Control Cells for Both Electrical Impedance and Light Scattering Automated Hematological Analyzers: Preparation from Normal and Thalassemic Blood Samples

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Abstract

The study on quality control of automated blood cell analyzers, Technicon H*1 and Coulter MAXM by using three separately self-prepared control cells was extensively investigated. The three parts of control cells are pseudo-leukocyte and fixed platelets, which are fixed by glutaraldehyde, and control red cells from normal and thalassemic patients preserved and anticoagulated in CPD or CPDA-1. The Technicon H*1 system was based on the principle of light scattering but the Coulter MAXM was based on the principle of electrical impedance for cell counting and measurement. The self-prepared control cells can be satisfactorily utilized as control for each system with statistically significant difference ($p < 0.05$) for both systems. The expirated dates for control cells are different in both systems and should be determined for each system specifically. The control red cells prepared from thalassemic patients were quite satisfactorily useful as an abnormal control for both systems during this study.

Manual procedures for cell counting are being replaced by automated instruments. Electronic counting has provided satisfactory outcome: analyzing comparatively large numbers of samples, reducing laboratory costs, improved turnaround time and accurate blood count. Two major principles of automated cell counters available: electrical impedance have been used nowadays and light scattering[1-3].

Calibration of automated counters is necessary for routine use of blood cell counting. Control preparations are used to ensure continued satisfactory performance after calibration. Ideally, the control material should be of identical nature to the samples being tested, not only in their cellular constituents but also in their physical properties such as viscosity, mixing characteristics and tendency to stick together[4].

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The difficulty is that a laboratory requires control materials with relatively good stability. The nature of cellular constituents are usually labile in both quantitative and qualitative means. In formulating the preparation of control materials the stability of the individual parameters of untreated blood should be the first to be considered.

The calibration of automatic machines in Thailand has still used commercial calibration controls. The drawbacks of commercial controls are different in nature from patient's blood, high cost and short shelftime after overseas transportation. Since thalassemic blood samples are common in our region, we have thus considered control material for thalassemia as well.

The aim of this study is to prepare control cells for automated blood cell analyzers using normal and thalassemic blood samples.

MATERIAL AND METHOD

Preparation of packed red blood cell

The CPD (Citrate Phosphate Dextrose) or CPDA-1 (Citrate Phosphate Dextrose Adenine) whole blood was centrifuged at 1,500 g for 10-15 minutes at room temperature (RT). Plasma and buffy coat layer were removed from packed red cell.

Preparation of pseudo-leukocyte cells

Approximately 20 ml of the packed red cell was washed three times with PBS (Phosphate Buffered Saline). One volume of washed cells was added slowly into ten volumes of PBS containing 0.5 per cent glutaraldehyde. After 18 hours at RT with occasional mixing with rotator, the cell suspension was washed three times with PBS and resuspended in PBS to yield the packed cell volume (PCV) of 0.50 l/l. EDTA (disodium 10 mg/ml), and sodium azide, (0.25 mg/ml), were then added to the cell suspension and the cells were resuspended using vortex mixer.

Preparation of fixed platelet

Units of human platelet concentrates were suspended overnight; those with excessive sedimented red cells were discarded. The accepted units were pooled after filtration through a standard platelet administration set. An equal volume of PBS containing 0.2 per cent glutaraldehyde was added and allowed to fix for 20 minutes. The suspension was then centrifuged at 700 g for 15 minutes and the supernatant was removed. Cells were resuspended in PBS and undergoing centrifugation. An aliquot of PBS (two-fold volume) was added to the cells. Excess leukocytes and red cells can be removed by sedimentation after standing overnight at 4°C and collection of the supernatant. The step might be repeated as necessary.

Preparation of composite control cells

The red blood cells, pseudo-leukocytes and platelets were combined and adjusted to be in normal, low and high ranges by calculation and continuously mixed during dispending into sterile vials for 8 hours at RT. The control cells were divided into aliquots, (1 ml per tube), and stored at 4°C.

Stability of control cells

The hematological parameters were determined by using Technicon H*1 and Coulter MAXM analyzers every week upto 2 months. The expiration of control cells was the week that the data ($X \pm 2SD$) was out of range of week 0.

Stability of thalassemic red blood cell

An aliquot of 2 ml of thalassemic blood was collected into 0.28 ml of CPD and stored at 4°C. The hematological parameters of the same blood specimen were determined by both analyzers every week from week 1 to week 8. The expiration time of thalassemic blood was the week that the data ($X \pm SD$) was out of range of week 0.

Statistic correlation of control cell between Technicon H*1 and Coulter MAXM analyzers

Wilcoxon Signed Rank test was used to analyze the correlation of parameters derived from different technology of blood cell analysis at a specified interval up to the end of experiment.

RESULTS

Evaluation of composite control cells

Table 1 shows the values of leukocytes, red blood cell (RBC) parameters and platelets in normal, low and high ranges of control cell from Technicon H*1 and Coulter MAXM analyzers.

The stability of control cells

The expiration date of open and closed vials of control cells is shown in Table 2.

Table 1. The hematological parameters in normal, low and high values of control cell determined by using Technicon H*1 and Coulter MAXM analyzers.

PARAMETER		LOW value±2SD	NORMAL value±2SD	HIGH value±2SD
WBC (10^3/μL)	H*1	5.06±0.3	14.2±1.4	22.4±1.2
	MAXM	3.3±0.3	14.60±0.4	27.6±0.7
RBC (10^6/μL)	H*1	2.03±0.1	5.43±0.04	5.57±0.2
	MAXM	2.05±0.1	5.65±0.13	5.57±0.13
HGB (g/dL)	H*1	6.2±0.3	12.5±0.2	17.5±0.1
	MAXM	6.6±0.2	12.6±0.1	18.2±0.3
HCT (%)	H*1	18.7±0.8	36.4±0.4	52.9±0.5
	MAXM	18.8±1.0	39.7±0.7	53.0±1.7
MCV (fL)	H*1	92.1±0.6	87.2±0.4	90.7±0.9
	MAXM	91.9±1.2	90.3±0.5	95.1±1.7
MCH (pg)	H*1	30.6±0.5	23.0±0.2	31.9±0.5
	MAXM	32.0±1.3	22.4±0.4	32.6±0.6
MCHC (g/dL)	H*1	33.2±0.7	34.3±0.3	35.1±0.4
	MAXM	34.8±1.7	31.9±0.6	34.2±1.0
RDW (%)	H*1	12.1±0.2	15.5±0.2	12.2±0.3
	MAXM	12.5±0.3	16.1±0.4	12.2±0.3
PLT (10^3/μL)	H*1	71±7.0	155±5.0	467±40.0
	MAXM	45±8.0	167±10.0	405±41.0

Table 2. The expiration date of open and closed vials of control cell determined by using Technicon H*1 and Coulter MAXM analyzers.

PARAMETER		OPEN VIAL	CLOSE VIAL
WBC (10^3/μL)	H*1	30	40
	MAXM	10	10
RBC (10^6/μL)	H*1	10	10
	MAXM	60	60
HGB (g/dL)	H*1	15	50
	MAXM	15	40
HCT (%)	H*1	1	10
	MAXM	5	40
MCV (fL)	H*1	1	1
	MAXM	1	1
MCH (pg)	H*1	15	15
	MAXM	60	60
MCHC (g/dL)	H*1	1	1
	MAXM	5	5

Precision of control cells

Intra-assay study

This study was performed using three different batches of control cells. Each sample was performed in ten replicated for each single run by both analyzers. The mean, SD and CV were calculated and shown in Table 3. The CVs of all parameters determined by both analyzers fall within the range of less than 5 per cent except CVs of pseudo-leukocyte in batch 1 and 3 (5.4% and 6.85%, respectively).

Inter-assay study

This study was performed using three different batches of control cells similar to those for the intra-assay precision test. Each sample was analyzed once a day, every 5 days, for 2 months or up to the day in which the measured parameter is still stable. The mean, SD and CV were calculated and shown in Table 4 .The CV of pseudo-leukocyte determined by Coulter MAXM is within the range of 1-3 per cent which is lower than that by Technicon H*1 (>10 %). The CVs of fixed platelets determined by both analyzers are within the similar range of less than 5 per cent. The CVs of all RBC parameters determined by both analyzers also fall within the range of less than 5 per cent except RBC count and MCH (Mean Cell Hemoglobin) in batch 3 by Coulter MAXM (9.33% and 12.84%, respectively).

Correlation of control cell between Technicon H*1 and Coulter MAXM analyzers

From Table 1 and 2, all parameters of control cells determined by both analyzers were significantly different with p-value < 0.05.

Table 3. The comparison of intra-assay data of hematological parameters in three normal control.

		WBC ($10^3/\mu L$)	PLT ($10^3/\mu L$)	RBC ($10^6/\mu L$)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)
Batch 1										
Mean	H*1	14.2	155	5.42	12.5	36.4	87.2	23.0	34.3	15.5
	MAXM	14.5	165	5.65	12.67	39.7	90.3	22.4	31.9	16.2
SD	H*1	0.77	2.5	0.02	0.1	0.2	0.2	0.1	0.15	0.1
	MAXM	0.14	5.58	0.03	0.05	0.25	0.38	0.14	0.24	0.17
%CV	H*1	5.4	1.6	0.37	0.8	0.55	0.23	0.4	0.44	0.65
	MAXM	0.97	3.38	0.53	0.4	0.63	0.42	0.63	0.75	1.05
Batch 2										
Mean	H*1	10.3	160	4.93	12.0	35.9	86.2	24.0	33.3	14.5
	MAXM	10.52	170	5.15	12.17	39.2	85.4	23.4	30.9	15.2
SD	H*1	0.45	2.13	0.03	0.1	0.2	0.3	0.2	0.2	0.1
	MAXM	0.14	5.44	0.03	0.05	0.3	0.6	0.2	0.22	0.2
%CV	H*1	4.4	1.33	0.61	0.83	0.56	0.35	0.8	0.6	0.69
	MAXM	1.33	3.2	0.58	0.41	0.77	0.7	0.86	0.71	1.32
Batch 3										
Mean	H*1	6.28	173	3.95	12.5	35.4	85.1	34.0	32.3	14.7
	MAXM	6.52	181	4.15	13.17	38.2	88.0	33.4	30.0	15.4
SD	H*1	0.43	3.21	0.03	0.09	0.3	1.4	0.2	0.15	0.1
	MAXM	0.14	6.5	0..04	0.06	0.25	0.39	0.33	0.13	0.17
%CV	H*1	6.85	1.86	0.76	0.72	0.85	1.65	0.59	0.46	0.68
	MAXM	2.15	3.59	1.0	0.46	0.65	0.44	1.0	0.43	1.11

Table 4. The comparison of Inter-assay data of hematological parameters in three normal control.

		WBC ($10^3/\mu L$)	PLT ($10^3/\mu L$)	RBC ($10^6/\mu L$)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)
Batch 1										
Mean	H*1	14.48	157	5..41	12.5	36.4	87.2	23.1	34.3	15.5
	MAXM	12.45	165	5.69	12.8	39.5	90.4	22.3	32.5	16.2
SD	H*1	1.74	4.23	0.09	0.1	0.18	0.31	0.49	0.15	0.06
	MAXM	0.15	9.05	0.07	0.16	0.47	0.42	0.36	0.47	0.17
%CV	H*1	12.01	2.7	1.66	0.79	0.5	0.35	2.13	0.45	0.41
	MAXM	1.2	5.48	1.28	1.26	1.19	0.46	1.6	1.44	1.05
Batch 2										
Mean	H*1	10.50	162	4.92	12.0	36.0	86.2	24.1	33.3	14.5
	MAXM	10.44	167	5.31	12.4	39.2	89.5	23.1	31.4	15.5
SD	H*1	1.67	4.25	0.09	0.14	0.19	0.31	0.49	0.15	0.06
	MAXM	0.15	7.46	0.21	0.23	0..46	0.58	0.51	0.83	0.42
%CV	H*1	15.87	2.62	1.91	1.16	0.52	0.35	2.033	0.47	0.44
	MAXM	1.46	4.48	3.9	1.85	1.18	0.65	2.21	2.64	2.73
Batch 3										
Mean	H*1	6.51	176	3.92	12.9	35.4	85.1	34.1	32.3	14.8
	MAXM	6.43	180	4.41	13.0	38.3	88.5	31.3	30.5	15.5
SD	H*1	1.65	3.79	0.1	0.25	0.25	1.39	0.49	0.16	0.07
	MAXM	0.17	8.64	0.4	0.44	0.47	0.55	4.01	0.6	0.35
%CV	H*1	25.41	2.15	2.4	1.96	0.71	1.63	1.44	0.48	0.51
	MAXM	2.68	4.91	9.33	3.35	1.22	0.62	12.84	1.98	2.27

The stability of thalassemic blood

The leukocyte and platelets count were unstable. The red cell parameters were stable for at least 6 weeks. The CVs in all genotypes of thalassemia fell within less than 5 per cent.

DISCUSSION

The major research in anticoagulant preservation has been directed to increasing the shelf life of blood(5,6). In this study, the cell preservation and fixation have been investigated. The pseudo-leukocytes can be utilized as a quantitative control for true leukocyte number but it is not appropriate as a qualitative or differential control for leukocytes since they are normal fixed red blood cells whose membrane resist lysis by lysing agent. The use of this parameter is more appropriate in Coulter MAXM. The Coulter MAXM's lysing reagent resulted in quite good cellular response giving the SD and CV values of pseudo-leukocyte close to those of true leukocyte. An excellent relationship was found between calculated and observed value of the pseudo-leukocyte count measured by both analyzers. There was no detectable deviation from linearity in the ranges tested. These results reflected the excellent consistency of the machine with the few numbers of the tested samples. We expect that the correlation coefficient is still very closely hanging around such number although more tested samples should be done.

The number of fixed control platelets is stable for 10 days when determined by Technicon H*1 analyzer and then gradually rises until day 30 on which it starts rising at a more accelerated rate. This phenomenon is observed to correlate well with the decline in pseudo-leukocyte number at the same period. This observation suggests that the

machine recognized those fragmented pseudoleukocytes and counted as platelets. Previous studies reported that microspherocytes, Pappenheimer bodies, leukocyte fragments, blood parasites, and bacterias can be the causes of spuriously high platelet counts generated by laser light scattering automated blood cell analyzer[7-11]. However, during the first 10 days, the stable fixed control platelets are quite useful for quality control due to low CV (<5%).

In control cells, the use of red cells in CPD solution has been recommended as a stable control material for standardization of the blood counting apparatus and performance checking throughout the day of RBC count, Hb (Hemoglobin) concentration and MCH. But the limitations of the use of blood in CPD as the way for control of the MCV (Mean Cell Volume) is that it reduces when compared with commercial control material as previously reported[5]. Decreasing of MCV in the first week showed the loss of water out of red cells. When red cell dehydration occurred, the MCHC (Mean Cell Hemoglobin Concentration) of red blood cells might be increased, which would influence the decrease of Hct (Hematocrit) value, thus the MCV value may increase aging owing to cell swelling.

All parameters for control blood determined by the two different cell counters are significantly different with p-value < 0.05. The principle of cell analysis is different so they must have their own values and expiration date of all parameters.

The results between open and closed vials showed longer stable period in the closed vial due to less loss of fluid from evaporation out of the vial causing small changes of parameters.

It was previously reported that for anticoagulant and preservative, CPD was better than EDTA in maintaining longer stabilities of preserved normal RBC parameters[5,12]. Thalassemic red cells, which are known as the abnormal cells in terms of their volume, shape, intracellular hemoglobin content, and osmotic fragility are recommended as abnormal control for automated cell analyzers[13].

(Received for publication on July 11, 1997)

REFERENCES

1. Warner BA, Reardon D. A field evaluation of the Coulter STKS. Am J Clin Pathol 1991; 95: 207-17.
2. Bollinger PB, Drewinko B, Brailas CD, et al. The Technicon H*1. An automatedhaematology analyser for today and tomorrow. Am J Clin Pathol 1987; 87: 71-8.
3. Fossat C, David M, Harle JR, et al. New parameters in erythrocyte counting. Arch Pathol Lab Med 1987; 111: 1150-4.
4. Hall R, Malia RG. Automated blood-cell analysis. In: Hall R, Malia RG, ed. Medical Laboratory Hematology. 2nd ed. Oxford: Butterworth-Heinemann Ltd., 1990: 193-218.
5. Lawrence ACK, Beving JM, Young M. Storage of blood and the mean corpuscular volume. J Clin Pathol 1975; 28: 345-9.
6. Hogman CF, Hedlund K, Akerblom O, Venge P. Red blood cells preservation in protein poor media-I. Leukocyte enzymes as a cause of hemolysis. Transfusion 1978; 18: 233-41.
7. Stass SA, Holloway ML, Peterson V. Cytoplasmic fragments causing spurious platelet counts in the leukemic phase of poorly differentiated lymphocytic leukemia. Am J Clin Pathol 1979; 71: 125-8.
8. Culati GL, Hyun BH. The Automated CBC: A Current Perspective. In: Hyun BH, ed. Hematology/Oncology Clinics of North America. Vol 8. Philadephia: W.B. Saunders Company, 1994: 593-603.
9. Gloster ES, Strauss RA, Jiminez JF, Neuberg RW, Berry DH, Turner EJ. Spurious elevated platelet counts associated with bacteremia. Am J Haematol 1985; 18: 329-32.
10. Akwari AM, Ross DW, Stas SA. Spurious elevated platelet counts due to microspherocytosis. Am J Clin Pathol 1982; 77: 220-1.
11. Morton DB, Orringer EP, La Hart LA, Stass AS. Pappenheimer bodies, an additional cause for a spurious platelet count. Am J Clin Pathol 1980; 74: 310-1.
12. Haradin AR, Weed RI, Reed CF. Changes in physical properties of stored erythrocytes. Relationship to survival in vivo. Transfusion 1969; 9: 229-37.
13. Bunyaratvej A, Ratanasritong S, Buthachat S. Thalassemic red cells determined by different technology of blood cell analysers. J Med Assoc Thai 1992; 75: 223-7.

การเตรียมเซลล์ควบคุมจากเลือดของคนปกติและธาลัสซีเมีย สำหรับเครื่องนับเม็ดเลือดอัตโนมัติ ซึ่งใช้หลักการกระจายแสงและการต้านกระแสไฟฟ้าจากเซลล์

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ได้ทำการเตรียมเซลล์ควบคุมขึ้นเป็น 3 ส่วน คือ ส่วนของเม็ดเลือดขาวหลอก เกล็ดเลือดตรึงสภาพ และส่วนของเม็ดเลือดแดงของคนปกติ และ ผู้ป่วยธาลัสซีเมีย โดยเก็บรักษาเม็ดเลือดแดงในน้ำยาซีพีดี หรือ ซีพีดีเอ-1 นำมาทดสอบกับเครื่องนับเม็ดเลือดอัตโนมัติ 2 เครื่อง คือ เทคนิคอน เอช-1 ใช้หลักการกระจายแสงจากเซลล์ และ คูลเตอร์ แมกซ์เอ็ม ใช้หลักการต้านกระแสไฟฟ้าจากเซลล์ จากการทดลองพบว่า เซลล์ที่เตรียมขึ้นสามารถนำมาใช้ควบคุมคุณภาพของเครื่องนับเม็ดเลือดอัตโนมัติได้ โดยค่าที่เตรียมได้มีความแตกต่างกันของวันหมดอายุ สำหรับเซลล์เม็ดเลือดแดงของผู้ป่วยธาลัสซีเมีย สามารถนำมาใช้เป็นเซลล์ควบคุมผิดปกติได้

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