Sample stability for complete blood cell count using the Sysmex XN haematological analyser

Massimo Daves[1], Elmar M. Zagler[1], Roberto Cemin[2], Flora Gnech[1], Alexandra Joos[1], Stefan Platzgummer[1], Giuseppe Lippi[3]

[1]Clinical Pathology Laboratory, Hospital of Merano, Merano; [2]Cardiology Division Hospital of Bolzano, Bolzano; [3]Laboratory of Clinical Chemistry and Haematology, University Hospital of Parma, Parma, Italy

Background. Sample stability is a crucial aspect for the quality of results of a haematology laboratory. This study was conducted to investigate the reliability of haematological testing using Sysmex XN in samples stored for up to 24 h at different temperatures.

Materials and methods. Haematological tests were performed on whole blood samples collected from 16 ostensibly healthy outpatients immediately after collection and 3 h, 6 h or 24 h afterwards, with triple aliquots kept at room temperature, 4 °C or 37 °C.

Results. No meaningful bias was observed after 3 h under different storage conditions, except for red blood cell distribution width (RDW) and platelet count (impedance technique, PLT-I) at 37 °C. After 6 h, meaningful bias was observed for mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) at room temperature, red blood cell (RBC) count, mean corpuscular haemoglobin concentration (MCHC), MCH, MCV and PLT-I at 4 °C, and RBC, RDW, MCHC, MCH and PLT-I at 37 °C. After 24 h, a meaningful bias was observed for MCHC, MCV, platelet count (fluorescent technique, PLT-F) and mean platelet volume (MPV) at room temperature, MCHC, MCV, PLT-I and MPV at 4 °C, and all parameters except RBC count and MPV at 37 °C.

Discussion. Great caution should be observed when analysing results of haematological tests conducted more than 3 h after sample collection.

Keywords: sample stability, haematological testing, storage conditions, haematological analysers.

© SIMTI Servizi Srl

Introduction

Several lines of evidence attest that the vast majority of laboratory errors (i.e., approximately 70%), emerge from manually intensive activities of the pre-analytical phase rather than from the analytical and post-analytical phases[1]. Inappropriate conditions for the transportation and storage of specimens create a tangible challenge for the quality of testing[2]. The receipt of unsuitable samples is relatively frequent in laboratory practice which can ultimately jeopardise clinical decisions because analysis of such samples may generate unreliable results, which do not reflect the real clinical condition. As such, detection and management of unsuitable samples should be regarded as essential actions, as recently emphasised by several national and international scientific societies[3,4]. The complete blood cell count (CBC) is a crucial test for the diagnosis and management of a variety of haematological disturbances, provided that the quality throughout the testing process can be guaranteed. Several articles have been published about the stability of whole blood specimens for CBC testing, but results are often contradictory and largely instrumentation-dependent[5]. Delayed sample analysis is not a rare circumstance in clinical and laboratory practice, especially when blood samples are shipped to distant centralised laboratories, when the analysis cannot be readily performed for organisational or technical reasons, or when sample retesting may be needed to verify test results[6]. The definition of accurate and appropriate criteria for sample stability is necessary for clinical purposes, but also for ancillary legal implications. A paradigmatic example is that of antidoping testing (e.g., for the athlete's blood passport)[7,8], when samples may be collected in the field and shipped to reference laboratories, which are often at long distances from the site of collection[9]. This study was aimed to investigate the stability of CBC using the new generation of Sysmex haematological analysers (i.e., Sysmex® XN-Series, Kobe, Japan), which is the most recent update of the reference Sysmex XT-2000 analyser used by numerous clinical laboratories and by the vast majority of antidoping facilities accredited by the ISO 17025 and World Anti-Doping Agency (WADA)[10].

All rights reserved - For personal use only
No other uses without permission

Material and methods

Instrument description

The newly developed automated haematology analyser XN (Sysmex, Kobe, Japan) enumerates and classifies blood cells by a combination of direct current detection and flow cytometry. More specifically, cell enumeration is based on semiconductor laser counting and cells are then classified by irradiation with a 633 nm laser beam and analysis of forward scattered light, side scattered light and side fluorescent light. The three signals are integrated to differentiate and count white blood cells (WBC), nucleated red blood cells (NRBC), reticulocytes and platelets, as well as to detect abnormal and immature cells. The RBC/PLT channel counts red blood cells (RBC) and platelets (PLT) using the sheath flow direct current detection method. After sample dilution, cell signals are captured and analysed to generate information on cell volume[11]. Along with the traditional impedance technique (PLT-I), the instrument is also equipped with a new dedicated channel for platelet analysis (PLT-F), which entails a fluorescent flow cytometry method using a novel fluorescent dye specifically developed for PLT staining[12]. The total imprecision of all parameters has been reported to be <4.0%, with the exception of the WBC count (<8.6%)[11].

Study design

The study cohort consisted of 16 ostensibly healthy outpatients (11 males, 5 females; aged 35-89 years), who had their blood collected for routine haematological testing. The blood was drawn into polyethylene terephthalate plastic tubes (Venosafe, Terumo Europe N.V, Leuven, Belgium) containing K2-EDTA by experienced nurses and immediately transported to the central laboratory, where the CBC was performed on Sysmex XN within 1 hour of collection.

After the baseline CBC had been performed (sample A), each patient's sample was divided into three identical aliquots (aliquots 1, 2 and 3) without further addition of anticoagulant. Aliquots 1 were stored at room temperature (20-23 °C), aliquots 2 were stored at 4 °C, whereas aliquots 3 were stored at 37 °C. Additional CBC were then performed on all stored aliquots at three defined time points, i.e., 3, 6 and 24 h after initial storage, as follows: 3 h at room temperature (sample B); 3 h at 4 °C (sample C); 3 h at 37 °C (sample D); 6 h at room temperature (sample E); 6 h at 4 °C (sample F); 6 h at 37 °C (sample G); 24 h at room temperature (sample H); 24 h at 4 °C, (sample I); and 24 h at 37 °C (sample J). All aliquots were tested on the same analyser, and with an identical lot of reagents and after an appropriate amount of time to stabilise the testing temperature, as recommended by the manufacturer.

The results are reported as mean, standard deviation and 95% confidence interval (CI) of the mean. The differences between the different parameters measured in paired aliquots were evaluated with the paired Student's t test after verification of normal distribution by the D'Agostino-Pearson test. The differences between samples not normally distributed were instead evaluated by Wilcoxon's test. P-values <0.05 were considered statistically significant. Percentage variations from the baseline value in samples with statistically significant differences were then analysed with Bland-Altman plots and compared with the current quality specifications for desirable bias, as derived from the intra-individual and inter-individual variations[13].

The investigation was based on pre-existing samples, the results were not reported and did not affect the clinical management of patients, so that ethical permission and informed consent were unnecessary. All the sample were made anonymous before analysis. The study was performed in accordance with the Declaration of Helsinki and under the terms of all relevant local legislations.

Results

The results of this study are shown in Table I for parameters with a normal distribution (i.e., WBC count, RBC count, red blood cell distribution width [RDW], mean corpuscular haemoglobin concentration [MCHC], mean corpuscular volume [MCV], platelets and mean platelet volume [MPV]) and in Table II for those not normally distributed (i.e., mean corpuscular haemoglobin [MCH]). After 3 h, which is the most frequent time window for routine assessment of CBC specimens, RDW, MCV and MPV displayed significant differences in samples stored at room temperature, RBC, MCV, PLT-I and PLT-F in samples stored at 4 °C, and RBC, RDW, PLT-I and PLT-F in samples stored at 37 °C. After 6 h, RBC, RDW, MCH, MCV and MPV displayed significant differences in samples stored at room temperature, RBC, MCHC, MCH, MCV, PLT-I and PLT-F in samples stored at 4 °C, and RBC, RDW, MCHC, MCH, PLT-I and PLT-F in samples stored at 37 °C. After 24 h, RDW, MCHC, MCV, MPV, PLT-I and PLT-F displayed significant differences in samples stored at room temperature, RDW, MCHC, MCV, PLT-I and PLT-F in samples stored at 4 °C, and WBC, RDW, MCHC, MCV, PLT-I and PLT-F in samples stored at 37 °C. Interestingly, when these statistically significant variations were compared with the current quality specifications by means of Bland and Altman plot analysis (Tables III and IV), no meaningful differences were observed for the various parameter tested after 3 h under the different storage conditions, except for RDW and PLT-I in specimens stored at 37 °C. After 6 h, a

© SIMTI Servizi S.r.l.

All rights reserved - For personal use only
No other uses without permission

Table I - Normally distributed parameters.

Parameters	Sample A mean ± SD 95% CI for mean	Sample B mean ± SD 95% CI for mean p *vs* sample A	Sample C mean ± SD 95% CI for mean p *vs* sample A	Sample D mean ± SD 95% CI for mean p *vs* sample A	Sample E mean ± SD 95% CI for mean p *vs* sample A
WBC (10^9/L)	10.46±5.23 7.67 to 13.24	10.57±5.29 7.67 to 13.24 p=0.0535	10.34±4.97 7.69 to 12.99 p=0.2108	10.31±4.99 7.65 to 12.98 p=0.1358	10.54±5.24 7.75 to 13.33 p=0.0534
RBC (10^{12}/L)	3.64±0.76 3.24 to 4.05	3.66±0.75 3.26 to 4.06 p=0.1607	3.68±0.76 3.29 to 4.10 p=0.0031	3.69±0.77 3.29 to 4.10 p=0.003	3.68±0.77 3.27 to 4.09 p=0.0025
RDW	17.04±2.69 15.61 to 18.4	17.24±2.75 15.77 to 18.71 p=0.003	17.02±2.75 15.60 to 18.45 p=0,6993	17.51±2.69 16.08 to 18.94 p<0.0001	17.34±2.70 15.90 to 18.78 p<0.0001
MCHC (g/dL)	31.83±1.76 30.89 to 32.77	31.91±1.46 31.13 to 32.69 p=0.6916	32.17±1.51 31.37 to 32.98 p=0.2122	31.50±1.70 30.59 to 32.40 p=0.1649	31.96±1.71 31.04 to 32.87 p=0.5391
MCV (fL)	92.43±8.68 87.80 to 97.06	91.46±8.48 86.94 to 95.97 p=0.0003	90.47±8.86 85.74 to 95.19 p<0,0001	92.64±9.10 87.79 to 97.49 p=0.4929	90.94±8.58 86.37 to 95.51 p=0.0002
PLT-I (10^9/L)	262±194.87 158.16 to 365.84	263.5±197.81 158.10 to 368.90 p=0.6232	250.37±189.28 149.51 to 351.23 p=0.0070	241.56±177.19 147.14 to 335.98 p=0.0057	262.69±197.56 157.41 to 367.96 p=0.8615
PLT-F (10^9/L)	284.56±219.83. 167.42 to 401.7	286.62±222.23 168.20 to 405.04 p=0.2557	271.56±204.77 162.45 to 380.68 p=0.0146	268.31±202.25 160.54 to 376.08 p=0.0043	280.75±217.93 164.62 to 396.87 p=0.1931
MPV (fL)	11.35±1.19 10.69 to 12.01	11.55±1.19 10.89 to 12.20 p=0.0214	11.37±1.10 10.76 to 11.98 p=0.8600	11.13±1.11 10.51 to 11.75 p=0.0748	11.55±1.28 10.84 to 12.26 p=0.0217
Parameters	**Sample F mean ± SD 95% CI for mean p *vs* sample A**	**Sample G mean ± SD 95% CI for mean p *vs* sample A**	**Sample H mean ± SD 95%CI for mean p *vs* sample A**	**Sample I mean ± SD 95% CI for mean p *vs* sample A**	**Sample J mean ± SD 95% CI for mean p *vs* sample A**
WBC (10^9/L)	10.32±4.99 7.65 to 12.98 p=0.0844	10.27±5.02 7.80 to 12.09 p=0.1957	10.64±5.27 7.83 to 13.45 p=0.1934	10.32±5.06 7.62 to 13.02 p =0.1771	9.55±5.06 6.85 to 12.24 p<0.0001
RBC (10^{12}/L)	3.78±0.79 3.36 to 4.20 p=0.0013	3.75±0.79 3.33 to 4.17 p=0.0002	3.65±0.75 3.25 to 4.05 p=0.6805	3.67±0.76 3.26 to 4.07 p=0.09	3.65±0.77 3.25 to 4.06 p=0.5104
RDW	16.93±2.68 15.50 to 18.36 p=0,0524	17.72±2.65 16.31 to 19.14 p<0,0001	17.69±2.68 16.26 to 19.12 p<0,0001	16.67±2.60 15.29 to 18.06 p=0,0005	19.30±2.366 18.04 to 20.56 p<0,0001
MCHC (g/dL)	32.50±1.76 31.56 to 33.44 p=0.0051	31.24±1.55 30.41 to 32.07 p=0.0347	30.87±1.94 29.84 to 31.91 p=0.0088	33.04±1.71 32.13 to 33.95 p=0.0001	26.72±1.48 25.93 to 27.51 p<0.0001
MCV (fL)	89.38±8.71 84.74 to 94.02 p<0.0001	93.22±9.12 88.36 to 98.08 p=0.0690	95.46±9.39 90.45 to 100.46 p=0.0004	88.82±8.66 84.21 to 93.44 p<0.0001	110.71±11.19 104.74 to 116.67 p<0.0001
PLT-I (10^9/L)	252.31±195.28 148.25 to 356.37 p=0.0020	239.44±175.95 145.68 to 333.19 p=0.0036	246.56±185.54 147.69 to 345.43 p=0.0023	249.81±190.15 148.49 to 351.14 p=0.0001	204.19±160.21 118.82 to 289.56 p=0.0014
PLT-F (10^9/L)	276.94±212.96 163.46 to 390.42 p=0.0354	266.75±203.02 158.57 to 374.93 p=0.0037	267.50±208.05 156.64 to 378.36 p=0.0022	273.37±213.08 159.83 to 386.92 p=0.0029	233.12±197.99 127.62 to 338.63 p=0.0012
MPV (fL)	11.48±0.99 10.93 to 12.03 p=0.1573	11.27±1.08 10.66 to 11.88 p=0.4375	11.90±1.40 11.12 to 12.67 p=0.0128	12.05±1.14 11.41 to 12.68 p=0.0009	11.55±1.28 10.84 to 12.26 p=0.3022

Mean ± standard deviation and 95% confidence interval for the mean for the normally distributed values.
SD: standard deviation; CI: confidence interval; WBC: white blood cells; RBC: red blood cells; RDW: red blood cell distribution width; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; PLT-I: platelet count using the impedance technique; PLT-F: platelet count using the fluorescent technique; MPV: mean platelet volume.

© SIMTI Servizi Srl

All rights reserved - For personal use only
No other uses without permission

Table II - Non-normally distributed parameters.

Parameters	Sample A Median (IQR) 95% CI for median	Sample B Median (IQR) 95% CI for median p *vs* sample A	Sample C Median (IQR) 95% CI for median p *vs* sample A	Sample D Median (IQR) 95% CI for median p *vs* sample A	Sample E Median (IQR) 95% CI for median p *vs* sample A
MCH (pg)	30.20 (27.45-32.25) 27.74 to 32.24	30.10 (27.45-32.25) 27.80 to 31.80 p=0.0906	30,10 (27.45-32.25) 27.58 to 32.14 p=0.0942	29.95 (27.80-32.05) 27.94 to 32.04 p=0.0676	29.95 (27.25-32) 27.40 to 32 p=0.0006
Parameters	**Sample F Median(IQR) 95% CI for median p *vs* sample A**	**Sample G Median (IQR) 95% CI for median p *vs* sample A**	**Sample H Median (IQR) 95% CI for median p *vs* sample A**	**Sample I Median (IQR) 95% CI for median p *vs* sample A**	**Sample J Median (IQR) 95% CI for median p *vs* sample A**
MCH (pg)	29.75 (27.80-31.65) 28.02-31.61 p=0.0026	29.70 (27.50-31.95) 27.72-31.91 p=0.0031	30.45 (27.70-32.30) 27.98 to 32.30 p=0.4973	30.30 (27.80-32.05) 28.02 to 32.04 p=0.7609	30.75 (26.90-32.70) 26.98 to 32.70 p=0.4637

Median and interquartile range and 95% confidence interval for the median of mean corpuscular haemoglobin, a non-normally distributed value.
IQR: Median and interquartile range; CI: confidence interval; MCH: mean corpuscular haemoglobin.

bias greater than the current quality specifications was observed for MCH and MCV in specimens stored at room temperature, RBC, MCHC, MCH, MCV and PLT-I in specimens stored at 4 °C, and RBC, RDW, MCHC, MCH and PLT-I in specimens stored at 37 °C. After 24 h, a bias greater than the current quality specifications was observed for MCHC, MCV, PLT-F and MPV in specimens stored at room temperature, MCHC, MCV, PLT-I and MPV in specimens stored at 4 °C, and for all parameters except RBC and MPV in samples stored at 37 °C (Tables III and IV).

Discussion

Sample stability is a crucial aspect for the quality of laboratory diagnostics[14], including haematological testing. This aspect is particularly relevant for clinical laboratories that receive a large number of samples from peripheral facilities within an integrated network[6], as well as for the current framework of antidoping testing, which entails collection of whole blood specimens in the field and delayed analysis in reference or accredited centres[15].

Various studies have been published on the reliability of delayed haematological testing on whole anticoagulated (EDTA) blood samples, using a vast array of haematological analysers. We focused our analysis on the new generation of Sysmex analysers, since it is predictable that these will widely replace the former XT series in clinical laboratories and, especially, in accredited antidoping centres.

In a previous evaluation of the Sysmex haematology XN modular system, Briggs *et al.* found that the values of WBC count, leukocyte differential and PLT-F did not vary significantly in samples stored at room temperature or 4 °C for over 72 h[11]. In a small study including three patients' samples, Tanaka *et al.* observed that PLT counts were stable up to 48 h in samples stored at either 4 °C or room temperature[12]. In another study the stability of WBC count, leukocyte differential, RBC count, PLT-F, PLT-I and others parameters (i.e., reticulocytes and immature platelet fraction) was evaluated in ten samples of which aliquots were stored at room temperature and 4 °C for 4, 8, 24, 48, and 72 h: the authors concluded that all parameters were stable at both room temperature and 4 °C for up 72 h except for PLT-F[16]. Our findings do, therefore, differ partially from those of Seo *et al.* for RBC analysis, whereas the stability of WBC count at 4 °C and room temperature was similar. It should be noted that Seo *et al.* did not study stability at 37 °C.

The results of these previous studies are not in accord with those obtained in our investigation, in that we found that virtually all parameters were substantially biased when samples were stored for 24 h, regardless of the storage condition (Tables III and IV). Even after 6 h, sample storage at 4 °C or 37 °C generated a substantial bias in four out of the nine parameters tested, whereas storage at room temperature only generated a significant bias for MCV and MCH. Interestingly, 37 °C was the temperature of storage that caused the broadest and largest changes in the nine parameters tested. This is an important aspect in countries with high environmental temperatures, making it necessary to use cooling systems that can maintain room temperature when samples are transported over long distances.

A second important implication of this study is the need to define reliable means of sample transportation, which should comply with the current recommended procedures issued by WADA for definition of an athlete's biological passport. This important strategy for identification of blood doping currently entails a double approach, based on the so-called OFF-hr Score (OFFS), which is a combination of haemoglobin and reticulocyte values, and the Abnormal Blood Profile Score (ABPS), which is a combination of haematocrit, haemoglobin,

© SIMTI Service Srl

All rights reserved - For personal use only
No other uses without permission

Table III - Desiderable Bias for parameters normally distributed.

Parameters	Desirable bias	Sample B p *vs* sample A % bias (95% CLA)	Sample C p *vs* sample A % bias (95% CLA)	Sample D p *vs* sample A % bias (95% CLA)	Sample E p *vs* sample A % bias (95% CLA)	Sample F p *vs* sample A % bias (95% CLA)
WBC (10^9/L)	±5.6%	p=0.0535	p=0.2108	p=0.1358	p=0.0534	p=0.0844
RBC (10^9/L)	±1.7%	p=0.1607	p=0.0031 −1.5 (1.6; −4.5)	p=0.003 −1.4 (0.9; −3.7)	p=0.0025 −1 (1.1; −3.1)	p=0.0013 −3.6 (3.1; −10.3)
RDW	± 1.7%	p=0.003 −1.14 (0.73; −3.01)	p=0,6993	p<0.0001 −2.8 (0.2; −5.3)	p<0.0001 −1.5 (0.22; −3.29)	p=0,0524
MCHC (g/dL)	±0.4%	p=0.6916	p=0.2122	p=0.1649	p=0.5391	p=0.0051 −2.1 (2.8; −7)
MCV (fL)	±1.2%	p=0.0003 1.05 (2.68; −0.58)	p<0,0001 −2.2 (5.1; −0.7)	p=0.4929	p=0.0002 1.6 (4; −0.8)	p<0.0001 3.4 (6.1;0.7)
PLT-I (10^9/L)	±5.9%	p=0.6232	p=0.0070 5.3 (17; −6.4)	p=0.0057 8.6 (22.3; −5.1)	p=0.8615	p=0.0020 7.8 (24.9; −9.4)
PLT-F (10^9/L)	±5.9%	p=0.2557	p=0.0146 5.3 14.4; −3.8)	p=0.0043 4 (14; −6.1)	p=0.1931	p=0.0354 2.1 (11.7; −7.5)
MPV (fL)	±2.3%	p=0.0214 −1.7 (3.5; −6.9)	p=0.8600	p=0.0748	p=0.0217 −1.7 (3.6; −7)	p=0.1573

Parameters	Desirable bias	Sample G p *vs* sample A % bias (95% CLA)	Sample H p *vs* sample A % bias (95% CLA)	Sample I p *vs* sample A % bias (95% CLA)	Sample J p *vs* sample A % bias (95% CLA)
WBC (10^9/L)	±5.6%	p=0.1957	p=0.1934	p=0.1771	p<0.0001 10.1 (21; −0.7)
RBC (10^9/L)	±1.7%	p=0.0002 −2.7 (1.5; −6.9)	p=0.6805	p=0.09	p=0.5104
RDW	±1.7%	p<0,0001 −4 (−0.4; −7.7)	p<0.0001 −3.8 (1; −8.6)	p=0,0005 2.1 (5.8; −1.5)	p<0,0001 −12.8 (2.7; −23)
MCHC (g/dL)	±0.4%	p=0.0347 1.8 (8; −4.3)	p=0.0088 3.1 (11; −4.8)	p=0.0001 −3.8 (1.9; −9.4)	p<0.0001 17.5 (21.9;13)
MCV (fL)	±1.2%	p=0.0690	p=0.0004 −3.2 (2.4; −8.8)	p<0.0001 4 (7;1)	p<0.0001 −17.9 (−13.9; −21.9)
PLT-I (10^9/L)	±5.9%	p=0.0036 8.4 (22.4; −5.6)	p=0.0023 4.8 (31.5; −22)	p=0.0001 6.9 (24.1; −10.3)	p=0.0014 21.9 (58.4; −14.6)
PLT-F (10^9/L)	±5.9%	p=0.0037 5.8 (16.4; −4.9)	p=0.0022 8.7 (25.1; −7.8)	p=0.0029 3.7 (15.6; −8.3)	p=0.0012 20.7 (51.3; −10)
MPV (fL)	±2.3%	p=0.4375	p=0.0128 −4.5 (7.3; −16.4)	p=0.0009 −6 (4.7; −16.7)	p=0.3022

Mean % bias and 95% confidence limit of agreement (95% CLA) of the samples that had a significant p *versus* sample A.
CLA: confidence limit of agreement; WBC: white blood cells; RBC: red blood cells; RDW: red blood cell distribution width; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; PLT-I: platelet count using the impedance technique; PLT-F: platelet count using the fluorescent technique; MPV: mean platelet volume.

© SIMTI Servizi Srl

All rights reserved - For personal use only
No other uses without permission

Table IV - Desiderable Bias for mean corpuscular haemoglobin (MCH).

Parameter	Desirable Bias	Sample B p *vs* sample A % bias (95% CLA)	Sample C p *vs* sample A % bias (95% CLA)	Sample D p vs sample A % bias (95% CLA)	Sample E P *vs* sample A % bia (95% CLA)	Sample F p *vs* sample A % bias (95% CLA)
MCH (pg)	±1.4%	p=0.0906	p=0.0942	p=0.0676	p=0.0006 1.8 (6;−2.5)	p=0.0026 1.5 (4.6;−1.7)
Parameter	**Desirable Bias**	**Sample G p *vs* sample A % bias (95% CLA)**	**Sample H p *vs* sample A % bias (95% CLA)**	**Sample I p *vs* sample A % bias (95% CLA)**	**Sample J p *vs* sample A % bias (95% CLA)**	
MCH (pg)	±1.4%	p=0.0031 1.3 (4.6;−2)	p=0.4973	p=0.7609	p=0.4637	

Mean % bias and 95% confidence limit of agreement of the sample that had a significant p *versus* sample A.
CLA: confidence limit of agreement; MCH: mean corpuscular haemoglobin.

reticulocytes, MCV, MCH, and MCHC[17]. The specific requirements for sample collection, transportation, storage and analysis are contained in the International Standard for Testing[18], which specifies that once a blood sample for the athlete's biological passport has been collected, it should be transported in a device that maintains the integrity over time notwithstanding changes in external temperature (i.e., refrigerator, insulated cool box, isotherm bag or any other device that possesses these capabilities). Even more importantly, it is also stated that if the sample is intended for use in connection with the biological passport program, it should be transported rapidly to the laboratory so that the analysis can be performed ideally within 36 hours of sample collection.

Interestingly, Ashenden *et al.* recently demonstrated that haemoglobin and reticulocytes were stable for at least 168 h using the Sysmex XT-2000i instrument, when maintained between 4 °C and 6 °C[19]. Nevertheless, a substantial and time-dependent variation (i.e., increase) of MCV could be observed, whereas data on MCH and MCHC were not reported. The results of our study clearly attest that three out of the six parameters included in the ABPS (i.e., MCV, MCH, and MCHC) were extremely vulnerable to the storage conditions. The values of MCHC and MCV were substantially biased (i.e., values exceeded the quality specification) after 24 h, regardless of the storage condition (Table III and IV). This clearly attests that the current WADA recommendation that sample analysis should be performed within 36 hours of collection is probably optimistic, at least using the Sysmex reference instrumentation. More reliably, this threshold should be reduced to 3 hours, since this was the only time frame in which the sample stability of MCV, MCH, and MCHC could be proven. It is also noteworthy that after 6 hours from collection, these parameters were unvaried when the sample was maintained at room temperature, whereas the refrigeration (i.e., 4 °C) was ineffective to prevent an increase of MCHC and a decrease of both MCV and MCH (Table I and III). This is in agreement with data reported in a previous review article by Lombardi *et al.*, who concluded that stability of haematological parameters is impaired by high storage temperatures, whereas RBCs and hematocrit may be affected by initial freezing followed by refrigeration[9]. As such, according to our data, we suggest that the use of isotherm bag should be preferred over refrigerators or insulated cool boxes when samples are shipped for purposes of ABP testing, provided that these devices fulfill quality criteria for sample shipment, as specified elsewhere[20].

Conclusions

The results of our study suggest that great caution should be observed when analysing results of haematological testing performed more than 3 h after sample collection, since a significant bias may emerge irrespective of the storage conditions. Medium to long-term storage of whole blood samples at high temperature (i.e., 37 °C) should be absolutely avoided, both during transportation and within the laboratory, because this temperature generates the largest and most clinically relevant bias in haematological test results. Finally, we raise the important concern that refrigeration of specimens is not an implicit guarantee that the quality of whole blood samples is preserved during extended periods of storage.

Authorship contributions

MD and GL conceived and designed the study, analysed the data, performed the statistical analysis and drafted the manuscript; EMZ, RC, FG, AJ and SP performed the literature revision, acquired data, interpreted the results and critically revised the manuscript. All Authors read and approved the final version of the manuscript.

© SIMTI Servizi Srl

All rights reserved - For personal use only
No other uses without permission

The Authors declare no conflict of interest.

References

1) Lippi G, Guidi GC, Matiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. Clin Chem Lab Med 2006; **44**: 358-65.
2) Adcock Funk DM, Lippi G, Favaloro EJ. Quality standards for sample processing, transportation and storage in hemostasis testing. Semin Thromb Hemost 2012; **38**: 576-85.
3) Tatsumi N, Miwa S, Lewis SM. International Society of Hematology and International Council for Standardization in Haematology. Specimen collection, storage and transmission to the laboratory for haematological tests. Int J Hematol 2002; **75**: 261-8.
4) Lippi G, Banfi G, Buttarello M, et al. Recommendations for detection and management of unsuitable samples in clinical laboratories. Clin Chem Lab Med 2007; **45**:728-36.
5) Zini G. International Council for Standardization in Haematology. Stability of complete blood count parameters with storage: toward defined specifications for different diagnostic applications (Editorial). Int Jnl Lab Hem 2014; **36**: 111-3.
6) Lippi G, Simundic AM. Laboratory networking and sample quality: a still relevant issue for patient safety. Clin Chem Lab Med 2012; **50**: 1703-5.
7) Lippi G, Plebani M, Sanchis-Gomar F, Banfi G. Current limitations and future perspectives of the Athlete Blood Passport. Eur J Appl Physiol 2012; **112**: 3693-4.
8) Sanchis-Gomar F, Martinez-Bello VE, Gomez-Cabrera MC, Viña J. Current limitations of the Athlete's Biological Passport use in sports. Clin Chem Lab Med 2011; **49**: 1413-5.
9) Lombardi G, Lanteri P, Colombini A, et al. Stability of haematological parameters and its relevance on the athlete's biological passport model. Sports Med 2011; **41**: 1033-42.
10) Robinson N. Fighting for fair competition: blood doping. A persistent challenge and smart approaches to detect it. Diagnostic Perspective. Available at: http://www.sysmex.de/fileadmin/media/f100/Diagnostic_Perspectives/Robinson_DiagPersp_Vol1_20-27.pdf. Accessed on 20/12/2014.
11) Briggs C, Longair I, Kumar P, et al. Performance evaluation of the Sysmex haematology XN modular system. J Clin Pathol 2012; **65**: 1024-30.
12) Tanaka Y, Tanaka Y, Gondo K, et al. Performance evaluation of platelet counting by novel fluorescent dye steining in the XN-series automated hematology analyzers. J Clin Lab Anal 2014; **28**: 341-8.
13) Ricos C, Alvarez V, Cava F, et al. Current databases on biologic variation: pros, cons and progress. Scand J Clin Lab Invest 1999; **59**: 491-500.
14) Lippi G, Becan-McBride K, Behúlová D, et al. Preanalytical quality improvement: in quality we trust. Clin Chem Lab Med 2013; **51**: 229-41.
15) Lippi G, Plebani M. Athlete's biological passport: to test or not to test? Clin Chem Lab Med 2011; **49**: 1393-5.
16) Seo JY, Lee ST, Kim SH. Performance evaluation of the new hematology analyzer Sysmex XN-series. Int J Lab Hematol 2015; **37**: 155-64.
17) World Anti-Doping Agency. Athlete Biological Passport - Operating Guidelines. Available at: https://www.wada-ama.org/en/files/wada-abp-operating-guidelinesv40-enpdf. Accessed on: 21/12/2014.
18) World Anti-Doping Agency. International Standard for Testing 2015. Available at: https://wada-main-prod.s3.amazonaws.com/resources/files/WADA-2015-ISTI-Final-EN.pdf. Accessed on: 21/12/2014.
19) Ashenden M, Clarke A, Sharpe K, et al. Stability of athlete passport parameters during extended storage. Int J Lab Hematol 2013; **35**: 183-92.
20) Lippi G, Lima-Oliveira G, Nazer SC et al. Suitability of a transport box for blood sample shipment over a long period. Clin Biochem 2011; **44**: 1028-9.

Arrived: 12 January 2015 - Revision accepted: 12 February 2015
Correspondence: Massimo Daves
Clinical Pathology Laboratory
Hospital of Merano
Via Rossini 5
39011 Merano, Italy
e-mail: massimo.daves@asbmeran-o.it

© SIMTI Servizi S.r.l.

All rights reserved - For personal use only
No other uses without permission