbreviations.

Table 5. Mean (standard deviation) and appropriate *P* values for CBC and reticulocyte parameters using Sarstedt tubes.

		0 hours	4 hours	8 hours	12 hours	18 hours	24 hours	30 hours	36 hours	42 hours	48 hours	60 hours	72 hours	*P* value
WBC	($x10^9$/L)	5.8 (1.1)	5.8 (1.1)	5.8 (1.1)	5.7 (1.1)	5.7 (1.2)	5.7 (1.2)	5.7 (1.3)	5.5 (1.2)	5.6 (1.2)	5.5 (1.2)	5.5 (1.2)	5.4 (1.2)	0.9958
RBC	($x10^{12}$/L)	4.35 (0.49)	4.35 (0.50)	4.35 (0.50)	4.32 (0.51)	4.36 (0.51)	4.35 (0.50)	4.35 (0.50)	4.34 (0.50)	4.36 (0.50)	4.36 (0.51)	4.36 (0.50)	4.36 (0.50)	1.0000
Hb	(g/dL)	13.1 (1.4)	13.0 (1.4)	13.1 (1.4)	13.0 (1.4)	13.1 (1.5)	13.1 (1.5)	13.1 (1.4)	13.1 (1.5)	13.1 (1.4)	13.1 (1.4)	13.1 (1.4)	13.1 (1.4)	1.0000
MCV	(fL)	88.2 (3.3)	88.3 (3.3)	88.4 (3.3)	88.5 (3.3)	88.6 (3.5)	88.6 (3.5)	88.9 (3.3)	88.7 (3.5)	88.8 (3.5)	88.6 (3.6)	88.8 (3.6)	89.2 (3.7)	0.9997
RDW	(%)	13.7 (1.2)	13.5 (1.2)	13.6 (1.2)	13.6 (1.2)	13.5 (1.2)	13.5 (1.2)	13.5 (1.3)	13.5 (1.2)	13.6 (1.2)	13.6 (1.2)	13.5 (1.2)	13.5 (1.2)	1.0000
PLT	($x10^9$/L)	212 (52)	219 (52)	219 (30)	214 (50)	217 (49)	217 (49)	216 (49)	215 (47)	215 (48)	213 (50)	210 (48)	205 (48)	0.999
MPV	(fL)	9.0 (1.2)	9.1 (1.2)	9.2 (1.2)	9.1 (1.3)	9.3 (1.2)	9.4 (1.3)	9.6 (1.3)	9.7 (1.3)	9.8 (1.3)	9.9 (1.3)	10.0 (1.3)	10.2 (1.3)	0.0268
RET	(%)	1.5 (0.6)	1.2 (0.6)	1.1 (0.7)	1.1 (0.6)	1.1 (0.6)	1.1 (0.7)	1.1 (0.6)	1.1 (0.6)	1.1 (0.7)	1.2 (0.6)	1.0 (0.6)	1.3 (0.6)	0.6767

See Table 1 for abbreviations.

In the context of sample stability, statistical analysis cannot differentiate between changes due to sample degradation and analytical variance within the limitations of the specified assay. Knowledge of the analytical specification and, in particular, manufacturer's precision guidelines is vital for meaningful evaluation of stability data (data versus information). However, very few researchers appear to take this on board, and give no definition of stability, no indication of how many samples were analysed, and gloss over the statistical methods used – a fact highlighted by Imeri *et al*.[4]

The method adopted in the authors' laboratory gives robust stability information based on the inherent imprecision of the assay. The method has parallels with that proposed by Livesey *et al*.[12] Although their methodology has been developed primarily for techniques such as enzyme-linked immunosorbent assay (ELISA), it relates sample stability to a maximum acceptable mean change defined as a percentage, and can provide reliable stability information on as few as 10 samples. Imeri *et al*.[4] recommend the method defined by Thiers[8] for assessing sample stability. However, this method requires a minimum of 15 samples before an analyte can be defined as stable (and may require upwards of 25 samples to confirm stability). Imeri *et al*.[4] used 64 volunteers for their study: this is costly in time, reagents and staff.

Sample stability assessment: analytical precision

The method developed in the authors' laboratory relies on the availability of suitable precision information relevant to the methodology. Beckman Coulter provides precision specifications in terms of SD/CV for a given level of the requisite parameter. Cumulative SD/CV were calculated at each time point to exclude variability between subjects so an assessment of the variability within subjects could be made.

Using the method developed in the authors' laboratory, all parameters matched or exceeded Beckman Coulter stated

Table 6. Mean (standard deviation) and appropriate *P* values for differential parameters using Sarstedt tubes.

		0 hours	4 hours	8 hours	12 hours	18 hours	24 hours	30 hours	36 hours	42 hours	48 hours	60 hours	72 hours	*P* value
NE	(%)	55.79 (8.10)	56.06 (8.36)	55.56 (8.34)	54.99 (8.62)	56.28 (8.95)	56.56 (8.89)	59.37 (8.81)	60.73 (8.89)	61.56 (9.29)	62.19 (8.72)	65.44 (8.55)	61.32 (13.22)	0.0034
LY	(%)	31.18 (8.70)	30.91 (8.47)	30.68 (8.55)	31.30 (8.65)	30.09 (8.81)	30.10 (8.93)	28.09 (8.85)	27.84 (8.43)	26.60 (9.42)	28.22 (8.87)	25.45 (7.58)	26.95 (12.78)	0.4981
MO	(%)	8.83 (2.22)	8.46 (1.71)	8.54 (1.65)	8.56 (1.55)	8.25 (2.33)	7.94 (1.82)	7.94 (2.65)	7.53 (2.78)	7.77 (2.80)	6.64 (1.93)	6.22 (2.41)	9.13 (4.94)	0.0174
EO	(%)	3.93 (3.31)	4.00 (3.23)	4.05 (3.32)	3.94 (3.44)	4.11 (3.42)	4.30 (3.06)	3.30 (3.09)	2.96 (1.76)	3.17 (2.32)	2.37 (1.40)	2.22 (1.54)	2.30 (1.73)	0.1397
BA	(%)	0.54 (0.20)	1.03 (0.77)	1.03 (0.77)	1.27 (1.71)	1.25 (0.98)	1.37 (1.80)	1.30 (1.16)	0.94 (0.89)	0.80 (0.92)	0.59 (0.36)	0.68 (0.93)	0.32 (0.51)	0.0206

See Table 1 for abbreviations.

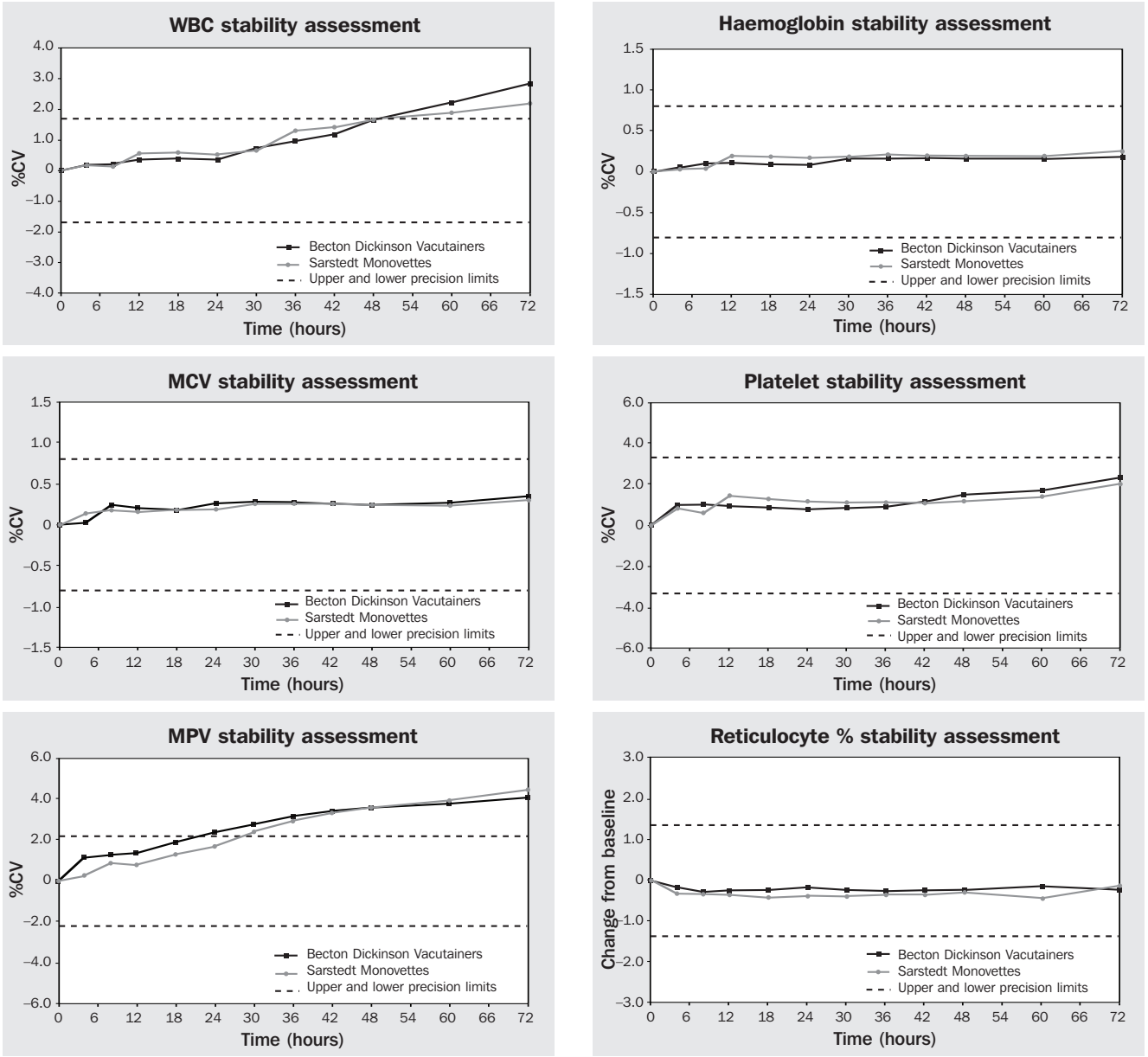

Fig. 1. Graphs showing calculated stability assessments for selected CBC and reticulocyte parameters using the Beckman Coulter LH750.

stability values, with both Sarstedt and BD tubes showing remarkably similar levels of stability for all parameters. The exceptions were that mean platelet volume (MPV) was only stable for 18 h in BD tubes and 24 h in Sarstedt tubes, and neutrophils (NE) was stable for 72 h in BD tubes, but only 48 h in Sarstedt tubes. However, it was noted that the degradation of the Sarstedt samples appears to be more predictable and follows a much more linear pattern.

Sample stability assessment: statistical analysis

In the current study, ANOVA tests were used to assess the suitability of the method developed in-house to generate robust stability data. ANOVA tests make simultaneous comparisons between two or more means, and the resultant *P* value gives an indication of the significance of any differences between these means. $P<0.05$ indicates that these differences are significant, and the parameter is not stable for the duration of the study.

There was a wide spread of *P* values for each tube type, ranging from $P=0.0034$ up to $P=1.00$. The findings of the statistical analysis indicate that WBC, RBC, Hb, MCV, RDW and PLT are stable for the duration of the study with both tube types; RET are stable for the duration of the study with Becton Dickinson tubes only; MPV, MO, BA are not stable for the duration of the study with either tube type; and NE is not stable for the duration of the study with Sarstedt tubes only.

Statistical methods were unable to confirm or refute stability on the remaining analytes, as the *P* values were between $P=0.05$ and $P=0.95$.

Sample stability assessment: comparison of methods

Overall, the current study found some correlation between the *P* values provided by ANOVA tests and the stability data calculated from the manufacturer's specifications for precision. This correlation was strongest for the red cell and

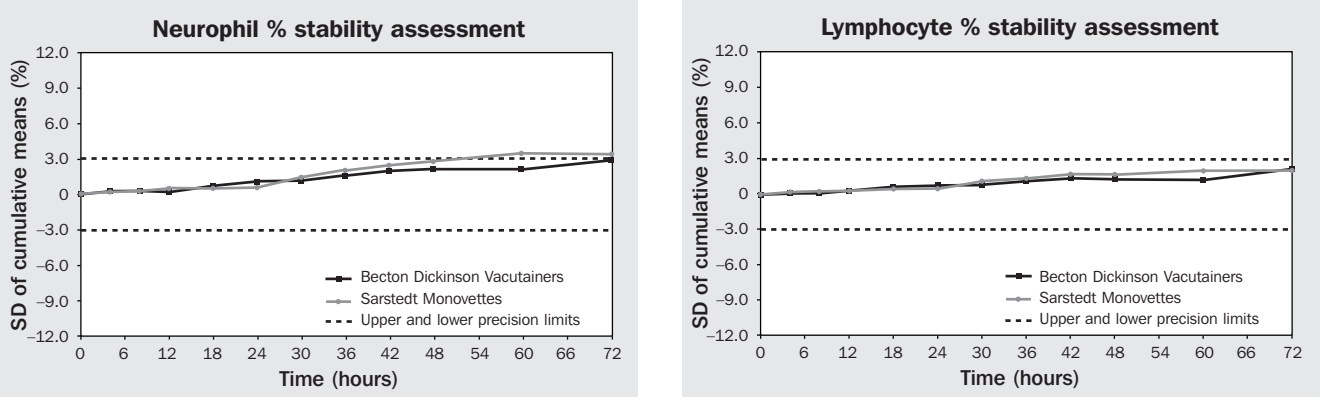

Fig. 2. Graphs showing calculated stability assessments for selected WBC differential parameters using the Beckman Coulter LH750.

platelet parameters, where all parameters showing $P>0.95$ showed stability to 72 h. However, the majority of differential parameters were calculated to be stable for 72 h, despite $P<0.7$. This weaker correlation noted between the statistical and calculated stabilities for the differential parameters is not unexpected. The manufacturer's stated precision limits are wider for these differential parameters, indicating that a greater level of variability is expected. However, this increased variation in results cannot be used as evidence that sample stability has deteriorated, provided that any changes are within the manufacturer's defined limits of precision, although inevitably it leads to lower P values when analysing the data using ANOVA.

Sample stability assessment: differential WBC parameters

Statistical analysis of the data indicated that the differential WBC parameters were unlikely to be stable for the duration of the study, as demonstrated by $P<0.7$ for both tube types. Closer examination of the raw data, in particular the WBC histogram and DataPlots, indicate that marked changes were evident for the majority of samples after 60 h, although these changes varied widely both within and between individuals. This is clearly reflected by the sudden increase in SD noted at 72 h (Tables 4 and 6).

There is negligible difference in stability between the two tube types for all differential parameters. The stability of the WBC differential depends on the stabilities of the two major cell types (i.e., neutrophils and lymphocytes). The numbers of other cell types (e.g., monocytes, eosinophils and basophils) are generally less than 10% of total WBC, and hence have minimal impact on the overall accuracy of the WBC differential. Consequently, the stability of the differential count is 48 h if Sarstedt tubes are used, and 72 h if BD tubes are used. Figure 2 indicates the variation in precision of neutrophils and lymphocytes over time.

However, when the mean results of each differential parameter are examined in more detail, differences become evident between the two tube types, particularly after 48 h. Neutrophil values show a steady rise until 48 h in BD tubes, and 60 h in Sarstedt tubes, after which there is a marked decrease in values: this decrease is more pronounced in BD tubes. The reverse pattern is noted with lymphocyte values.

Table 7. Comparative stability data (hours refrigeration) for FBC.

	Manufacturer's data	Study data		Literature review			
	Beckman Coulter stated values[7]	Becton Dickinson tubes	Sarstedt tubes	Imeri *et al.* 2008[4]	Buttarello 2004[2]	Bourner *et al.* 2005[5]	Jackson *et al.* 2008[9]
WBC	48	48	48	72	24	72	36
RBC	48	72	72	72	24	72	36
Hb	48	72	72	72	24	72	36
MCV	48	72	72	72	24	72	36
RDW	48	72	72	NDA	24	72	36
PLT	48	72	72	24	24	72	36
MPV	48	18	24	NDA	24	72	36
NE%	48	72	48	40	24	72	36
LY%	48	72	72	4	24	72	36
MO%	48	72	72	4	24	72	36
EO%	48	72	72	NDA	24	72	36
BA%	48	72	72	NDA	24	72	36
RET%	72	72	72	72	72	72	36

NDA: no data available. See Table 1 legend for abbreviations.

Patterns of monocyte, eosinophil and basophil values are more variable than those for neutrophils and lymphocytes, but any changes are mirrored with both tube types.

Storage of EDTA samples results in characteristic changes in white cell morphology over time. Neutrophils show cytoplasmic vacuolation accompanied by degranulation and nuclear separation and fragmentation. Ultimately, this leads to a single rounded nuclear mass prior to complete disintegration of the neutrophil. Monocytes and lymphocytes show cytoplasmic vacuolation together with irregular lobulation of the nucleus, giving a typical 'cloverleaf' appearance. Small lymphocytes tend to lose all cytoplasm. The degree of these changes varies within and between the different white cell populations, with some completely degenerate cells present alongside cells that exhibit only relatively minor changes. These changes in size and granularity give rise to a distinctive pattern on the WBC histogram and DataPlot, and lead to the numeric changes in the differential values produced by the analyser.

Conclusions

The current study showed that the original hypothesis – that the type of EDTA specimen tube impacts on sample stability – is only partially correct. Although the tube type has little impact on the stability of the majority of haematological parameters, both statistical and calculated stability assessments demonstrate evidence of a difference in stability for WBC and MPV between the two tube types tested. This difference only has an impact when a delay of >30 h between collection and analysis is anticipated.

Sarstedt Monovettes show slightly better stability for total WBC. Although BD tubes have been shown to have slightly longer stability for the individual differential parameters, the degradation of the Sarstedt samples appears to follow a much more linear pattern. This permits easier compensation when interpreting results and suggests that Sarstedt tubes should be regarded as the system of choice when expecting delays in analysis of >30 h.

Correlation between statistical evidence and available literature confirms that the in-house technique for calculating sample stability provides robust information within the inherent imprecision of the assay. However, it must be remembered that changes due to sample degradation and analytical variance within the limitations of the specified assay cannot be differentiated by statistical analysis alone. The current study provides important information on the stability of haematological samples, and gives confidence that the current laboratory practice for the analysis of haematology samples is fit for purpose.

The two tube types selected for this study are representative of currently preferred sample collection systems. However, other sample collections systems are available that may have a greater or lesser impact on sample stability than the systems tested here. Selection of blood sample collection systems should not be based solely on sample stability information. Familiarity with the system together with available tube sizes are also important factors to consider.

Results from the current study confirm that reliable haematological results can be provided on either of the two tubes tested up to 48 h after collection, and can also be used to make informed judgements on results provided on stored samples. □

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