Evaluation of the Reticulocyte Count Portion of Technicon H*3 Blood Analyzer: Lowering the Test Expense by Reducing the Reticulocyte Reagent

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Abstract

Automated reticulocyte counting has become an essential instrument of the hematology laboratory. This automatic technique has lead to diminishing labour tasks and to significant improvements in accuracy and precision compared with the manual microscopic methods. In any event, it adds a considerable expense to the laboratory budget. Here, we report the modified method of applying the new mixture of 1 μL of whole blood with 1 mL of reticulocyte reagent, which we evaluated for its accuracy and precision, instead of using the mixture of 3 μL of whole blood with 3 mL of reticulocyte reagent recommended by the company. We demonstrated the accepted accurate and precise results of percentage and absolute number of retculocyte count, low-stained reticulocyte count and its corpuscular indices; the mean reticulocyte corpuscular volume (MCVr), mean reticulocyte corpuscular hemoglobin concentration (CHCMr), and mean reticulocyte hemoglobin content (CHr). These suggested that, for every red cell assessed, the number, the cell volume, hemoglobin content and concentration are accurately and precisely measured by the modified method while the sub-populations of reticulocyte count and distribution width of reticulocyte indices are variable.

In conclusion, our results provided the information that 1) the modified method can be used as a routine test and it provides accurate and precise results; 2) with the modified method, two-thirds of the expense spent for reticulocyte reagent can be saved; 3) it should not be used for research purposes.

The reticulocyte count is clinically important for 1) the pathophysiological classification of anemias based on bone marrow efficiency 2) the early identification of the normalization of erythropoiesis after therapeutic intervention (with iron, folic acid, B_{12}, erythropoietin, etc.), after spontaneous or pharmacologically induced aplasia of the marrow, or following bone marrow transplantation. Although the reticulocyte count is clinically valuable, its reliability is limited by both physiological

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and technical factors[1]. The physiological factors include diurnal and daily variations in the bone marrow's mitotic activity[2,3]. The technical factors relate to the laboratory procedures used to perform the count. These factors include distributional variability of reticulocytes in the blood smear, variations in staining techniques and quality, limited number of reticulocytes counted, and variations of the criteria used for the identification of reticulocytes[4]. However, the method most widely used is a microscopic count of reticulocytes per 1,000 red cells. Aside from being a laborious task, this manual method tends to be inaccurate and imprecise[4-6]. Recently, the automated alternative has been introduced and evaluated for its accuracy and precision. Due to the large numbers of cells analyzed by flow cytometry, there is a clear improvement as to the precision of reticulocyte counts compared with the manual method[7-9]. However, because of economic difficulties many laboratories cannot justify establishing the automated method.

The new H*3 (Miles, Diagnostics Division, Tarrytown, New York) blood analyzer is a further refinement of the established Technicon H*1/ H*2 technology, having the additional reticulocyte counting capability. This is based on a new reticulocyte staining method, which utilizes the light-absorptive properties of the nucleic acid dye Oxazine 750[8]. The Technicon H*3 reticulocyte method preliminarily requires manually mixing 3 μL of whole blood with 3 mL of reticulocyte reagent. To reduce the cost of the test, the modified method was conducted by manually mixing 1 μL of whole blood with 1 mL of reticulocyte reagent. This method requires a lower amount of the reagent but still the same ratio of whole blood to reagent is maintained. We have evaluated this modified method for its accuracy and precision.

MATERIAL AND METHOD

Samples:

The 10 K3EDTA-anticoagulant treated peripheral blood samples were selected from the specimens with normal reticulocyte count and 10 K3EDTA-anticoagulant treated peripheral blood samples were selected from the specimens with high reticulocyte count more than 2 per cent. All samples were sent to the Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University. All the samples were analyzed with the Technicon H*3 within 4 hours after phlebotomy.

Method and Instrument:

The Technicon H*3 was used to analyze all the samples. According to company recommendations, the cell suspension to be analyzed must be prepared manually, adding 3 μL of K3EDTA-anticoagulant treated peripheral blood to 3 mL of reticulocyte reagent. To reach our purpose, mixing 1 μL of K3EDTA-anticoagulant treated peripheral blood with 1 mL of reticulocyte reagent was also performed three times per sample in order to evaluate the precision. The accuracy was tested by comparing the results obtained after mixing 3 μL of K3EDTA-anticoagulant treated peripheral blood with 3 mL of reticulocyte reagent, as recommended by the company, with the average value obtained from the modified method. The reagent contained a surfactant, which renders each cell globular, and a nucleic acid-binding basic dye, oxazine 750, which stains the reticulocyte. The vial is capped and inverted three times to allow the reagent mix completely with the blood. After incubation at room temperature for 15 minutes, the sample can be analyzed. The approximately 20,000 cells counted per sample are identified on a cell-by-cell basis applying three criteria: low-angle scatter (2° to 3°), primarily correlated to the cell volume; high-angle scatter (5° to 15°), correlated to the hemoglobin concentration; and absorbance, correlated to the intensity of staining and therefore to the RNA content[10]. All parameters provided by the instrument included the percentage and absolute number of reticulocytes counted, and its corpuscular indices; mean reticulocyte corpuscular volume (MCVr), mean reticulocyte corpuscular hemoglobin concentration (CHCMr), mean reticulocyte hemoglobin content (CHr), reticulocyte distribution width (RDWr), reticulocyte hemoglobin distribution width (HDWr) and reticulocyte corpuscular hemoglobin concentration distribution width (CHDWr) were analyzed. Based on the reticulocyte staining intensity of Oxazine 750, reticulocytes were attributed to one of three categories, i.e., low-stained-reticulocytes (L-ret), medium-stained-reticulocytes (M-ret), and high-stained-reticulocytes (H-ret). Immature reticulocytes were determined as the aggregate of M-ret and H-ret.

Statistical Analysis:

Accuracy was evaluated by the coefficient of the correlation between the method recom-

mended by the company and the modified method. Difference between company method and modified method was calculated using paired student *t* test, $p<0.050$ is considered as statistical significance. Precision was evaluated by the coefficient of the variation observed with triplicates of all samples prepared by the modified method.

RESULT

Accuracy:

Table 1 shows the comparison between the results obtained with the method recommended by the company and the modified method of normal reticulocyte count samples. The high correlation was demonstrated for the results regarding percentage of reticulocyte count (r, 0.89; intercept, 0.18; slope, 0.85), absolute number of reticulocyte count (r, 0.88; intercept, 12543; slope, 0.78), absolute number of L-ret (r, 0.84; intercept, 12812; slope, 0.74), MCVr (r, 0.88; intercept, 16.04; slope, 0.87), CHCMr (r, 0.96; intercept, -2.88; slope, 1.10), and CHr (r, 0.99; intercept, 0.64; slope, 0.99). The moderate correlation was demonstrated for the results with respect to the absolute number of M-ret (r, 0.66; intercept, 1888; slope, 0.73). No correlation was found between the RDWr's obtained from both methods. The low correlation was demonstrated for the left parameters. There is no statistical significant difference for all parameters between both methods.

Table 2 shows the comparison between the results obtained with the method recommended by the company and the modified method of high reticulocyte count (>2%) samples. The high correlation was demonstrated for the results regarding percentage of reticulocyte count (r, 0.99; intercept, 0.29; slope, 0.90), percentage of L-ret (r, 1.00; intercept, -0.03; slope, 1.01), percentage of M-ret (r, 0.80; intercept, 4.85; slope, 0.74), absolute number of reticulocyte count (r, 1.00; intercept, 4783; slope, 0.97), absolute number of L-ret (r,0.99; intercept, 2112; slope, 1.00), absolute number of M-ret (r, 0.98; intercept, 680; slope, 0.99), MCVr (r, 0.99; intercept, -9.08; slope, 1.08), CHCMr (r, 0.92; intercept, -3.93; slope, 1.14), HDWr (r, 0.92; intercept, -0.87; slope, 1.28), CHr (r, 0.98; intercept, -5.42; slope, 1.19) and CHDWr (r, 0.88; intercept, 1.11; slope, 0.74). The moderate correlation was demonstrated for the results with respect to the percentage of H-ret (r, 0.73; intercept, -0.87; slope, 0.90), absolute number of H-ret (r, 0.77; intercept, 18; slope, 0.74), and RDWr's (r, 0.55; intercept, -7.48; slope, 1.43). There is no statistical significant difference for all parameters between both methods.

Precision:

Tables 3 and 4 demonstrate the precision of the triplicate determinations of 10 normal reticulocyte count samples using the modified method. The mean values, standard deviation (SD), and coefficient of variation (CV), were calculated. The analytic precision, as CV was less than 10 per cent, was demonstrated for all samples regarding the results of percentage of L-ret, MCVr, CHCMr, and CHr and for most of the samples regarding the results of percentage and absolute number of reticulocyte count, RDWr, HDWr, and CHDWr. Most of the left parameters and absolute numbers of M-ret and H-ret demonstrated a CV higher by more than 10 per cent.

Tables 5 and 6 demonstrate the precision of the triplicate determinations of 10 high reticulocyte count (>2%) samples using the modified method. The mean values, standard deviation (SD), and coefficient of variation (CV), were calculated. The analytic precision, as CV was less than 10 per cent, was demonstrated for all samples regarding the results of percentage of L-ret, MCVr, CHCMr, HDWr, CHr and CHDWr and for most of the samples regarding the results of percentage and absolute number of reticulocyte count, absolute number of M-ret and RDWr. Most of the samples of absolute numbers of M-ret and H-ret demonstrated a CV higher by more than 10 per cent.

DISCUSSION

The reticulocyte count represents a very interesting assay of erythropoiesis activity. The limitation of its clinical application is probably due to the inaccuracy and imprecision of the conventional microscopic method[4-6]. Nowadays, the alternative automatic method could provide more accurate and precise results[7-9]. However, in Southeast Asian Country the automatic method is considered expensive. To justify a possible way to obtain accurate and precise results by paying less, the modified method was introduced and evaluated for its accuracy and precision.

Table 1. Accuracy test of reticulocyte count and indices by 3 mL reticulocyte reagent and 1 mL reticulocyte reagent in 10 normal recticulocyte count samples.

Parameter	mean±SD		P value	Correlation	Intercept	Slope
	3 mL	1 mL				
Ret. count (%)	1.3±0.31	1.4±0.32	0.678	0.89h	0.18	0.85
Ret. count (cells/μL)	61265±14508	62648±16339	0.590	0.88h	12543	0.78
L-ret (%)	85.8±4.98	85.2±3.78	0.693	0.45l	35.85	0.59
M-ret (%)	12.2±4.43	12.1±2.95	0.983	0.36l	5.60	0.54
H-ret (%)	2.0±0.94	2.6±1.09	0.108	0.39l	1.13	0.33
L-ret (cells/μL)	52304±11349	52991±12780	0.763	0.84h	12812	0.74
M-ret (cells/μL)	7683±3837	7924±3457	0.807	0.66m	1888	0.73
H-ret (cells/μL)	1273±837	1731±1037	0.176	0.46l	626	0.37
MCVr (fL)	108.2±5.03	107.5±5.22	0.057	0.88h	16.04	0.87
CHCMr (g/dL)	25.6±1.37	25.8±1.20	0.120	0.96h	-2.88	1.10
RDWr (%)	17.2±1.24	17.9±0.89	0.170	0.12n	14.21	0.17
HDWr (%)	3.05±0.27	3.02±0.31	0.769	0.30l	2.27	0.26
CHr (pg)	27.3±1.99	27.0±2.01	0.054	0.99h	0.64	0.99
CHDWr (pg)	4.3±0.32	4.4±0.16	0.502	-0.01n	4.34	-0.01

p<0.050 is considered as statistical significance

correlation; h, 0.8-1.0 = high correlation
m, 0.5-0.8 = moderate correlation
l, 0.2-0.5 = low correlation
n, 0.0-0.2 = no correlation

Table 2. Accuracy test of reticulocyte count and indices by 3 mL reticulocyte reagent and 1 mL reticulocyte reagent in 10 samples with reticulocyte count is >2%.

Parameter	mean±SD		P value	Correlation	Intercept	Slope
	3 mL	1 mL				
Ret. count (%)	2.7±0.29	2.7±0.32	0.081	0.99h	0.23	0.90
Ret. count (cells/μL)	124220±23522	123005±24144	0.073	1.00h	4783	0.97
L-ret (%)	71.5±19.81	70.6±19.53	0.303	1.00h	-0.03	1.01
M-ret (%)	17.6±1.13	17.4±1.22	0.497	0.74m	4.85	0.74
H-ret (%)	4.3±1.71	5.7±1.39	0.143	0.73m	-0.87	0.90
L-ret (cells/μL)	97009±18174	94540±17921	0.054	0.99h	2112	1.00
M-ret (cells/μL)	21991±5049	21420±4963	0.178	0.98h	680	0.99
H-ret (cells/μL)	5247±2204	7053±2275	0.102	0.77m	18	0.74
MCVr (fL)	108.0±8.07	108.0±7.43	0.252	0.99h	-9.08	1.08
CHCMr (g/dL)	26.7±1.27	26.8±1.02	0.491	0.92h	-3.93	1.14
RDWr (%)	17.6±2.27	17.5±0.88	0.854	0.55m	-7.48	1.43
HDWr (%)	3.26±0.40	3.23±0.28	0.593	0.92h	-0.87	1.28
CHr (pg)	27.9±2.63	28.0±2.17	0.687	0.98h	-5.42	1.19
CHDWr (pg)	4.3±0.42	4.3±0.50	0.575	0.88h	1.11	0.74

p<0.050 is considered as statistical significance

correlation; h, 0.8-1.0 = high correlation
m, 0.5-0.8 = moderate correlation
l, 0.2-0.5 = low correlation
n, 0.0-0.2 = no correlation

Table 3. Precision test of reticulocyte count by triplicate determinations of 10 samples with normal reticulocyte count. (S-1 to S-10)

Sample	Ret(%)	L-ret(%)	M-ret(%)	H-ret(%)	Ret(#)	L-ret(#) (cells/μL)	M-ret(#) (cells/μL)	H-ret(#) (cells/μL)
S-1								
mean	1.2	88.9	8.9	2.1	55920	49713	4996	1193
SD	0.00	1.84	1.47	0.37	0	1028	822	206
CV(%)	0.00*	2.07*	16.46	17.26	0*	2.07*	16.46	17.26
S-2								
mean	1.3	87.4	10.7	1.7	752000	59710	10101	2690
SD	1.05	5.00	3.38	1.81	8938	5250	3502	260
CV(%)	3.54*	5.72*	31.50	109.26	12.33	8.79*	34.68	9.69*
S-3								
mean	1.7	82.7	13.5	3.7	72500	59710	10101	2690
SD	0.20	3.06	3.24	0.29	8938	5250	3502	260
CV(%)	21.50	3.70*	23.91	7.68*	12.33	8.79*	34.68	9.69*
S-4								
mean	1.3	85.7	12.2	2.1	63333	54068	7963	1276
SD	0.29	2.70	3.24	0.25	13620	10658	3279	131
CV(%)	20.41	3.15*	22.36	12.07	20.41	19.71	41.18	10.28
S-5								
mean	1.6	89.0	9.6	1.4	66240	58396	6790	1054
SD	0.33	4.38	3.75	0.78	13521	9368	3943	696
CV(%)	20.41	4.92*	39.06	54.74	20.41	16.04	58.07	66.05
S-6								
mean	1.0	85.1	12.4	2.6	40197	34261	4939	1009
SD	0.09	2.21	1.22	1.07	3668	3840	388	327
CV(%)	5.10*	2.60*	9.89*	41.19	9.12*	11.21	7.85*	32.45*
S-7								
mean	1.6	79.8	17.4	2.9	81280	64745	14165	2399
SD	0.08	2.68	1.62	1.13	4148	2019	1953	1057
CV(%)	5.10*	3.36*	9.34*	39.11	5.10*	3.18*	13.79	44.04
S-8								
mean	1.9	81.3	14.5	4.4	84930	68521	12568	4020
SD	0.36	3.33	1.64	1.60	15909	9762	3855	2243
CV(%)	18.73	4.09*	11.3	36.20	18.73	14.25	30.70	55.80
S-9								
mean	1.0	81.5	14.3	4.1	44700	36481	6331	1857
SD	0.08	2.42	1.98	0.93	3650	3760	76	460
CV(%)	8.16*	2.97*	13.86	22.46	8.16*	10.31	9.10*	24.77
S-10								
mean	1.0	90.8	7.7	1.5	42147	38240	3341	581
SD	0.12	2.47	2.42	1.08	5438	4770	1402	346
CV(%)	12.90	2.72*	31.33	72.01	12.90	12.47	41.97	59.48

* CV < 10%, accepted CV

Table 4. Precision test of reticulocyte indices by triplicate determinations of 10 samples with normal reticulocyte count.

Sample	MCVr (fL)	CHCMr(g/dL)	RDWr (%)	HDWr(g/dL)	CHr (pg)	CHDWr(pg)
S-1						
mean	111.0	26.5	18.1	2.81	28.6	4.5
SD	0.98	0.05	1.7	0.13	0.21	0.26
CV(%)	0.88*	0.18*	9.39*	4.57*	0.72*	5.88*
S-2						
mean	99.6	25.5	18.5	2.80	24.7	4.2
SD	1.67	0.37	1.92	0.14	0.00	0.50
CV(%)	1.67*	1.47*	10.37	4.89*	0.00*	11.97*
S-3						
mean	107.8	26.5	18.2	3.57	27.7	4.6
SD	1.15	0.26	2.21	0.61	0.31	0.29
CV(%)	1.06*	0.99*	12.12	17.04	1.11*	6.40*
S-4						
mean	105.1	26.7	18.2	2.93	27.4	4.3
SD	2.63	0.09	0.46	0.06	0.59	0.12
CV(%)	2.50*	0.35*	2.55*	2.05*	2.15*	2.88*
S-5						
mean	106.1	27.9	17.8	3.00	28.2	4.2
SD	2.15	0.24	0.73	0.42	0.36	0.29
CV(%)	2.02*	0.88*	4.10*	14.11	1.26*	6.77*
S-6						
mean	115.3	25.2	16.0	3.00	28.2	4.2
SD	1.59	0.54	0.90	0.42	0.36	0.29
CV(%)	1.38*	2.16*	5.61*	14.11	1.26*	6.77*
S-7						
mean	112.8	26.0	17.7	2.90	28.6	4.5
SD	1.60	0.36	0.41	0.07	0.36	0.17
CV(%)	1.41*	1.37*	2.32*	2.43*	1.26*	3.80*
S-8						
mean	101.7	23.9	17.2	3.10	23.7	4.2
SD	1.77	0.26	0.40	0.11	0.19	0.22
CV(%)	1.74*	1.10*	2.35*	3.39*	0.80*	5.14*
S-9						
mean	103.3	24.2	19.4	3.50	24.1	4.3
SD	0.29	0.12	1.59	0.08	0.08	0.49
CV(%)	0.28*	0.52*	8.19*	2.38*	0.34*	11.36
S-10						
mean	112.2	25.7	17.5	2.56	28.1	4.60
SD	2.52	0.16	0.91	0.09	0.54	0.20
CV(%)	2.24*	0.64*	5.20*	3.62*	1.93*	4.50*

* CV < 10%, accepted CV

Table 5. Precision test of reticulocyte count by triplicate determinations of 10 samples with reticulocyte count >2.0%. (HS-1 to HS-10)

Sample	Ret(%)	L-ret(%)	M-ret(%)	H-ret(%)	Ret(#)	L-ret(#) (cells/μL)	M-ret(#) (cells/μL)	H-ret(#) (cells/μL)
HS-1								
mean	2.4	78.7	17.3	4.0	101057	79486	17484	4086
SD	0.05	1.62	0.78	0.90	2013	1841	898	962
CV(%)	1.99*	2.06*	4.50*	22.3	1.99*	2.31*	5.14*	23.53
HS-2								
mean	2.8	77.5	18.3	4.2	116733	90427	21427	4879
SD	0.05	1.6	2.16	1.21	1942	2289	2753	1346
CV(%)	1.66*	2.06*	11.80	28.80	1.66*	2.53*	12.85	27.58
HS-3								
mean	3.0	75.5	18.0	6.5	123300	93141	22102	8057
SD	0.08	1.63	1.56	1.11	3356	4456	1331	1386
CV(%)	2.72*	2.16*	8.70*	17.00	2.72*	4.78*	6.02*	17.21
HS-4								
mean	2.2	75.8	17.9	6.3	106530	80605	19220	6705
SD	0.29	1.13	1.99	1.41	13678	9192	4044	1719
CV(%)	12.80	1.50*	11.1	22.40	12.84	11.40	21.04	25.64
HS-5								
mean	2.3	75.9	18.6	5.6	114770	87122	21262	6386
SD	0.08	1.91	1.58	0.39	4074	4851	1406	452
CV(%)	3.55*	2.51*	8.49*	6.93*	3.55*	5.57*	6.61*	7.08*
HS-6								
mean	2.5	76.3	15.6	8.1	127750	97575	19711	10464
SD	0.24	1.28	2.61	1.40	12517	10573	1918	2653
CV(%)	9.80*	1.68*	16.70	17.30*	9.80*	10.84	9.73*	25.35
HS-7								
mean	2.7	77.9	15.3	6.8	114253	88991	17574	7688
SD	0.12	1.35	1.85	0.96	5213	3102	2946	788
CV(%)	4.56*	1.74*	12.10	14.10	4.56*	3.49*	16.76	10.25
HS-8								
mean	2.6	79.3	17.0	3.7	115700	91861	19528	4311
SD	0.22	0.82	1.87	1.27	9613	8539	1702	1506
CV(%)	8.31*	1.03*	11.00	34.30	8.31*	9.30*	8.72*	34.93
HS-9								
mean	2.6	78.2	17.2	4.6	103693	81072	17947	4742
SD	0.09	0.99	2.05	1.72	3809	2685	2832	1654
CV(%)	3.67*	1.27*	11.90	37.10	3.67*	3.31*	15.78	34.89
HS-10								
mean	3.2	76.0	18.5	5.5	184320	140066	34005	10249
SD	0.08	2.08	1.31	1.42	4703	4731	2053	2863
CV(%)	2.55*	2.74*	7.08*	25.7	2.55*	3.38*	6.04*	27.94

* CV < 10%, accepted CV

Table 6. Precision test of reticulocyte indices by triplicate determinations of 10 samples with reticulocyte count >2.0%. (HS-1 to HS-10)

Sample	MCVr (fL)	CHCMr(g/dL)	RDWr (%)	HDWr(g/dL)	CHr (pg)	CHDWr(pg)
HS-1						
mean	108.2	27.8	18.2	3.23	28.7	4.4
SD	1.31	0.25	0.61	0.08	0.16	0.17
CV(%)	1.21*	0.90*	3.37*	2.49*	0.57*	3.83*
HS-2						
mean	113.3	25.7	17.6	3.54	27.9	4.4
SD	0.70	1.03	1.10	0.07	0.12	0.17
CV(%)	0.62*	4.03*	6.24*	1.92*	0.45*	3.83*
HS-3						
mean	115.6	26.6	18.1	3.47	29.3	4.4
SD	0.87	0.51	0.82	0.04	0.33	0.25
CV(%)	0.76*	1.92*	4.51*	1.21*	1.13*	5.63*
HS-4						
mean	104.6	27.2	16.9	3.01	27.5	4.6
SD	0.85	0.98	1.33	0.08	0.33	0.12
CV(%)	0.81*	3.61*	7.84*	2.52*	1.19*	1.19*
HS-5						
mean	114.4	26.2	17.9	2.91	28.8	4.7
SD	0.86	0.57	1.56	0.16	0.25	0.17
CV(%)	0.76*	2.19*	8.69*	5.39*	0.87*	3.59*
HS-6						
mean	105.2	26.7	16.9	3.7	26.3	3.4
SD	0.37	0.69	1.60	0.13	0.49	0.09
CV(%)	0.35*	2.60*	9.43*	3.56*	1.87*	2.75*
HS-7						
mean	113.1	27.5	16.9	3.0	30.0	4.5
SD	0.31	0.71	1.60	0.07	0.33	0.29
CV(%)	0.27*	2.59*	9.43*	2.21*	1.10*	6.42*
HS-8						
mean	102.6	26.7	17.9	3.0	29.0	3.4
SD	1.16	1.00	1.12	0.15	1.46	0.17
CV(%)	1.13*	3.76*	6.27*	4.87*	5.04*	4.95*
HS-9						
mean	109.7	29.0	16.4	3.1	30.3	4.4
SD	1.63	0.71	2.20	0.08	0.50	0.16
CV(%)	1.49*	2.45*	13.40	2.64*	1.64*	3.71*
HS-10						
mean	92.6	25.7	19.3	3.35	23.3	4.5
SD	1.55	1.13	0.86	0.12	1.56	0.29
CV(%)	1.68*	4.41*	4.46*	3.70*	6.70*	6.42*

* CV < 10%, accepted CV

The accurate and precise study of our modified method has demonstrated that there are accepted accurate and precise results as to percentage, absolute number of reticulocyte count and L-ret, MCVr, CHCMr, and CHr determinations. This has suggested that, regarding every red cell assessed, the number, the cell volume, hemoglobin content and concentration are accurately and precisely measured by the modified method, while the sub-populations of reticulocyte count and distribution width of reticulocyte indices are variable. In addition, in high reticulocyte cont (>2%) samples, the accuracy and precision of our modified method is better than in normal reticulocyte cont samples. The explaination is an increase of the percentage leads to a significantly narrower 95 per cent confidence-interval and thus higher statistical accuracy in counting and an improvement in the precision of the results[11]. The accuracy and precision of our modified method is lower than that of the recommended method[8]. One reason for this is the accuracy of pipetting 1 μL of K3EDTA-anticoagulant treated peripheral blood into 1 mL of reticulocyte reagent. However, the modified method can provide precise results with a degree of accuracy acceptable for routine tests and permit better quantitative and qualitative results than the conventional manual method which purportedly has a higher degree of imprecision as demonstrated by a higher coefficient of variation (CV, 68.6%)[8].

In conclusion, our results supplied the information that 1) the modified method can be used as a routine test and provides accurate and precise results; 2) with the modified method, two-thirds of the expenses spent on reticulocyte reagent can be saved; 3) it should not be used for research purposes.

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การประเมินการตรวจวิเคราะห์เรติคูโลไซท์โดยเครื่องตรวจวิเคราะห์เลือดอัตโนมัติ Technicon H*3: การลดค่าใช้จ่ายโดยการลดปริมาณน้ำยาที่ใช้ลง

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การตรวจวิเคราะห์เรติคูโลไซท์โดยเครื่องอัตโนมัติได้ทวีความสำคัญขึ้นในการตรวจทางห้องปฏิบัติการทางการแพทย์ เพราะช่วยลดแรงงานและมีความถูกต้องแม่นยำสูงขึ้นเมื่อเปรียบเทียบกับการใช้กล้องจุลทรรศน์ อย่างไรก็ตาม การตรวจวิเคราะห์เรติคูโลไซท์โดยเครื่องอัตโนมัติยังมีปัญหาในเรื่องค่าใช้จ่ายที่สูงขึ้น คณะผู้วิจัยจึงประยุกต์การตรวจโดยการลดปริมาณน้ำยาที่ใช้ลง โดยใช้เลือด 1 μL ผสมกับน้ำยา 1 mL แทนการใช้เลือด 3 μL ผสมกับน้ำยา 3 mL ตามวิธีที่บริษัทแนะนำ คณะผู้วิจัยพบว่าความถูกต้องแม่นยำของค่าร้อยละและจำนวนนับสัมบูรณ์ของค่า retculocyte count และ low-stained reticulocyte count และค่าดัชนีของเม็ดเลือดแดงตัวอ่อนเรติคูโลไซท์ คือค่า mean reticulocyte corpuscular volume (MCVr), mean reticulocyte corpuscular hemoglobin concentration (CHCMr), และ mean reticulocyte hemoglobin content (CHr) เป็นที่ยอมรับได้ ส่วนค่า medium และ high-stained reticulocyte count และค่าการกระจายของขนาดและฮีโมโกลบินของเม็ดเลือดแดงตัวอ่อนเรติคูโลไซท์เชื่อถือได้ต่ำ

คณะผู้วิจัยสรุปว่า 1.) วิธีการประยุกต์การตรวจวิเคราะห์เรติคูโลไซท์นี้สามารถนำมาใช้ในงานประจำ โดยมีความถูกต้องแม่นยำที่ยอมรับได้ 2.) ด้วยวิธีการประยุกต์การตรวจวิเคราะห์เรติคูโลไซท์นี้สามารถลดค่าใช้จ่ายในส่วนของน้ำยาที่ใช้ตรวจ คือ reticulocyte reagent ลงได้ถึงสองในสาม 3.) วิธีการนี้ไม่เหมาะสมกับงานวิจัยที่ต้องการความถูกต้องแม่นยำสูง

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