

First comparative evaluation of leucocyte differential and reticulocyte count between Abbott Cell -DYN 3700 and Beckman-Coulter Gen S

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Abstract. Background: The Cell-Dyn 3700 has been launched with the same CBC and flagging performance specifications as the Cell-Dyn 3500. The primary goal of our evaluation was to evaluate the new algorithms of its reticulocyte method and the performance capabilities in comparison to our routine analyser (Coulter Gen S).

Methods: The WBC differential and reticulocyte counts were evaluated on the new Cell-Dyn 3700 instrument (Abbott Laboratories) on 758 normal and 727 pathological samples.

Results: When compared to the routine laboratory analyser (Gen S, Beckman Coulter) in the normal population, similar WBC differential results were found. In contrast, we noted some differences within the pathological samples. A bias of 0,643 Giga/L WBCs (white blood cells) was found between Cell-Dyn 3700 and Gen S measurements in samples with WBC counts of less than 3 Giga/L (no. 89). For WBC counts less than 1 Giga/L the Cell-Dyn 3700 residual plots of linear regression analysis provided the closer fit to its regression

line. FWBC (Fragile White Blood Cells) flag on the Cell-Dyn 3700 is a good indicator of the osmotic fragility of lymphocytes from CLL (chronic lymphocyte leukaemia). This highlights the limited linearity of impedance methods in these situations at a level of 100 Giga/L and above. The Cell-Dyn 3700 KWOC (Corrected White Optical Count) reported value is higher than the impedance and microscopy counts.

This study also evaluated the new Cell-Dyn 3700 reticulocyte algorithm method. In the evaluation of 400 random samples, the Cell - Dyn 3700 analyser showed a significant bias compared to Gen S (0,4 %) in a population ranging from 0,37 to 8 % for Gen S and 0,17 to 9,44 % for Cell-Dyn 3700 (with the exception of one value at 14,7 %).

Conclusions: These results suggest a clear improvement of the reticulocyte methodology on the Cell-Dyn 3700.

Key words: Cell-Dyn 3700, Reticulocytes, Fragile lymphocytes, WBC differentiation, Gen S.

Introduction

The Cell-Dyn 3700 has recently been launched with the same CBC and flagging performance specifications as the Cell-Dyn 3500¹⁻⁵. The reticulocyte algorithms have been modified, the veterinary differential has been refined and veterinary applications have been extended (but these latter modifications have not been considered in this study). Instrument hardware modifications (e.g., integrated sample loader) and upgraded data processing capabilities have been implemented.

The primary goal of our evaluation was to evaluate the new algorithms of its reticulocyte method. In this study, we will also consider the Cell-Dyn 3700 performance capabilities in comparison to our routine analyser (Coulter Gen S), and analyse the meaning of the FWBC flag in abnormal lymphoid cell disorders (particularly in CLL).

Materials and Methods

One-thousand eight-hundred and sixty-nine K₃ EDTA blood samples were collected from the main laboratory of the general hospital including a haematology department. In this setting, a high proportion of the laboratory workload comes from patients undergoing oncology treatment and chronic lymphocytic disorder.

Samples were analysed on the Abbott Cell-Dyn 3700 immediately after undergoing routine analysis on the Gen S (Beckman-Coulter). Instrument maintenance, calibration and quality control were all performed in accordance with the manufacturer's recommendations. There was a prior harmonization of the two systems.

To evaluate the linearity of WBC instrument and platelet measurements, 5 samples were diluted using instrument diluents immediately before counting.

Appropriate serial dilutions (10 to 100, each in triplicate) were made to cover the overall WBC and platelet ranges. Results were converted to graph form for visual examination and tested further in accordance with ICSH recommendations⁶.

In order to assess the automated leucocyte differentiation and flagging systems, we performed microscopic differential counts on 400 cells (performed by two investigators) on May-Grunwald-Giemsa stained films in accordance with NCCLS guideline H20-A⁷.

All the qualitative flagging system has been taken in our study on the two analysers, in particular we considered INE 2 for immature granulocytes, blasts, variant lymphs, large platelets, small platelets, giant platelets and platelet clumps (Gen S Beckman-Coulter), NRBC for nucleated red blood cells.

IG flag, Blasts, Variant lymph, FWBC, URI (Upper Region Interference) for large or giant platelets and aggregates, NRBC and NWBC (Non White Blood Cell) (Abbott Cell-Dyn 3700).

Samples were divided in two categories:

The "Diff normal" population, with no abnormal cells detected by microscopy and no diagnosed haematological disorder (no. 758), was used to compare the automated leucocyte differentiation between the two instruments and to estimate the flagging specificity.

The "Diff pathological" population, with hematological disorders and abnormal cells identified by microscopy, (no. 727), was used to evaluate the flagging sensitivity. Microscopy was considered positive if any abnormal WBC cells were present or if platelet morphology was abnormal (band cells are not estimated in our laboratory practice).

All WBC and platelet instrument flagging were considered and flagging assessment was performed following Galen and Gambino method⁸. Hematology outpatients and pediatric samples have systematic slide reviews due to the fact that Gen S automated leucocyte differentials are not routinely reported. Consequently, these populations are not included in our flagging evaluation and performance, but they have been included to compare the automated leucocyte differentiation.

400 samples were randomly collected for evaluation of the Cell-Dyn 3700 new reticulocyte algorithms. Manual reticulocyte counting was performed according to 1993 NCCLS standards H44-A⁹ and reticulocytes enumeration by Gen S and Cell-Dyn 3700 were performed according to the operations manual provided by the manufacturers. Both instrument use new methylene blue to stain the blood cells. The stained reticulocytes are recognized and isolated from other cells by the VCS technology (Volume, Conductivity, and light Scatter) on the Gen S and by optical light scattering on Cell-Dyn 3700. Discrepancies in the reticulocyte absolute values obtained with the Gen S and Cell-Dyn 3700 were considered if they fell in the different pre-defined groups: £18-20 Giga/L, between 18-20 and ≥ 90-100 Giga/L and >90-100 Giga/L.

We looked for potential interferences due to the presence of platelet aggregates or macroplatelets, rou-

leaux, Howell-Jolly bodies, basophil stippings and malaria parasites on the smear.

Results

Overall WBC correlation and analysis of discrepant results (no. 1869)

The linear regression for Gen S and Cell-Dyn 3700 WBC data was found to be $R^2=0,89$ (Cell-Dyn 3700 = Gen S - 0,5). Fourty four discrepant results were noted:

- 23 samples triggered a FBWC flag and the KWOC (Corrected White Optical Count) was reported on the Cell-Dyn 3700. 17 cases of these 23 cases were characterized by a lymphoid pathology (1 ALL, 1 prolymphocyte leukaemia, 15 CLLs). The WIC (White Impedance Count) from the Cell-Dyn 3700 was similar to Gen S Impedance Count (excluding WBC values higher than 100 Giga/L) and microscopy WBC estimates. The KWOC gave higher WBC counts in these cases.
- 17 discrepancies within leucopenic patients (9 FWBC flags).
- 1 discrepancy with 23 % NRBCs.
- 3 discordant samples from a cirrhosis patient, a patient with eosinophilia and a severe iron deficiency anaemia.

Analysis of WBC differential in leucopenia (no. 89)

In the leucopenic samples assessment (<3 Giga/L), we observed no difference ($p=0,96$) in terms of mean comparisons (Cell-Dyn 3700 mean = Gen S mean = 1,115) between the 2 instruments. The correlation remains good ($R^2 = 0,97$; Cell-Dyn 3700 = 1,06 Gen S - 0,06). Nevertheless, Bland and Altman method in WBC low range (<3 Giga/L) showed a bias between the 2 instruments (- 0,643) (fig.1).

Overall linearity (Cell-Dyn 3700: from 0,03 to 26,6 Giga/l) did not show any significant difference between the 2 instruments. The linearity was excellent, actual values were similar to expected values, with an insignificant intercept and a slope near to 1 but the Gen S regression linear model demonstrated higher

Figure1: In the low WBC range (<3 Giga/L), the Bland and Altman plot demonstrated a bias between the Cell-Dyn 3700 and GenS.

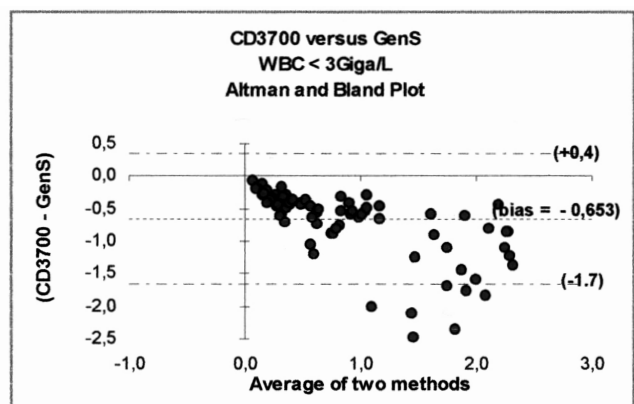
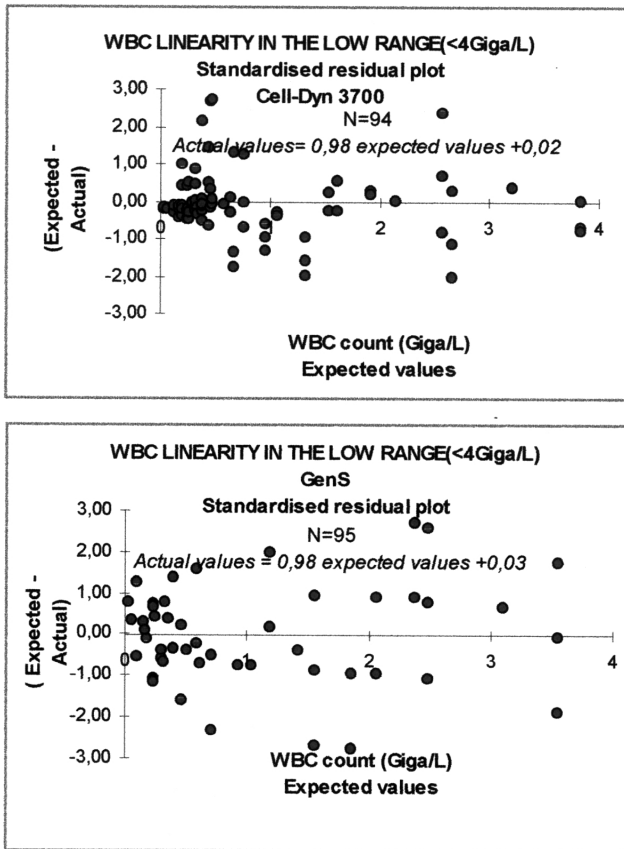


Figure 2: WBC linearity study: The lack of fit with the linear regression line is more accentuated with the GenS than the Cell-Dyn 3700 in the low WBC range (<1Giga/L).

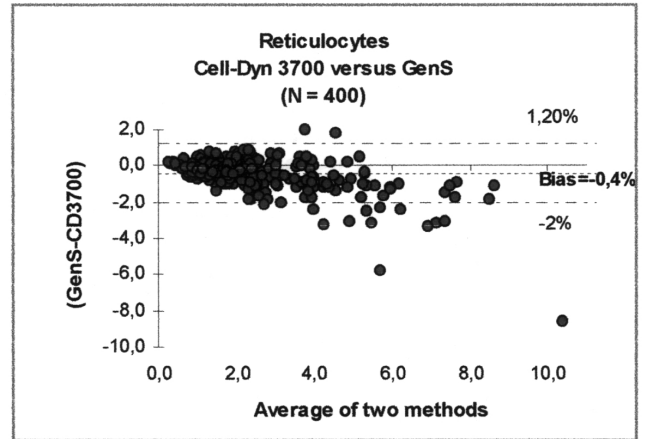


differences with WBC values (<1 Giga/L) (fig. 2). The actual values are less accurate than Cell-Dyn 3700's in this range. The 99 % confidence interval of the neutrophil and lymphocyte absolute values are smaller with the Cell-Dyn 3700 when compared to microscopy in 89 leucopenic patients (<3 Giga/L) (table I).

Analysis of the differential in the " Diff normal" population (no. 758)

The linear regression coefficients are better for the two instruments than for the instruments and microscopy individually. R² coefficients have no statistical significant difference for all parameters within this normal population.

Figure 3: There is a bias of (-0,4% or - 12,4 Giga/L) between Cell-Dyn 3700 and GenS reticulocyte estimates (n= 400). This bias becomes significant when referred to as reticulocyte levels over 3% or 100 Giga/L.



Platelets and RBC parameters in the overall population (no. 1869)

The platelets count through the overall range (1,17 to 1242 Giga/L with Cell-Dyn 3700 and 1 to 1130 Giga/L with the Gen S) show very good agreement between the 2 instruments (R² = 0,9; Cell-Dyn 3700 = Gen S - 0,5).

The linearity studies (Platelet actual values range from 1,28 to 382 Giga/L for the Cell-Dyn 3700 and 2 to 364 Giga/L for the Gen S) demonstrated identical slopes for both instruments (0,99), an intercept of 0,03 was found for the Gen S and 0,43 for the Cell-Dyn 3700. In the low end (<50 Giga/L), a similar pattern of regression line and residual plots was observed.

Linear correlation coefficients (R²) for Cell-Dyn 3700 and Gen S were 0,99, 0,99 and 0,95, respectively, for RBC, HGB and MCV.

Reticulocytes (no. 400)

The reticulocyte percentages of 400 blood samples ranged from 0,17 % to 9,44 % (one value at 14,7 %) when estimated by the Cell-Dyn 3700 vs Gen S 0,37 to 8 %. The mean reticulocyte percentages obtained from Gen S and Cell-Dyn 3700 were 2 % and 2,4 % respectively. The bias observed between the two me-

Table I: 99% confidence intervals for neutrophil, lymphocyte and monocyte populations among 89 leucopenic samples (<3 Giga/L) for Cell-Dyn 3700 and Gen S. Microscopy absolute values are derived from the relative microscopy estimates and the Cell-Dyn 3700 WBC counts.

		Neutrophil #	Lymphocyte #	Monocyte #
Cell-Dyn 3700 – Gen S	Lower Limit	-0,164	-0,343	-0,087
	Bias	0,004	-0,018	0,021
	Upper Limit	+0,172	+0,307	+0,129
(Cell-Dyn 3700 - Microscopy)	Lower limit	-0,171	-,0182	-,0117
	Bias	0,012	0,001	0,015
	Upper Limit	+0,147	+0,183	+0,148
Gen S – Microscopy	Lower Limit	-0,238	0,263	-0,124
	Bias	-0,015	0,019	-0,006
	Upper Limit	+0,208	+0,3	+0,112

thods was not significant (0,4 %) through the overall range. Nevertheless, we noted that the agreement decreased when the reticulocyte percentages were abo-

Table II: False negative flags and repartition by abnormality types (minor abnormalities: immature granulocytes < 1% and activated lymphocytes < 1%; NRBCs: nucleated red blood cells).

	CELL DYN 3700	Gen S
False negative flags excluding minor abnormalities	66 / 727 (9,1%)	105 / 727 (14,4%)
Macrothrombocytes	23	26
Blasts (<0,5%)	3	5
Blasts (<0,5% - 4 %)	0	3
Blasts (> 1%)	2	1
NRBCs	7 (< 1% NRBCs)	19 (0,5 - 5% NRBCs)
Immature Granulocytes (1 - 3%) 23	29	35
Minor abnormalities	266 / 727	340 / 727
False negative flags including minor abnormalities	332 / 727 (46%)	445 / 727 (61%)

ve 3 or 4 % (fig.3). The bias reaches (- 1 %) for reticulocyte levels over 3 %.

According to our clinical criteria, 14 % (i.e., 56/400) of discrepant results between instruments were identified. Half of these samples contained Howell-Jolly bodies, basophilic stipplings or platelet abnormalities (aggregates or macrothrombocytes).

Macrothrombocytes or platelet aggregates and RBC inclusions were found in 16 cases out of the 23 higher discrepant results. Out of 33 low discrepant results, 7 were found to have macrothrombocytes. A number of samples still showed discrepancies in the reticulocyte results between the 2 instruments without clear explanation.

We observed within these 56 discrepant results that Cell-Dyn 3700 was closer to microscopy findings than Gen S (Cell-Dyn 3700, bias = 0 % and 99 % confidence interval (CI) between 3,5 and 3,6 %; Gen S, bias = 1,1 % and 99 % CI between 3,9 and 6,1 %).

Flagging system

65 % (i.e., 40/62) of Cell-Dyn 3700 false positive flags were identified as Band Flags. Gen S NRBCs detection rate was lower than Cell-Dyn 3700's. Smudge cells were well detected on Cell-Dyn 3700 (16 out of 18 cases). Gen S flagging system was not evaluated for this population (Gen S Differential and flags are not reported for patients coming from Hematology unit). Cell-Dyn 3700 FWBC flag was also triggered in 9 samples coming from leucopenic patients undergoing chemotherapy.

Both instruments were unable to appropriately flag macrothrombocytes. (Table II)

Discussion

This study reports and compares results of the new Cell-Dyn 3700 with our routine hematology analyser (Gen S) in *Diff normal* population from outpatient consultation and in *Diff pathological* population associated with routine hospital workload (haematology unit excluded).

In *Diff normal* population (no. 758), both instruments can be indifferently used. Note that the Cell-Dyn 3700 flagging system is less specific than the Gen S regardless of the flags nature. Band flag was triggered in absence of Cell abnormality (Band cells are not considered in our haematology practice).

In *Diff pathological* population, (no. 727), Cell-Dyn 3700 flagging system appears more sensitive than the Gen S specifically for NRBC detection. The Cell-Dyn 3700 alerted the operator of smudge cells presence in CLL samples. As CLL patients were part of the hematology unit, the flagging evaluation of the Gen S was not studied for this population.

In the Oncology population, patients with neutrophil counts less than 0,8 Giga/l are considered at greatly increased risk of infection and are separately nursed in isolation, with specific antibiotic and anti-fungal treatments. WBC and neutrophil count accuracy is paramount to evaluate the infectious risk. Within 89 leucopenic samples, Cell-Dyn 3700, when compared to microscopy, gave excellent WBC Diff precision and accuracy. These findings prove to be similar to those that have been extensively demonstrated for Cell-Dyn 3500^{2,3} in low WBC counts.

1 Giga/L is the lowest value of the working range of haematology analyzers using impedance principle as the Gen S² does.

Some authors have shown a 10% discrepancies in the WBC counts between impedance methods and the Cell-Dyn 3500 when WBCs are below 4,5 Giga/L and 20 % within WBCs below 1Giga/L². This low-end linearity of Cell-Dyn 3700 WBC Diff count can also be beneficial to an HIV population for CD4 lymphocyte subset enumeration and for leucodepletion monitoring³.

In CLLs: WBC count discrepancies between the two instruments were mainly due to CLLs. Lymphocytes from patients with CLL are typically fragile and morphologically appear as "smudge" cells. They probably are less resistant to stress or in process of "cell agony". This phenomenon has been long considered as an artefact and disregarded in the interpretation of blood film¹⁰. Nevertheless, it is known to create variations in the WBC count estimations according to the methodology used. The lymphocytes are destroyed by mechanical compression during smearing, while with the Cell-Dyn 3700 these cells are destroyed during hydrodynamic focusing and may fall below the dynamic threshold of WOC (White Optical Count). Therefore, they are potentially omitted from the reported WOC count. KWOC is usually reported in CLL patients on Cell-Dyn 3700 because the instrument is implementing a

mathematical correction according to the slope of WBC count rate. This count is higher than the impedance count reported by Cell Dyn 3700 and Gen S. In cases where FWBC alert is given, WIC count must be reported instead of KWOC on Cell-Dyn 3700.

WIC count seems to be more stable than KWOC although non-WBC particles may be included into the WBC counts. However, attention must be given to WBC counts in CLL around 100 Giga/L due to the limited linearity of impedance WBC >100 Giga/L.

During the automated WBC dilution process, the lymphocytes are in contact with a hypotonic solution (sheath); they swell and eventually lyse. Increased or decreased resistance to osmotic pressure of CLL cells was reported, according to various methodological approaches¹¹. In Cell-Dyn 3700 working conditions, there is apparently an increased osmotic fragility of B-lymphocytes from CLL. They are destroyed progressively during automated measurements and the Cell-Dyn 3700 generates a FWBC flag. Even if there is no clear explanation of this phenomenon, assumption is made that there is decreased membrane potassium permeability, a tendency to retain water and a failure to adapt to hypotonic solutions¹⁰. FWBC flag is present every time smudge cells are detected by microscopy, except in 2 cases, but does not seem to be affected by the number of lymphocytes present in peripheral blood. Even if this phenomenon is not clearly explained, FWBC flag suggests the presence of osmotic fragile cells typically in CLL. This flag is an additional parameter to diagnose altered lymphocyte conditions.

The result of the reticulocyte study highlighted that Gen S and Cell-Dyn 3700 both employing a new methylene blue stain, gave similar results and therefore can be classified in the same category of methods. These methods improved precision in comparison to routine reticulocyte manual counts (7,13 % for Cell-Dyn 3700 and 16 % for Gen S) even though the influence of interference on all reticulocyte methods is theoretically the same. Our results also suggest a clear improvement in the reticulocyte methodology of Cell-Dyn 3700 over Cell-Dyn 3500. In a recent publication, the correlation between Cell-Dyn 3500 and reticulocyte manual method was found unsatisfactory. The Cell-Dyn 3500 yielded consistently and significantly higher counts than both Gen S and manual methods within 170 random samples. The Gen S was considered comparable to the manual method and the light scattering measurement was incriminated¹². Monitoring of hematopoietic regeneration is typically undertaken using the absolute neutrophil count in combination with the platelet count, serial determinations of IRF and reticulocyte count in the early period post chemotherapy often gives an earlier indication of bone marrow regeneration. We found that the IRF derived from Gen S and Cell-Dyn 3700 showed an insignificant bias between methods but the R^2 value was not satisfactory enough ($R^2 = 0,28$) to use this parameter as an indicator of bone marrow regeneration. The best IRF agreement has been demonstrated between automated fluorescent methods such as Cell-Dyn 4000 and the Sysmex

R-series¹³. In conclusion, Cell-Dyn 3700 and Gen S may be equally used for WBC differentiation and reticulocyte enumeration in a normal population. These 2 automated reticulocyte counters can equally provide clinical results to evaluate decreased or increased erythropoietic activity in anaemia classification (regenerative or aplastic anemia). They may be less suitable for samples with reticulocytopenia (e.g. drug-induced aplasia, transplantation recovery, etc.) and IRF clinical utilisation. The Cell-Dyn 3700 has showed good performance in leucopenic samples and its superiority to the Gen S. In particular, the Cell-Dyn FWBC flag is a good indicator of the presence of fragile cells in CLLs.

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