

EVALUATION OF THE CONTRAVES AUTOLYZER 801

YC LIM DipMLT, SK CHEONG MBBS, FRCP. JA JOYCE DipMLT, O AINOON MBBS, DCP, V JEYASEKERAN DipMLT* and N MENAKA MBBS, FRCPA*

*Division of Haematology, Faculty of Medicine, National University of Malaysia and *Clinical Diagnostic Laboratories, University Hospital, Kuala Lumpur.*

Summary

The Contraves Autolyzer 801 is a 20 parameter cell counter that operates on the principle of impedance. This evaluation study found that the within-batch precision for 5 of its parameters – WBC, RBC, Hb, Hct and Plt – had coefficients of variation below 5%, while the counts for red cell parameters were reproducible for up to 3 days. The analyser exhibited good linearity for all the parameters. When compared with 2 other counters – the Coulter S-Plus and the Coulter M530, some degree of proportional and constant bias due to unsuitable calibration were detected in the WBC, Hct and Plt parameters. Otherwise it showed generally good correlation in all the parameters and also with the manual PCV method. Carry over was negligible in the red cell parameters. The Contraves Autolyzer 801 is easy to operate and readily accepted by operators.

Keywords: Haematology analyser, cell counter, evaluation study.

INTRODUCTION

Over the years, different types of electronic counters have been developed for the automatic counting of blood cells. Total red cell counts have become increasingly more reliable and reproducible, thus increasing their diagnostic value. Early instruments only provided basic information on the blood sample such as the total white cell count (WBC), total red cell count (RBC) and the haemoglobin (Hb) level. Later instruments could also provide inter-related red cell parameters, namely, the mean cell volume (MCV) and packed cell volume (PCV). The more sophisticated automatic counters today can measure up to 20 parameters of a single sample of whole blood. They can also provide differential percentages and absolute counts for white cells, total platelet count (Plt), mean platelet volume (MPV) and packed platelet volume (Pct) as well as produce histograms on the distributions of white cells, red cells and platelets.

The Contraves Autolyzer 801 is an example of these powerful modern cell counters. It operates on the principle of impedance and counts, using a series of valves and electrodes, the number of cells in a fixed volume of sample.

An evaluation of the above counter was carried out in the Division of Haematology, Department of Pathology, Universiti Kebangsaan Malaysia, based on the report prepared by BCSH General Haematology Task Force.¹

However this was not a "full evaluation" due to constraints of time and the availability of suitable samples.

The aims of this study were to:

- (i) determine the within-batch and day-to-day precision of the instrument under local conditions.
- (ii) determine the linearity of 3 cell parameters – WBC, RBC and Plt.
- (iii) determine carryover on the RBC and Hb parameters. However, for lack of suitable samples, this study was not carried out on the WBC and Plt.
- (iv) compare the values of 6 parameters – WBC, RBC, Hb, haematocrit (Hct) MCV and Plt – with the values from the Coulter S-Plus, a 18-parameter cell counter.
- (v) compare the values of 5 parameters determined – WBC, RBC, Hb, Hct and MCV – with the results from the Coulter M530, a 7-parameter analyser.
- (vi) compare the Hct values with those obtained by a manual pack cell volume micro-method.

MATERIALS AND METHODS

Preparation of samples

Whole blood from normal individuals were used as normal-value samples. High RBC, Hb-value samples were prepared by packing normal whole blood² and low-value samples by diluting normal blood with autologous plasma. The buffy coat of a normal blood sample was

collected and resuspended in saline to give a high WBC count. A sample from a patient with acute myeloid leukaemia (AML) was also used. To prepare platelet concentrate for the linearity study, platelet-rich plasma with aspirin added to prevent clumping was centrifuged at 4000 rpm and the platelet button obtained was resuspended in saline.³ It was then diluted to different proportions with saline.

Within-batch precision

At least 8 samples each of normal, high and low RBC and Hb levels were aspirated to obtain the means, standard deviations and coefficients of variation (CV)⁴ of their respective parameters. For the high WBC and platelet precision study, WBC and platelet concentrates were first prediluted with iso-osmol (the diluent for the counter, dilution 1:150) before being counted for 8 and 10 times respectively.

Day-to-day precision

Samples with normal as well as high RBC and Hb levels were sampled once a day for 4 consecutive days. The counts for each day were compared with the values of their respective within-batch means and standard deviations.

Linearity study

To test the linearity of the red cell parameters, packed red cells were diluted to different proportions with saline. For WBC and platelet parameters, a whole blood sample from a patient with AML and platelet concentrate were diluted to different proportions with saline before further dilution with iso-osmol (dilution 1:150) and aspiration directly into the analyzer.

Carryover study

Samples with both high RBC, Hb and low RBC, Hb values were used. For each parameter, the high value control was aspirated 3 times giving values a_1 , a_2 and a_3 , followed immediately by 3 aspirations of low control to give b_1 , b_2 and b_3 .

Carryover percentage was calculated by: $100(b_1 - b_3)/(a_3 - b_3)$. For low to high carryover, the order of aspiration was reversed. A carryover study on WBC and Plt parameters was not conducted as there were no suitable blood samples available on the day of study.

Comparison study

WBC, RBC, Hct, MCV and Plt counts of 124 cases of "routine blood samples", representing a general cross-section of samples of different diseases⁵ seen in a haematology laboratory were compared with those from the Coulter M530 (the counter in use in the Division of Haematology, UKM) and the Coulter S-Plus (from the University Hospital, Kuala Lumpur). Both Coulter counters were calibrated using the Coulter 4C controls while the Contraves Autolyzer 801 was calibrated using the Contraves controls. The results from the Contraves Autolyzer 801 were plotted against those from the Coulter M530 and Coulter S-Plus.

A comparison study was also carried out between the Hct values of the Contraves Autolyzer 801 and those obtained by manual micro method.⁶ Regression analysis was carried out and the coefficients of correlation calculated.^{7,8}

RESULTS

Tables 1 and 2 show the results of the within-batch and the day-to-day precision studies. Generally, the within-batch CV's of the red cell parameters were less than 2%, those for WBC about 3% while those for Plt were about 5%. Table 2 shows that the counts for red cell parameters were reproducible for up to 3 days while those of the WBC only for 1 day.

Table 3 shows the carryover percentages of RBC and Hb parameters from low to high and from high to low controls. Carry-over was generally negligible with the percentages below 2%.

The Contraves Autolyzer 801 exhibited good linearity within the ranges tested for all the 5 parameters. Table 5 shows that the measuring ranges stipulated by the manufacturers are similar to those established in the linearity study. There was good correlation of the results with those of the 2 Coulter counters as well as with the manual PCV. Tables 4a and 4b show the correlation coefficients and the linear regressions for the parameters in the comparison study. Figures 1 and 2 are examples of the scatter plots obtained for each of the parameters.

DISCUSSION

The Contraves Autolyzer 801 showed good precision for normal, high and low control samples. It also showed good linearity in

TABLE 1
WITHIN-BATCH PRECISION

Parameter	$\bar{x}$	S.D.	CV%	Parameter	$\bar{x}$	S.D.	CV%
WBC H	144.4	4.35	3.0	RBC H	8.77	0.10	1.1
N	6.8	0.21	3.0	N	4.76	0.04	0.9
				L	1.28	0.02	1.4
Plt H	610	26.42	4.3	Hb H	25.1	0.48	1.9
N	265	6.60	2.5	N	13.9	0.20	1.4
L	74	3.44	4.7	L	3.2	0.05	1.7
H = high N = normal				Hct H	77.4	1.17	1.5
L = low				N	43.5	0.37	0.9
				L	11.4	0.11	1.0

*CV's for all parameters were less than 5% for high, normal and low samples.

TABLE 2
DAY-TO-DAY PRECISION

Parameter	No. of days (n)	Mean value for nth days	Within-batch mean $\pm$ 2SD
WBC	1	6.7	6.8 $\pm$ 0.42
RBC H	4	8.67	8.77 $\pm$ 0.20
N	4	4.74	4.76 $\pm$ 0.08
Hb H	3	25.2	25.1 $\pm$ 0.56
N	3	14.1	13.9 $\pm$ 0.40
Hct H	3	78.2	77.4 $\pm$ 2.34
N	3	45.0	43.5 $\pm$ 0.74

* Mean value of counts for different parameters for n days were compared to their respective within-batch means $\pm$ 2 std deviations.

TABLE 3
CARRYOVER STUDY

Parameter	Carryover %	
	High to low	Low to high
RBC	0.1	1.1
Hb	1.0	0

TABLE 4a
COMPARISON STUDY

Autolyzer 801 vs Coulter S-Plus		
Parameter	r	Regression
WBC	0.98	$y = 0.84x + 0.16$
RBC	0.97	$y = 1.00x + 0.20$
Hb	0.98	$y = 1.06x - 0.44$
MCV	0.98	$y = 1.04x + 1.05$
Hct	0.97	$y = 1.01x + 3.10$
Plt	0.97	$y = 0.84x + 2.51$

all the parameters tested. Although the results are comparable to those of the Coulter Systems ($r \geq 0.94$), the regressions showed significant bias in some of the parameters.' When compared with the Coulter M530, constant bias were detected in MCV (4.72 fl), Hct (1.31%) and manual Hct (1.32%) while with the Coulter S-Plus there was significant proportional bias in the WBC count ($= -16\%$), constant bias in Hct (3.10%) and both proportional bias ($= -16\%$) and constant bias ($2.5 \times 10^9/l$) in Plt..

Since bias generally originates from poor calibration, the Autolyzer 801 was recalibrated using the Contraves' calibrants. However, the same bias when compared to the Coulter M530 were still detected. The Autolyzer was next calibrated with a sample of normal whole blood whose values were predetermined by repeated sampling ($n = 11$) on the Coulter M530. This calibration eliminated bias and suggested that the Contraves' calibrants may, in some way, differ from blood cells and hence are not suitable calibrants for this system.

TABLE 4b
COMPARISON STUDY

Autolyzer 801 vs Coulter M530		
Parameter	r	Regression
WBC	1.00	$y = 0.94x - 0.51$
RBC	0.97	$y = 1.02x + 0.04$
Hb	0.99	$y = 0.98x + 0.15$
MCV	0.97	$y = 0.95x + 4.72$
Hct	0.97	$y = 1.00x + 1.31$
Hct (manual)	0.94	$y = 1.04x + 1.32$

Although both the Coulter S-Plus and the Autolyzer 801 provided differential analyses, a comparison on this was not done as the results were incomplete for most of the samples. Comparison was also not done on the differential results with a manual differential count as it 'was felt' that a 100-200 cell manual count is not comparable to that of the system which counts all the cells in a sampling.

Daily start-up begins with the automatic checking of the electronic components of the system as well as the performance and accuracy of the dilutor. This is followed by an automatic FILL/CHECK cycle which provides a printed report on the status of the Autolyzer at start-up. FILL/CHECK can be carried out easily at any time between runs to assess the status of the System. Total start-up time is 2 minutes.

Maintenance of the Autolyzer 801 involves flushing with Digiclean solution after each batch run as well as repeated washings with Digipur (a special cleaning solution) and Digiclean before shutdown for the day. Protein build-up was not encountered during the testing period.

TABLE 5
LINEARITY STUDY: RANGES ESTABLISHED IN THE STUDY
COMPARED TO THOSE CLAIMED BY THE MANUFACTURER

Parameter	Linearity range established	Manufacturer's range
WBC	0 - 270 $\times 10^9 /l$	0 - 200 $\times 10^9 /l$
RBC	0 - 8.6 $\times 10^{12} /l$	0 - 10 $\times 10^{12} /l$
Hb	0 - 250 g/l	0 - 250 g/l
Hct	0 - 78 %	Not available
Plt	0 - 5151 $\times 10^9 /l$	0 - 5000 $\times 10^9 /l$

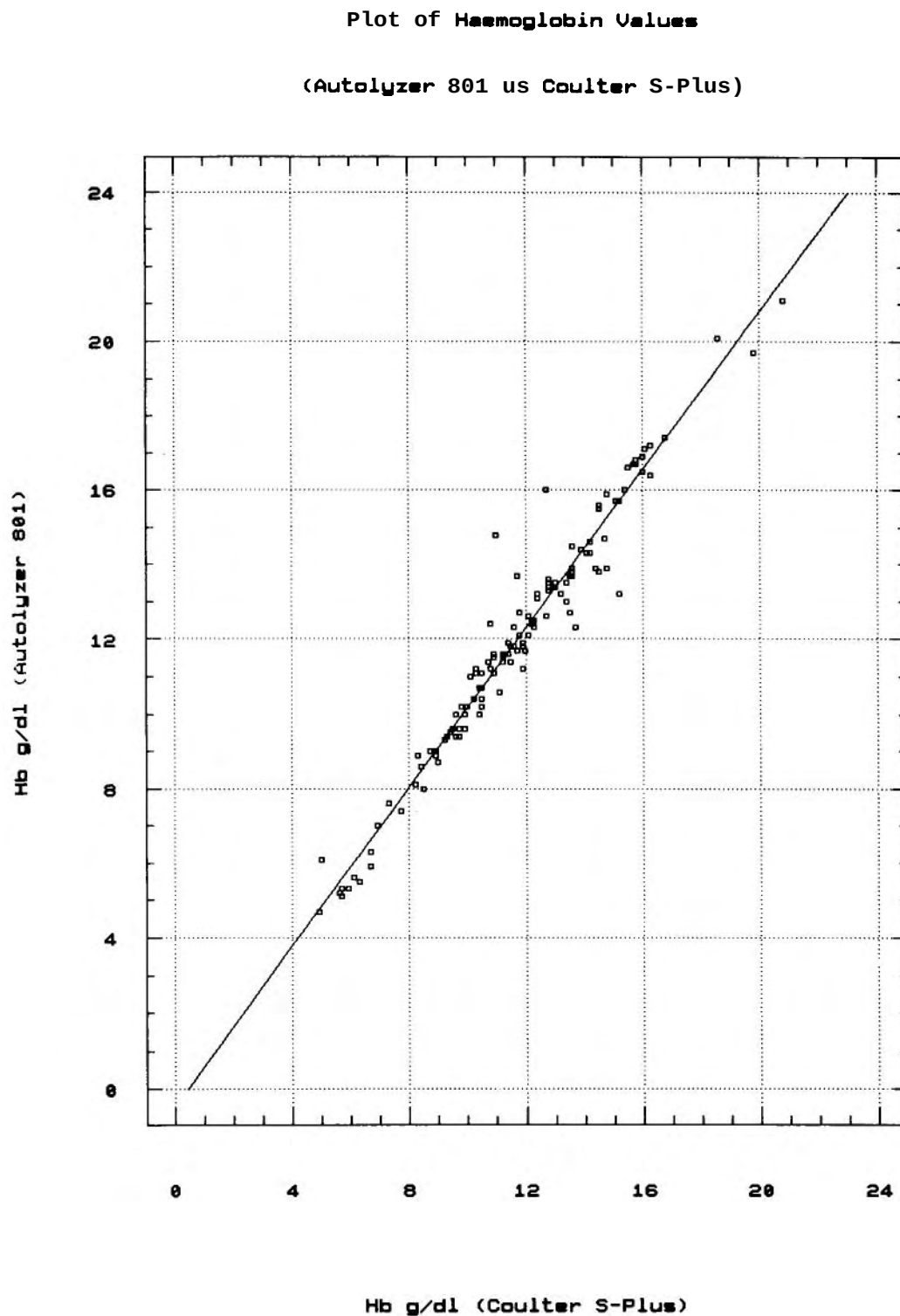


FIG. 1: Scatter plot of haemoglobin values determined by the Contraves Autolyzer vs the Coulter S-Plus. $n = 127$, $y = 1.06x - 0.44$, $r = 0.98$.

Plot of Haemoglobin Values

(Autolyzer 801 vs Coulter M530)

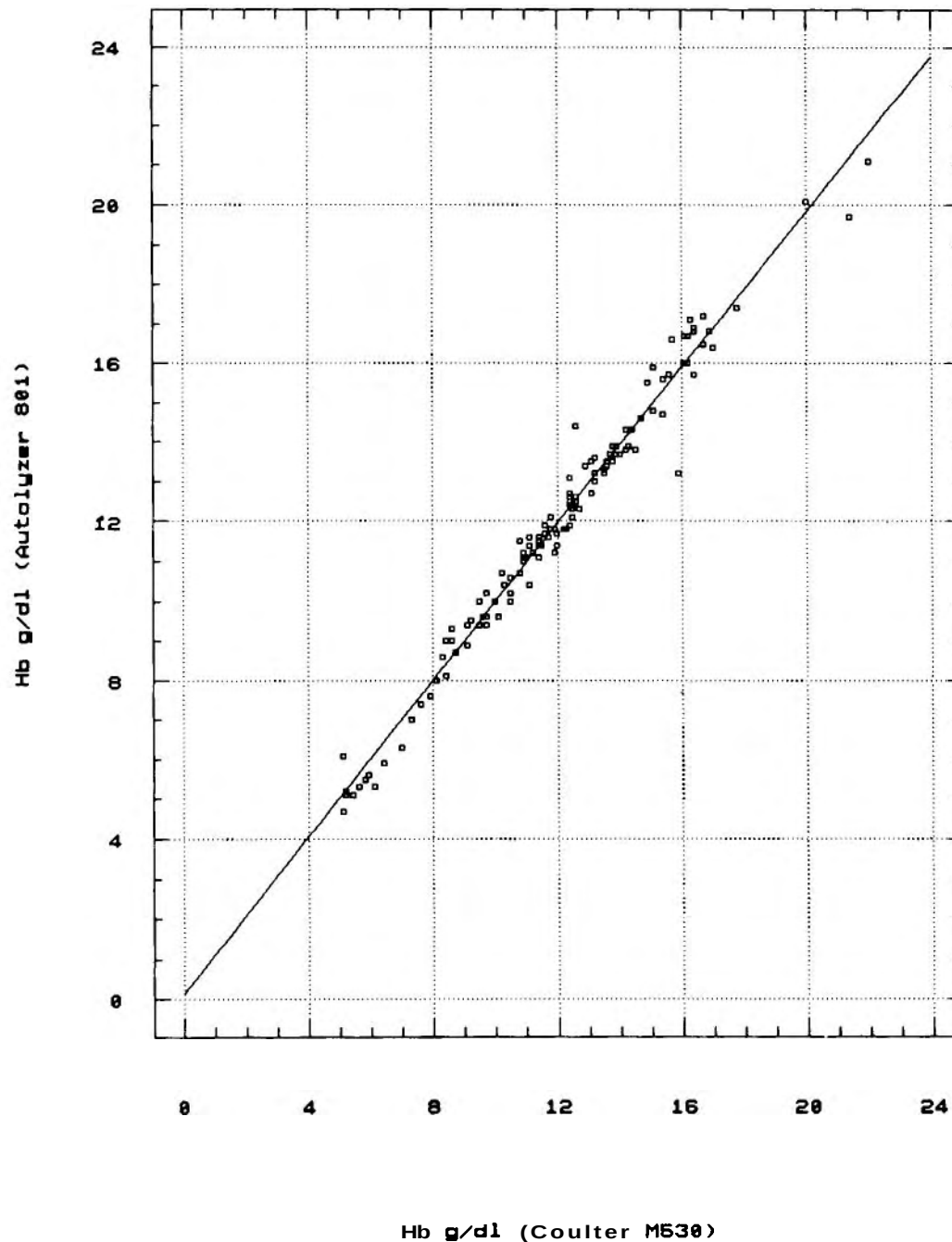


FIG. 2: Scatter plot of haemoglobin values determined by the Contraves Autolyzer 801 vs the Coulter M530. $n = 127$, $y = 0.98x + 0.15$, $r = 0.99$

For trouble shooting, the printer will, on keyboard command, print out the faults diagnosed with debugging recommendations which are comprehensive and easy to carry out. No error was encountered during the test period.

To test the ease of operation, all 14 technical staff of the Division were requested to select and run 6 tests from 23 tests available using the Contraves Autolyzer 801. The instrument was readily accepted by all the operators.

ACKNOWLEDGEMENTS

We wish to thank Kemajuan Abadi Sdn. Bhd. for loaning us the instrument and providing the reagents for this study.

We also wish to thank the staffs of the Haematology Division, Department of Pathology, UM and the Division of Haematology, Department of Pathology, UKM for their cooperation in making this study a success.

Lastly, we also thank Dr. Chong Siew Meng for statistical advice on the analysis of the data.

REFERENCES

1. A Summary of Recent British Evaluations of Automated Blood Cell Counters: Report prepared by BCSH General Haematology Task Force, 1984: 1–26.
2. Dacie JV, Lewis SM. Practical Hematology. 6th ed. Churchill Livingstone, 1984: 16–7.
3. Dacie JV, Lewis SM. Practical Hematology. 6th ed. Churchill Livingstone, 1984: 382.
4. Strike PW. Medical Laboratory Statistics. Wright PSG, 1981: 73–95.
5. Shinton NK, England JM, Kennedy DA. Guidelines for the evaluation of instruments used in haematology laboratories. *J Clin Pathol* 1982, 35: 1095–102.
6. Dacie JV, Lewis SM. Practical Hematology. 6th ed. Churchill Livingstone, 1984: 32–4.
7. Strike PW. Medical Laboratory Statistics. Wright PSG, 1981: 173–9.
8. Mack C. Essentials of Statistics for Scientists and Technologists. Heinemann Educational Books Ltd. London. 1966: 116–25.