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Evaluation of Prior-Mixing Effect Using Technicon H*2

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Abstract: The effect of prior cumulative mixing was evaluated by means of a simple regression model which used as a dependent variable percentage(%) change of each repeated observed value for the initial value. The red blood cell count (RBC), hematocrit (Hct), and mean corpuscular volume(MCV) showed a significant decrease ($p<.01$), but % of basophils had a significant increase ($p<.05$). The ratios of rate of change (regression coefficient/SD) were. 0012 for RBC, .0020 for Hct, .0024 for MCV, and .0011 for % of basophils. Of the 19 parameters evaluated, 21% changed.

Key Words: Technicon H*2, Prior-mixing effect.

INTRODUCTION

It is an important issue during simultaneous comparison of two or three instruments which use automated tube inversions to determine whether the cumulative number of mixings (tube inversions) alters the results when the same specimen tube are run several times consecutively from one instrument to another. If the changes depend on the cummulative mixing number, the study design must be changed if the same specimen tube is used for multiple instrument evaluation. We evaluated the effect of prior-mixing using a Technicon H*2. The Technicon H*2 mixes samples more times than the Sysmex NE-8000 or Coulter STKs even though the degrees of rotation of shaking of the NE-8000 are greater than Technicon H*2.

METHODS

3 randomly selected patient specimens were used because we wanted to include samples with parameters of different levels. The 19 parameters evaluated using Technicon H*2 included: white blood cell count (WBC), red blood cell count (RBC), platelet count (PLt), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglonin (MCH), absolute and percentage WBC differential counts including neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells (LUC). The mean WBC ($\times 10^3$/ul) was 6.22 for specimen I, 12.68 for specimen II, and 8.52 for specimen III. Table 1 shows the number of cumulative mixings for 3 patient specimens run 13 consecutive times. The simple regression model used for evaluation of the prior-mixing effect and the formula are as follows:

The percent(%) change of each observed

$$Y=b_0 + b_1X$$

Y: % change$^+$ of each observed value
X: number of cumulative mixing
b_0: intercept value of Y at X=0
b_1: slope or trend of % change$^+$

+ : (observed repeat value/initial value)$\times$100

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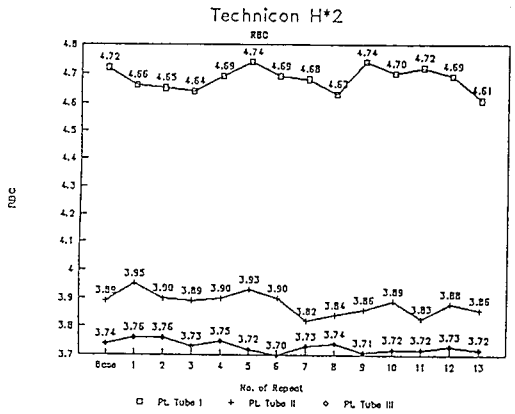

Fig. 1-1. Red blood cell count (RBC) values.

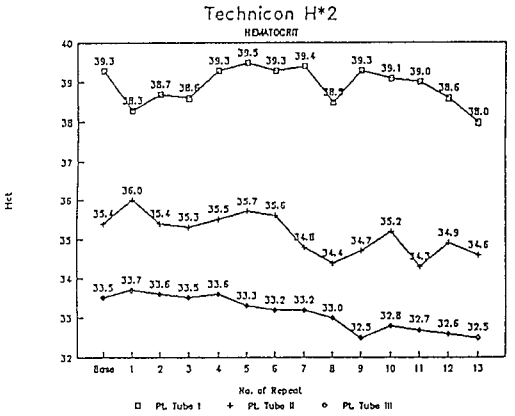

Fig. 2-1. Hematocrit (Hct) values.

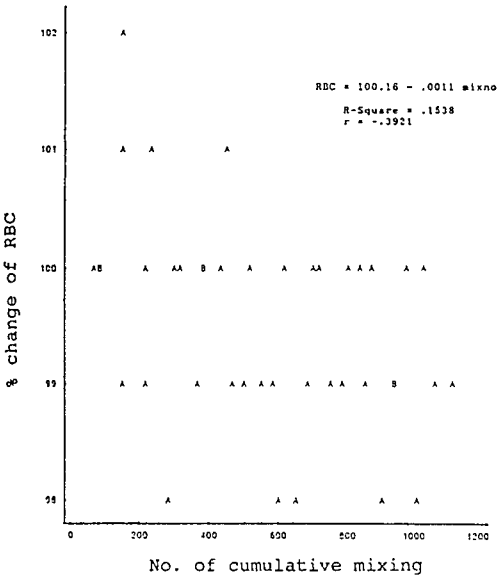

Fig. 1-2. Correlation between % change of RBC and no. of cumulative mixings (Legend: A=1, B=2 observations).

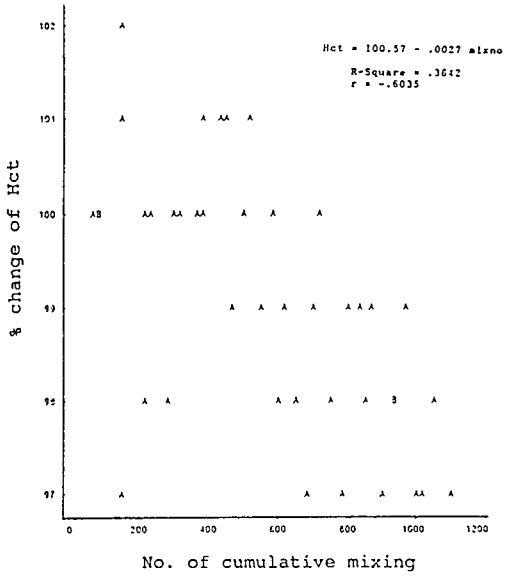

Fig. 2-2. Correlation between % change of Hct and no. of cumulative mixings (Legend: A=1, B=2 observations).

value was calculated as a dependent variable for the purpose of adjusting the difference of parameter levels of 3 patient specimens. The independent variable was the number of cumulative mixings. To compare the amount of change, the ratios of rate of change (regression coefficient/SD) were also calculated.

RESULTS

Table 2 shows the percent change of the 13th repeat value from the initial value. The results of simple regression analysis for the evaluation of prior-mixing effect are shown in Table 3. The RBC, Hct, MCV, and percent(%) of basophils had significantly changed. The ratios of the rate of change were. 0012 for RBC, .0020 for Hct, .0024 for MCV, and .0011 for % of basophils. Figure 1-1 shows RBC values of tubes assayed 13 times. There is a slight trend to decrease in all 3 patient specimens. The correlation coefficient between % change of RBC and the number of cumulative mixings was-.3921 (Fig. 1-2), and the regression

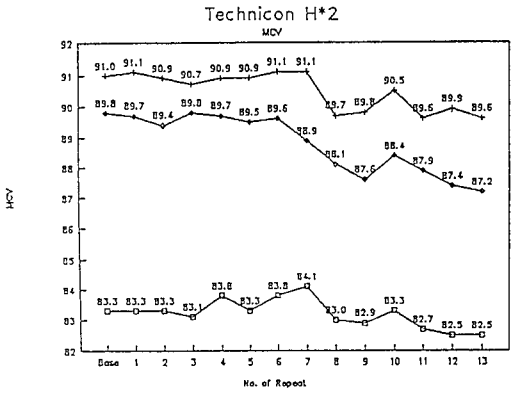

Fig. 3-1. Mean corpuscular volume(MCV) values.

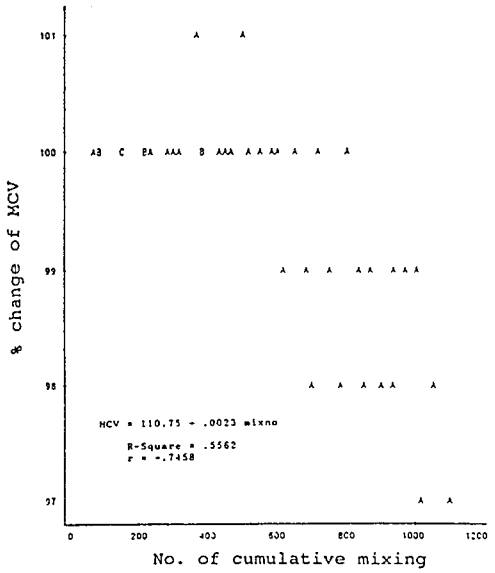

Fig. 3-2. Correlation between % change of MCV and no. of cumulative mixings (Legend: A=1, B=2, C=3 observations).

model was significant (p<.50). Figure 2-1 shows Hct values from tubes repeated 13 times displayed a tendency to decrease. The slope of % change (regression coefficient) was significant (p<.01). The correlation coefficient between % change of Hct and the number of cumulative mixing was-.6035 (Fig. 2-2).

Figure 3-1 shows a marked tendency to decrease of MCV. The regression coefficient was significant (p<.01). The correlation coefficient between % change of MCV and the number of cumulative mixings was-.7458 (Fig. 3-2).

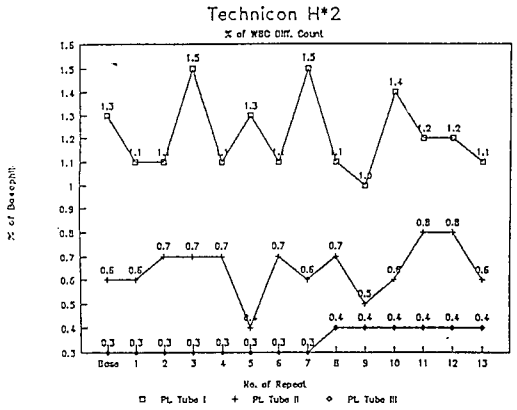

Fig. 4-1. Percent of basophils values.

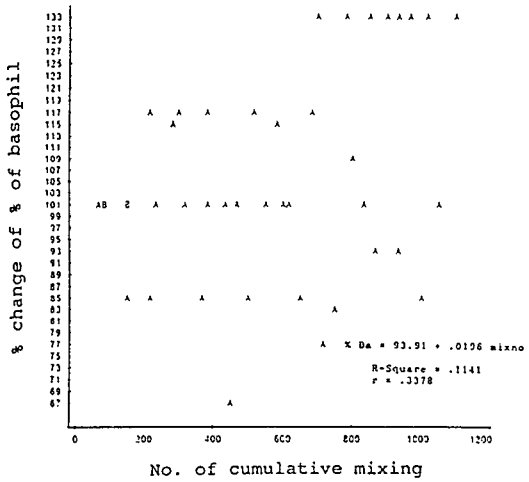

Fig. 4-2. Correlation between % change of basophils % and no. of cumulative mixings (Legend: A= 1, B=2 observations).

The % of basophils in samples run 13 times are shown in figure 4-1. There were no consistent increased or decreased trends. However, the slope of % change (regression coefficient) was significant (p<.05), and the correlation coefficient between % of basophils is and the number of cumulative mixings was. 3378(Fig. 4-2). There fore, the change of % of basophils is considered a variation of the instrument itself, rather than a real effect of prior-mixing.

In summary of the evaluation of prior-mixing effect, there were 21.1% changes: 1 increased change (5.3%) and 3 decreased changes (15.8%) among 19 evaluated parameters (Table 4). The coefficients of variation for

13 replications are in Table 5.

DISCUSSION

Our results were comparable with the studies of Bollinger, et al (1987) and Kershaw, et al (1987)[2] (Table 6). In this study, there were

Table 1. Number of cumulative mixings for 3 patient specimens on a Technicon H*2 instrument

No. of	Pt. I	Pt. II	Pt. III
Initial	72	75	78
lst	144	150	156
2nd	216	225	234
3rd	288	300	312
4th	360	375	390
5th	432	450	468
6th	504	525	546
7th	576	600	624
8th	648	675	702
9th	720	750	780
10th	792	825	858
11th	864	900	936
12th	936	975	1014
13th	1008	1050	1092

Table 2. The percent (%)+ change of 13th repeat value from the initial value

Variables	Initial value	percent change		
		Pt. I	Pt. II	Pt. III
WBC	100	117	101	99
RBC	100	98	99	99
PLt	100	91	100	95
Hgb	100	101	99	100
Hct	100	97	98	97
MCV	100	99	98	97
MCH	100	103	100	101
# Neu	100	116	101	99
% Neu	100	100	100	100
#Lym	100	114	104	105
% Lym	100	97	102	106
# Mono	100	140	94	94
% Mono	100	121	93	93
# Eo	100	120	69	80
% Eo	100	107	83	70
# Ba	100	88	125	100
% Ba	100	85	100	133
# LUC	100	127	94	110
% LUC	100	105	117	100

+ : (observed repeat value/initial value) $\times 100$

Table 3. Simple regression analysis for the prior-mixing effec

Dependent variable+	Independent Variable	n	Regression		Interpretation	|r| ++ SD
			Coeff.	SE		
WBC	No.of mixing	42	.0019	.0025	Stable	
RBC	No.of mixing	42	-.0011*	.0004	Decrease	.0012
PLt	No.of mixing	42	.0001	.0017	Stable	
Hgb	No.of mixing	42	.0001	.0006	Stable	
Hct	No.of mixing	42	-.0027**	.0006	Decrease	.0020
MCV	No.of mixing	42	-.0023**	.0003	Decrease	.0024
MCH	No.of mixing	42	0.008	.0007	Stable	
# neu	No.of mixing	42	.0019	.0025	Stable	
% neu	No.of mixing	42	.0001	.0006	Stable	
# lym	No.of mixing	42	.0017	.0030	Stable	
% lym	No.of mixing	42	-.0002	.0026	Stable	
# mono	No.of mixing	42	.0055	.0085	Stable	
% mono	No.of mixing	42	.0024	.0063	Stable	
# eo	No.of mixing	42	.0015	.0070	Stable	
% eo	No.of mixing	42	-.0016	.0054	Stable	
# ba	No.of mixing	42	.0142	0081	Stable	
% ba	No.of mixing	42	.0196*	.0086	Increase	.0011
# luc	No.of mixing	42	.0076	.0096	Stable	
% luc	No.of mixing	42	.0037	.0086	Stable	

+ : % change, (observed repeat value/initial value) $\times 100$, ++ : Ratios of rate of change (r = regression coefficient) * : $p < .05$, ** : $p < .01$

Table 4. Summary of evaluation of prior-mixing effect

Evaluation		No. of parameter (%)
Stable		15 (78.9)
Unstable		4 (21.1)
	Increased	1 (5.3)
	Decreased	3 (15.8)
Total		19 (100.0)

Table 5. Coefficients of Variation (13 replications)

Parameters	Pt. I		Pt. II		Pt. III	
	$\bar{X}$	CV%	$\bar{X}$	CV %	$\bar{X}$	CV%
WBC ($\times 10^3$/ul)	6.22	3.58	12.68	1.18	8.52	2.35
RBC ($\times 10^6$/ul)	4.68	.85	3.88	1.03	3.73	.54
PLt ($\times 10^3$/ul)	251	3.35	212	2.77	149	2.68
Hgb (9/ul)	13.0	.69	11.8	.59	10.8	.74
Hct (%)	38.9	1.16	35.1	1.42	33.1	1.27
MCV (fL)	83.2	.55	90.5	.66	88.8	1.07
MCH (pg)	27.2	1.01	30.3	.86	28.9	.97
Absolute no. of WBC diff. ($\times 10^3$/ul)						
Neutrophils	3.17	3.79	9.68	1.14	6.81	2.64
Lymphocytes	2.20	3.64	2.19	2.74	.91	5.49
Monocytes	.37	10.81	.49	6.12	.48	8.33
Eosiniphils	.27	7.41	.11	9.09	.14	7.14
Basophils	.08	12.50	.04	25.00	.06	16.67
LUC	.13	7.69	.17	17.65	.12	8.33
% of WBC diff.						
Neutrophils	50.9	1.38	76.4	.48	80.0	.98
Lymphocytes	35.4	1.98	17.3	2.25	10.7	4.67
Monocytes	5.9	8.64	3.8	6.58	5.7	9.82
Eosinophils	4.4	7.73	1.6	7.50	.9	11.11
Basophils	1.2	13.33	.6	16.67	.3	16.67
LUC	2.1	8.57	1.4	11.43	1.3	16.15

Table 6. Comparison of coefficients of variation

Parameters	Bollinger, et al (1987)+		Kershaw, et al (1987)+		
	x (n=20)*	CV%	n*	x	CV%
WBC ($\times 10^3$/ul)	8.0	2.1	7	7.4	2.3
RBC ($\times 10^6$/ul)	4.0	0.8	7	4.7	0.6
PLt ($\times 10^3$/ul)	255	2.2	5	228	2.7
Hgb (g/dl)	12.9	0.7	8	13.8	0.9
Hct (%)	-	-	5	41.0	1.2
MCV (fL)	98	0.8	11	85	1.1

+ : Technicon H*1 * : No. of replications

significant changes in RBC, Hct, MCV, and % of basophils. The Hct is measured indirectly as the product of the ×red cell count in automated instruments[3]. Our study results reflect a consistent relationship among RBC, Hct, and MCV.

The basophils are counted by the basophil/lobularity method in the Technicon System[4,5,6]. The coefficients of variation were 13.3～16.7% for % of basophils and 12.5～25.0% for the absolute number of basophils (Table 5). Even though the slope of % change of basophils % had increased significantly ($p<.05$, Table 3), this result reflects not only real change but also variation of the instrument itself.

We concluded that the RBC, Hct and MCV must be considered and monitored when the same specimen tube is run several times consecutively for the purpose of instrument evaluation. According to this study, approximatly 600 times cumulative mixings (tube inversions) except a few observations made 2% of decreasing changes form the initial value for RBC (Fig. 1-2), Hct (Fig. 2-2). and MCV (Fig. 3-2).

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= 국문초록 =

자동혈구분석기(Technicon H*2)에 의한 혈액검체의 측정전 Mixing 횟수가 검사결과에 미치는 영향에 관한 평가

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신 인 순 · 양 용 석

자동혈구분석기(Technicon H*2)에 의한 혈액검체의 측정전 mixing 횟수가 검사결과에 미치는 영향을 평가하기 위하여 19가지 parameter를 대상으로 초기측정치에 대한 반복측정치의 변화율을 종속변수로 하여 단순회귀분석을 실시하였다. Mixing횟수가 증가할수록 RBC, Hct, MCV는 통계적으로 유의하게 ($p < .01$) 감소하였고, 호염기구 백분율은 유의하게 ($p < .05$) 증가하였으므로 평가된 19가지 parameter의 21%인 4가지 검사항목에서 변화가 있었다.

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