CHAPTER 3: OBJECTIVES

From the above background and problems the following objectives were identified for the purpose of this study.

1. To determine the accuracy of the total white blood cell count of the Cell-Dyn 3500 by comparing it to established and commonly used instruments.

2. To determine the acceptability of the automated leukocyte differential count generated by the Cell-Dyn 3500 by comparing it to the reference method as described by the National Committee for Clinical Laboratory Standards.

3. To determine if the Cell-Dyn 3500 gives a linear relationship over the physiological range, and usually encountered pathological range of white blood cell counts.

4. To establish if the carry-over of the Cell-Dyn 3500 is clinically significant.

5. To determine if the Cell-Dyn 3500 operates with sufficient precision, by running the same sample a number of times.

CHAPTER 4: MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Instruments Used in the Evaluation

- Cell-Dyn 3500[a]
- Serono-Baker System 9000[b]
- Coulter Model FN[c]

4.1.2 Sample Collection

Canine blood samples were collected according to the method and standard of the NCCLS[51]. Venous blood was collected in standard 4.5 mℓ evacuated vials containing 0.054mℓ of 15% K_3EDTA[d]. Depending on the size of the animal and the preference of the clinician collecting the samples, blood was either collected from the jugular or cephalic veins. The samples were analysed as soon as possible after collection and they were kept at room temperature for no more than 4 hours before analysis, as prescribed by the NCCLS[51].

Samples were rejected if macroscopically visible clots were present. The presence of microscopically visible platelet clumps was acceptable, but was recorded by the examiners. Any abnormal conditions of the specimen, such as lipaemia or haemolysis were recorded[51].

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[a] Abbott Diagnostics Division SA, 149 Samuel Evans Drive, Aeroton, Johannesburg, RSA
[b] Serono-Baker Diagnostics, Inc, 100 Cascade Drive, Allentown, Pennsylvania, USA
[c] Coulter-Beckman Stand 1A, Fedsure Park, Tonetti Street 1685, Halfway House, RSA
[d] Radem Laboratory Supplies, Sandton, RSA

The NCCLS prescribes that 100 normal and 100 abnormal samples should be evaluated. In order to achieve this, the maximum number of samples that could be collected over the time period of the trial, i.e. two months were used. A total number of 361 canine blood samples were collected and found to be of sufficient quality to be included in the evaluation. Samples were collected from patients in the Onderstepoort Veterinary Academic Hospital, i.e. patients on which haematology analysis was requested, the South African Police, the South African Defence Force and one private veterinary hospital (Sinoville Animal Clinic, Pretoria, RSA). The 100 normal samples were collected from clinically normal dogs from the South African Police Force and South African Defence Force. The rest of the samples were from ill animals or animals that were reported to be ill by their owners.

4.2 METHODS

4.2.1 Total White Cell Count Evaluation

Sample analysis on the Cell-Dyn 3500 and the Serono-Baker System 9000 was performed. During this study the Cell-Dyn 3500 was used in the open mode, where the EDTA tube stopper is opened and the sample is aspirated with the open mode probe. The Coulter (Model FN) electronic cell counter was used to analyse 31 samples to double-check the bias of the reference Serono-Baker System 9000. The Cell-Dyn and the Baker System 9000 were compared by the t-test using the mean difference of paired data and regression analysis was done. The same method was applied for the comparison of the Cell-Dyn 3500 and the Coulter Model FN. The Cell-Dyn 3500, Baker System 9000 and the Coulter Model FN were compared with each other using the Friedman test as the same blood sample was evaluated on each of these instruments (i.e. more than one comparison was made).

Calculation of the sensitivity ratio (SR) as described by Mandel[22] was done. The SR is calculated as follows: SR= [mean standard deviation of the reference method ÷ mean standard deviation evaluated method]. This takes account of the slope of the linear regression as well as the analytical error or variability associated with each method[22].

The reference method is more sensitive if the SR is more than 1 and the method under evaluation is more sensitive if the SR is less than 1. If the SR is close to or equal to 1 then both methods are equally sensitive[22].

4.2.2 Five-Part Leukocyte Differential Count Determination

The five-part leukocyte differential count was evaluated according to the method described by The National Committee for Clinical Laboratory Standards (NCCLS)[51], although it was slightly modified to make it more suitable and practical for the examiners involved.

The examiners involved were the investigator (examiner 1) and Dr J Cullum (examiner 2), a post-graduate student at the Faculty of Veterinary Science, University of Pretoria, currently enrolled as an MMedVet (Laboratory Diagnostics) student.

Four blood films were prepared from each specimen on clean glass microscope slides. The wedge-pull film technique was used to prepare the blood films. The films were stained within one hour after preparation with Cams Quick[e] dye, a Romanowsky-type stain. The blood films were placed in four different containers. Examiner 1 did the differential counts on the slides in one container, examiner 2 used another container. The third container with slides was given to the arbitrator, who evaluated the slides in which there were a statistically significant difference in the results of the two examiners. The fourth container was kept as a backup.

The two examiners both did a manual differential count on 200 cells, each using the slide in the container given to her, thus counting a total of 400 cells on each sample. The individual subpopulations were expressed as a percentage of the total white cell count. Distorted cells that were clearly identifiable were included, but unidentifiable cells were reported as "smudge cells". Comments were made if any abnormalities were observed. These were

[e] CA Milsch, PO Box 943, Krugersdorp, 1730 RSA

semi-quantitatively expressed on a scale from 1+ to 6+, where 1+ represented a subtle change and 6+ a very severe change.

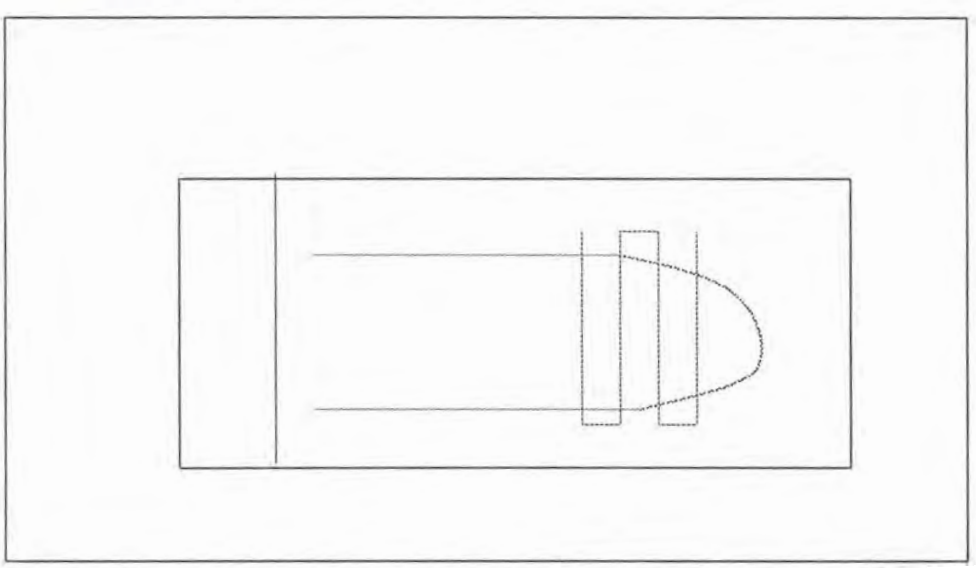
The qualitative comments which the examiners were asked to report were the presence of the following:

- microthrombi: clumps of thrombocytes present on the blood smear
- band cells: neutrophils with curved or sausage-shaped nuclei[51], a smooth nuclear membrane[18, 27] and parallel sides[27].
- smudge cells: broken lymphocytes with nuclear material that is clearly visible, but the cell is unrecognisable, due to storage of blood in EDTA for 30 to 60 minutes or longer[27].
- monocyte activity: monocytes that are larger and rich in intracellular organelles[40], giving them a foamy appearance.
- blast transformed lymphocytes: increased cytoplasmic basophilia[18, 27].
- atypical lymphocytes: lymphocytes with abundant, foamy or vacuolated cytoplasm[51]
- lymphocyte rafting: groups of lymphocytes clumping together in circulating blood.
- toxic neutrophils: neutrophils with granular and cytoplasmic abnormalities, including the presence of large, reddish purple granules, Döhle bodies, or cytoplasmic basophilia and vacuolation[27].
- agglutination: erythrocyte grouping in irregular clumps due to the presence of anti-erythrocyte antibodies[30, 40].
- Rouleaux formation: spontaneous stacking of red blood cells like a pile of coins[40]
- Lipaemia: elevated concentration of lipids in plasma[30], obviously only reported when there was any visible milky appearance of the plasma.
- Haemolysis: the presence of free haemoglobin in blood due to the destruction of erythrocytes. In this case it refers to either haemolysis due to a pathological process in the patient or haemolysis as a result of improper handling of the sample after collection.
- *Babesia canis* parasites
- *Ehrlichia canis* parasites
- Any other abnormality.

Nucleated red cells were reported as a number per 100 leukocytes counted.

The results of the two examiners were added to obtain a total manual cell count of 400. The counting pattern used by the examiners was a modification of the Battlement method, as shown in Figure 4.1. The total leukocyte counts of the Cell-Dyn 3500 were used to calculate the absolute differential counts from the percentages obtained by the examiners. These values were used for comparison with the absolute differential counts generated by the Cell-Dyn 3500. The Cell-Dyn and the Manual differential counts were compared by the t-test using the mean difference of paired data and regression analysis. Due to the low numbers of basophils present in canine samples and the difficulty of identifying these cells, one examiner did not report any basophils, therefore a 200 manual cell count was used instead of a 400 manual cell count to compare the results. If the 400 manual cell count had been used here, it would have falsely decreased the mean basophil count and caused an even greater discrepancy between the manual count and the count obtained with the Cell-Dyn 3500. The comments generated by the Cell-Dyn 3500 and the human evaluators were compared by means of frequency tables and the Chi-square was calculated.

Figure 4.1: Method used for the differential cell counts



4.2.3 Linearity studies

Ten samples were used for the linearity study, selected to include the normal physiological white cell counts and to include a wide range of pathological white cell counts. Tests were performed to give results at ten concentrations, evenly spaced, i.e. 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10% concentrations of each sample. Three replicate measurements were made on each dilution and the average of the three results taken, as is recommended for the evaluation of automated blood cell counters[38]. Saline (0.9%)[f] was used as the diluent. MLY calibrated pipettes[g] were used for the diluting technique.

For analyses, the mean of the counts obtained was plotted against the dilution factor. Regression analysis was then performed and the slope and intercept were calculated as well as the correlation coefficient value.

[f] Sabax Ltd, Evans Road, Aeroton, Johannesburg, RSA
[g] Medical Laboratory Automation, Pleasantville, New York, USA

4.2.4 Carry-over assessment

A sample with high leukocyte count (H) was analysed three consecutive times, followed by a sample with a low leukocyte count (L), also analysed three times consecutively, giving six results, i.e. h_1, h_2, h_3, l_1, l_2, l_3. Twelve sets of samples were analysed.

The carry-over was calculated using the following formula[9, 10]:

$$K = \frac{l_1 - l_3}{h_3 - l_3}$$

The carry-over is expressed as a percentage. Generally a carry-over of more than 5% is considered to be unacceptable, as this has an adverse effect on precision[10]. Most chemistry systems have carry-over values of about 2% and that is considered to have little effect on the precision[10]. It was tested to see if the carry-over is significantly less than 5% and 2% in order to evaluate the acceptability of the Cell-Dyn 3500's carry-over.

4.2.5 Precision

Precision studies were be done by testing samples on 2 or more occasions. The idea was to do at least ten repetitions per sample, but due to sample size and work load this was not possible in all the cases. Since it is preferable to assay more samples fewer times than to assay fewer samples more times[38, 67], a total number of 41 samples were analysed. Some samples were analysed 12 times and others only 5 times. The samples were chosen to include as wide as possible a range of white cell counts. Precision was quantified as the coefficient of variation after calculation of the standard deviation[9, 38, 67].

CHAPTER 5: RESULTS

5.1 COMPARISON OF THE TOTAL WHITE BLOOD CELL COUNTS

In the descriptions below the following abbreviations are used:

CDTCount: Cell-Dyn 3500 total white blood cell count;

BA: Serono Baker total white blood cell count;

CO: Coulter total white cell count.

5.1.1 Total White Blood Cell Count of Cell-Dyn 3500 compared to that of the Serono Baker System 9000

5.1.1.1 Comparison of all the data

A total number of 361 samples were analyzed. The total white cell counts were compared by the t-test using the mean difference of paired data. The mean difference was -2.703 (t = -16.448). Therefore there was a statistically significant difference between the total cell counts of the Cell-Dyn 3500 and the Baker System 9000 (p = 0.0001). Figure 5.1 revealed that a large component of this difference is attributable to a slope difference. The regression equation being CDTCount = 0.838BA + 0.308. The correlation coefficient was 0.989.

There were a number of samples (six clearly identifiable) which gave markedly different results. In order to establish whether these differences were associated with specific classes of abnormal samples, the total white cell counts of the two analyzers were compared again, every time leaving out a different class of abnormal samples. The results of these comparisons follow.

5.1.1.2 Comparison after samples with "WBC Diff Alert" and "WBC Count Alert" flags have been omitted

A total number of 248 samples were present in this class. The data were compared by the t-test using the mean difference of paired data. The mean difference was -2.699 (t = -14.198). Therefore there is a statistically significant difference between the total cell counts of the Cell-Dyn 3500 and the Baker System 9000 under these conditions as well (p = 0.0001). Figure 5.2 reveals that a large component of this difference is attributable to a slope difference. The regression equation being CDTCount = 0.845BA + 0.403. The correlation coefficient was 0.973.

5.1.1.3 Comparison after samples with "WBC Count Alert" flags have been omitted

There were 349 samples in this class. The data were compared by the t-test using the mean difference of paired data. The mean difference was -2.355 (t = -19.027). Therefore there is a statistically significant difference between the total cell counts of the Cell-Dyn 3500 and the Baker System 9000 also under this condition (p = 0.0001). Figure 5.3 reveals that a large component of this difference is attributable to a slope difference. The regression equation being CDTCount = 0.875BA - 0.095. The correlation coefficient was 0.979.

5.1.1.4 Comparison after samples with "WBC Data Invalid" flags have been omitted

There were a total number of 354 samples in this class. The data were compared by the t-test using the mean difference of paired data. The mean difference was -2.382 (t = -18.355). Therefore there is a statistically significant difference between the total cell counts of the Cell-Dyn 3500 and the Baker System 9000 (p = 0.0001). Figure 5.4 reveals that a large component of this difference is attributable to a slope difference. The regression equation being CDTCount = 0.869BA - 0.033. The correlation coefficient was 0.981.

For a comparison of the results of the Cell-Dyn 3500 compared to the Baker System 9000 and the Cell-Dyn 3500 compared to the Coulter Model FN, see Table 5.1

5.1.1.5 Comparison after samples with "Monocytosis" flags have been omitted

A total number of 339 samples were in this class. The data were compared by the t-test using the mean difference of paired data. The mean difference was -2.663 (t = -15.391). Therefore there is a statistically significant difference between the total cell counts of the Cell-Dyn 3500 and the Baker System 9000 (p = 0.0001). Figure 5.5 reveals that a large component of this difference is attributable to a slope difference. The regression equation being CDTCount = 0.838BA + 0.328. The correlation coefficient was 0.972.

Table 5.1 Comparison of the Total White Blood Cell Counts measured on the Cell-Dyn 3500 and the Baker System 9000 and the Cell-Dyn 3500 and the Coulter Model FN compared by regression analysis and paired *t*-test

	n	Slope	Intercept	p model	r	Mean Difference	t	p (t)
CD/BA	361	0.838	0.308	0.0001	0.989	-2.703	-16.448	0.0001
CD/CO	31	0.932	-1.616	0.0001	0.968	-3.029	-6.434	0.0001

Figure 5.1: Total White Cell Count of the Cell-Dyn 3500 Compared with the Total White Cell Count of the Baker System 9000.

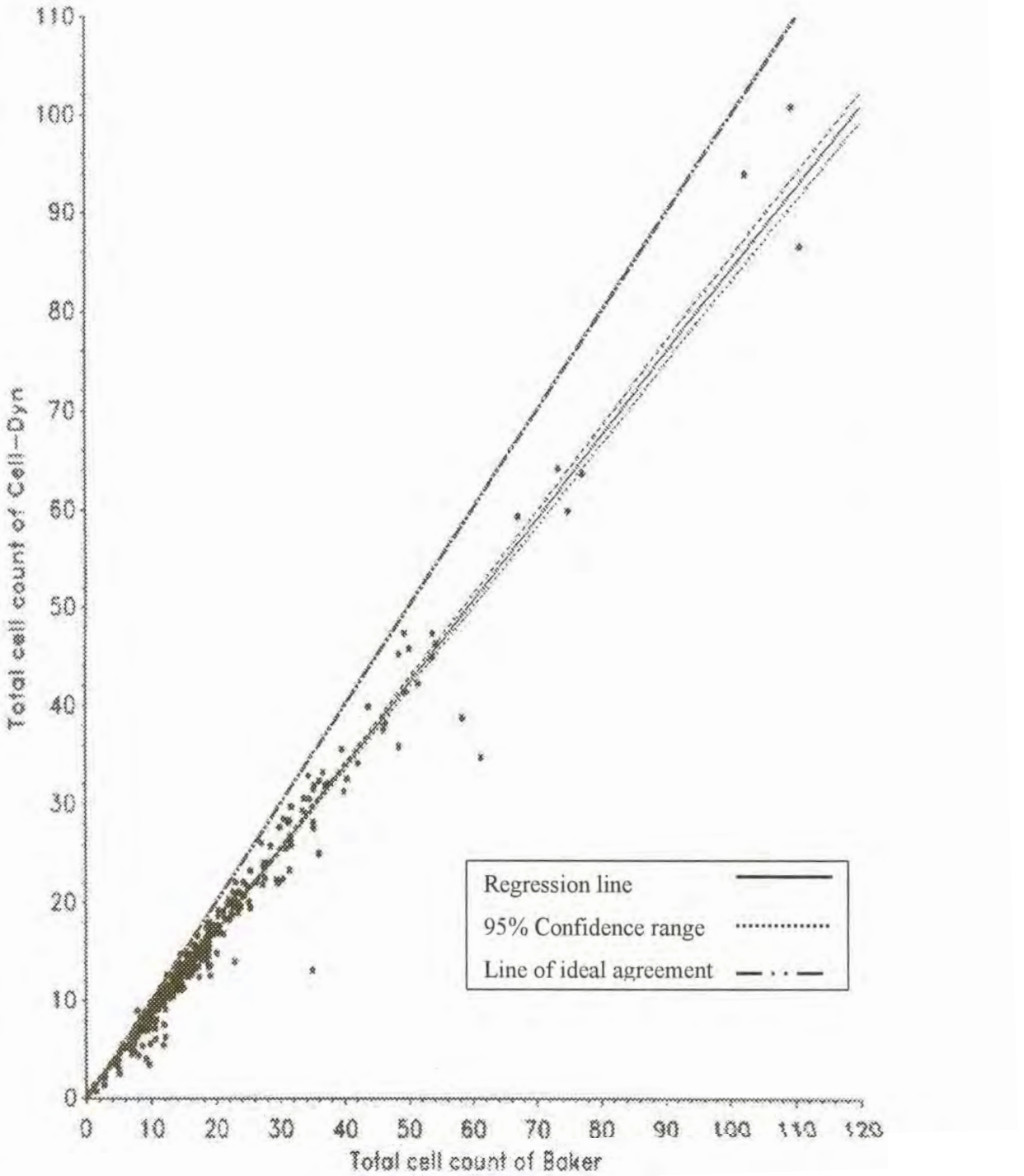


Figure 5.2: Total White Cell Count of the Cell-Dyn 3500 Compared with the Total White Cell Count of the Baker System 9000 after samples with "WBC Count Alert" and "WBC Diff Alert" Flags have been Omitted.

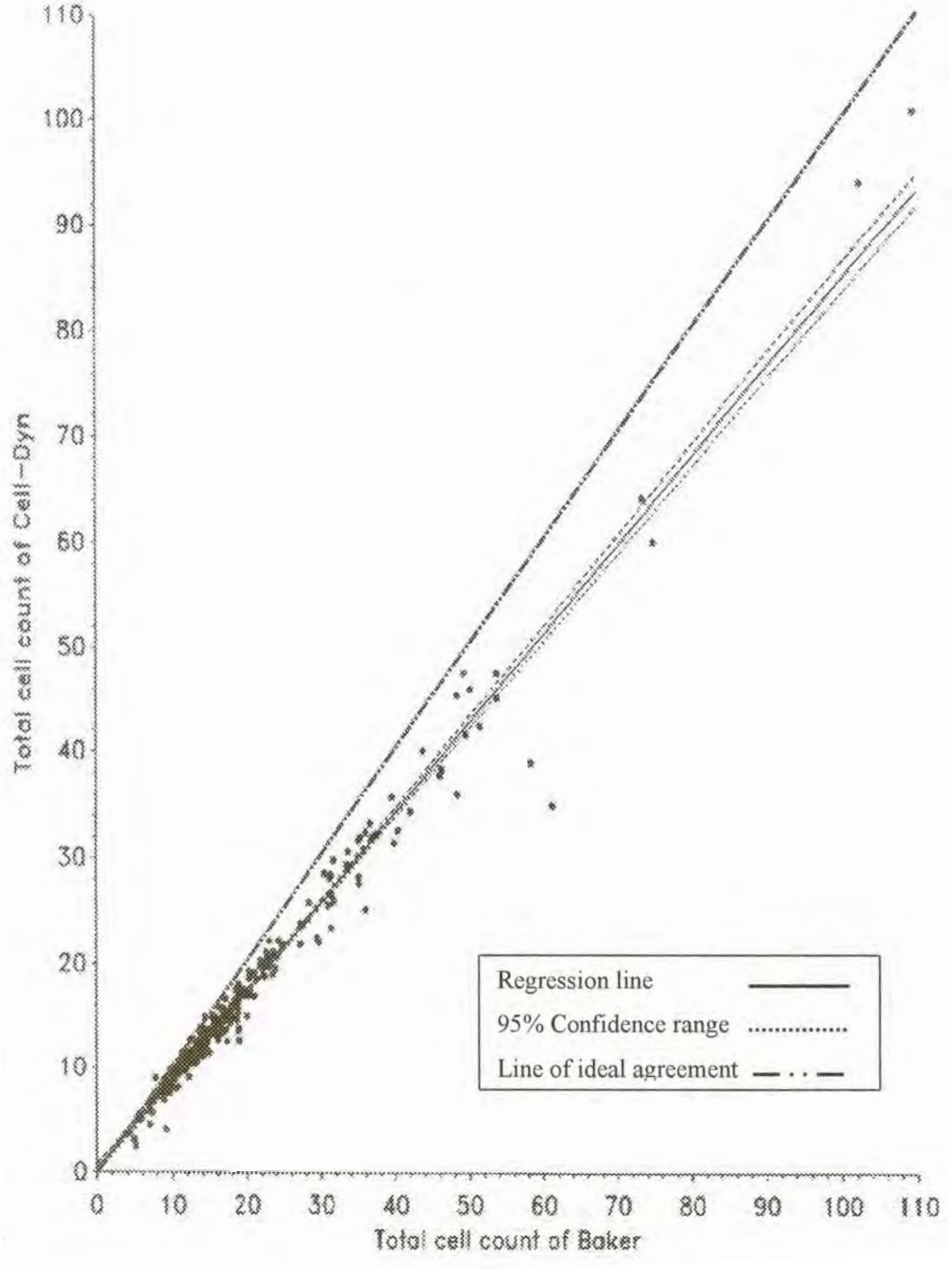


Figure 5.3: Total White Cell Count of the Cell-Dyn 3500 Compared with the Total White Cell Count of the Baker System 9000 after samples with "WBC Count Alert" Flags have been Omitted.

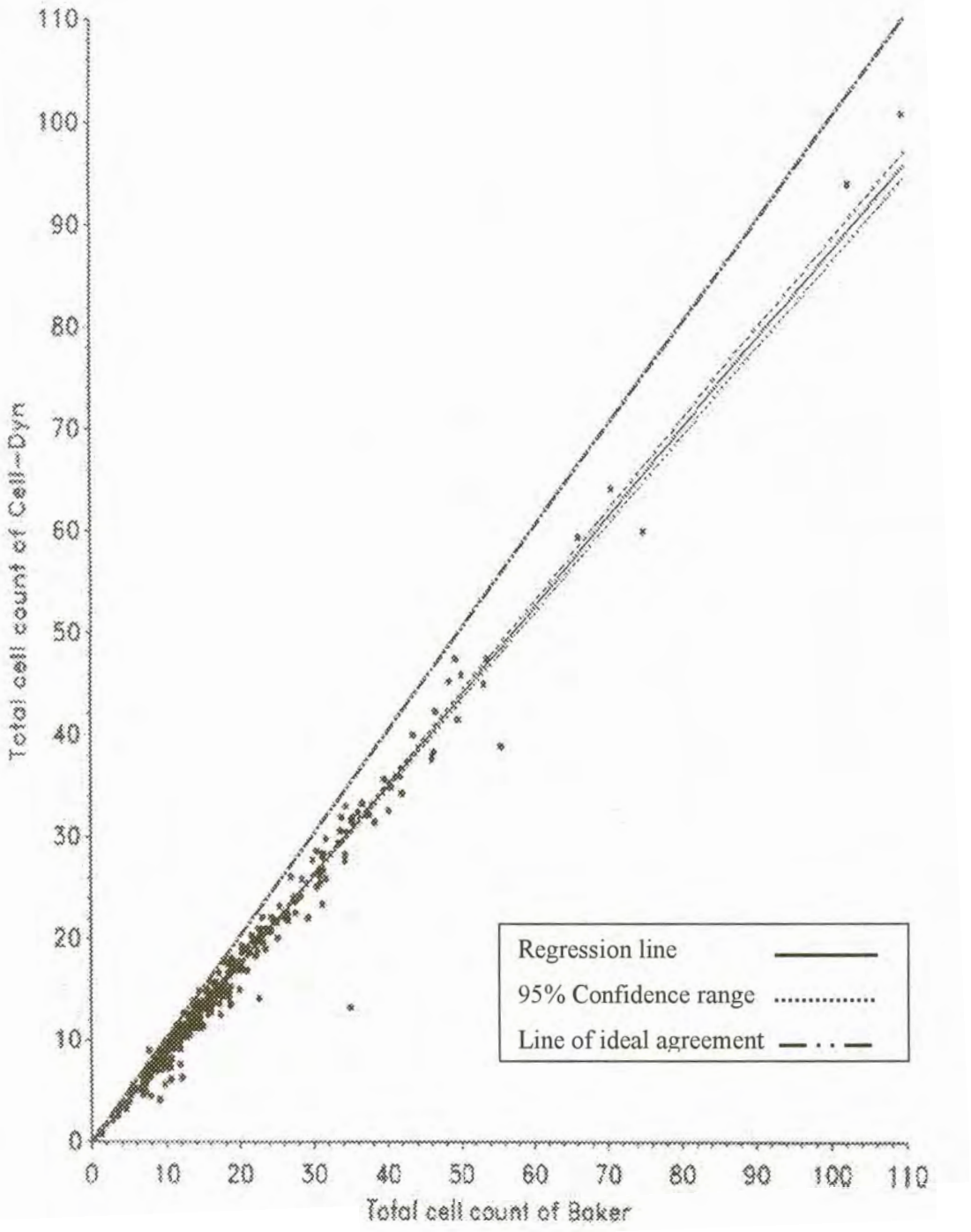


Figure 5.4: Total White Cell Count of the Cell-Dyn 3500 Compared with the Total White Cell Count of the Baker System 9000 after samples with "WBC Data Invalid" Flags have been Omitted.

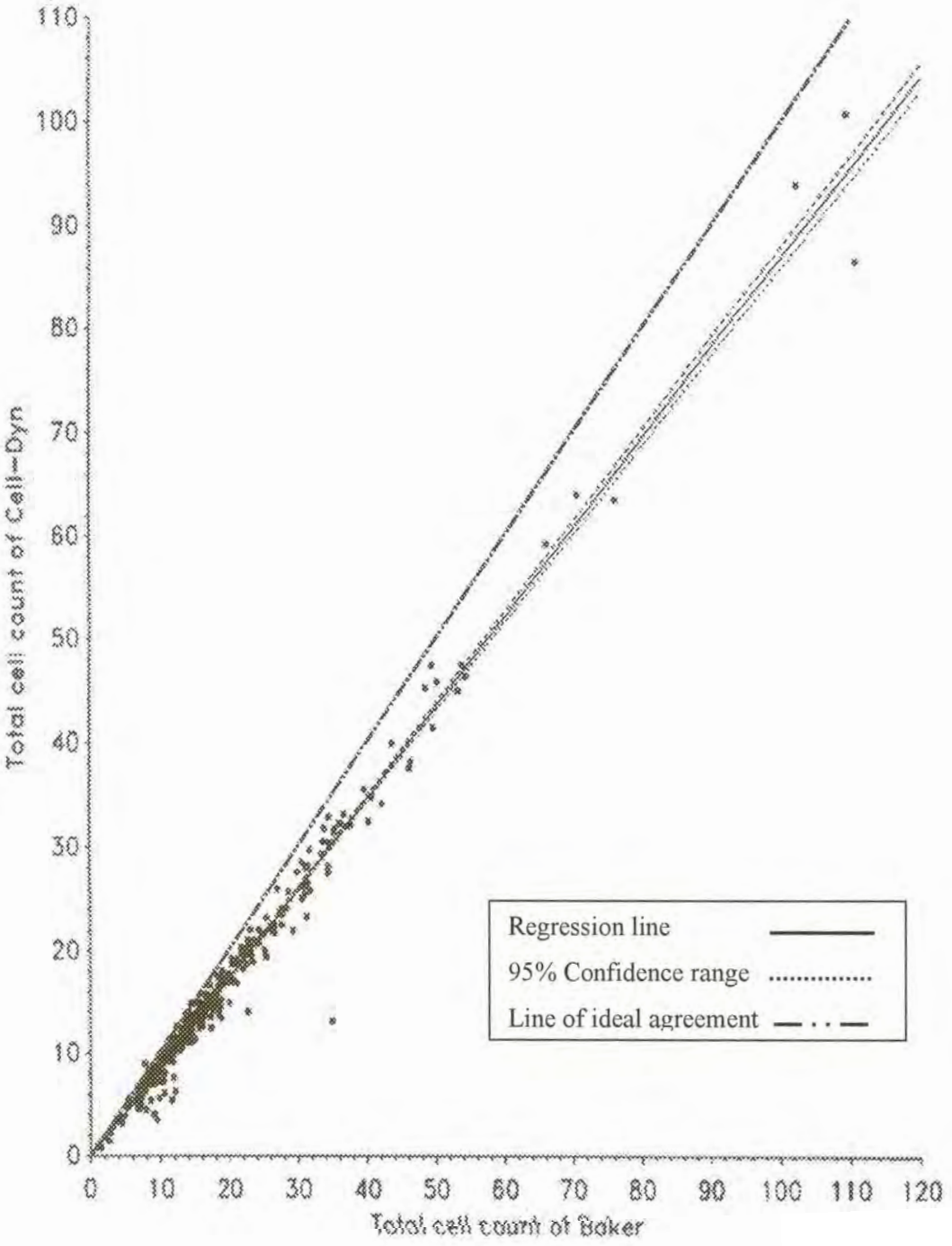
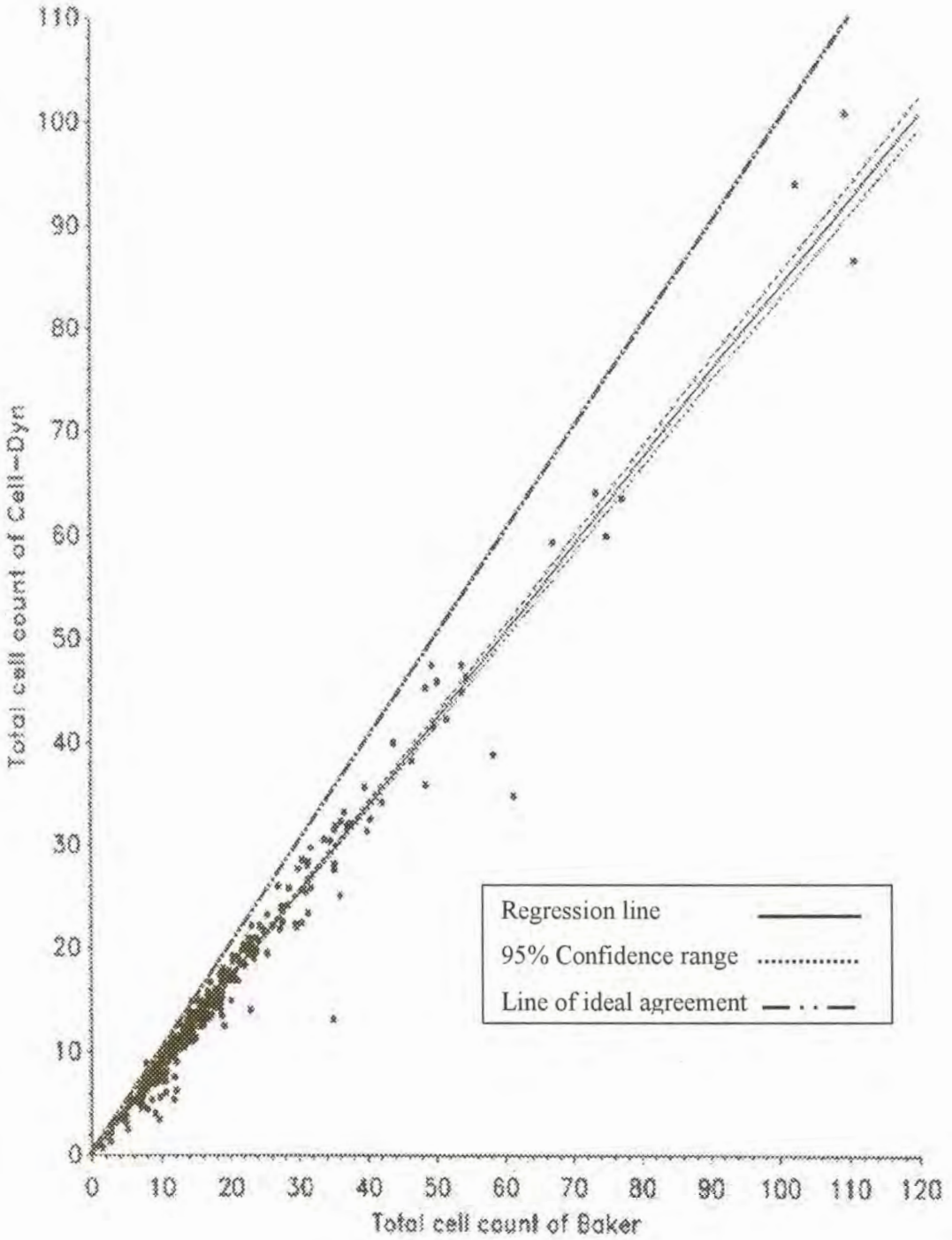


Figure 5.5: Total White Cell Count of the Cell-Dyn 3500 Compared with the Total White Cell Count of the Baker System 9000 after samples with a Monocytosis have been Omitted.



5.1.2 Total White Blood Cell Count of Cell-Dyn 3500 compared to that of the Coulter Model FN

A total number of 31 samples were analyzed. The data were compared by the t-test using the mean difference of paired data. The mean difference was -3.029 (t = -6.434). Therefore there is a statistically significant difference between the total cell counts of the Cell-Dyn 3500 and the Coulter Model FN (p = 0.0001). Figure 5.6 reveals that a large component of this difference is attributable to both an intercept as well as a slope difference. The regression equation being CDTCount = 0.932CO -1.616. The correlation coefficient was 0.968.

5.1.3 Comparison of the Total White Blood Cell Count of the Cell-Dyn 3500, Baker System 9000 and Coulter Model FN

There were a total number of 31 samples. The mean total white blood cell count on the Cell-Dyn 3500 is 17.681, the mean total white blood cell count on the Baker System 9000 is 20.097, and the mean total white blood cell count on the Coulter Model FN is 20.478. The Friedman test statistic is 47.81 and the p-value is 0.0000. There is a statistically significant difference between the Cell-Dyn 3500 and the Baker System 9000. This is also true for the Cell-Dyn 3500 and the Coulter Model FN. However, there is not a significant difference between the Baker System 9000 and the Coulter Model FN, see Table 5.2.

Figure 5.6: Total White Cell Count of the Cell-Dyn 3500 Compared to the Total White Cell Count of the Coulter Model FN.

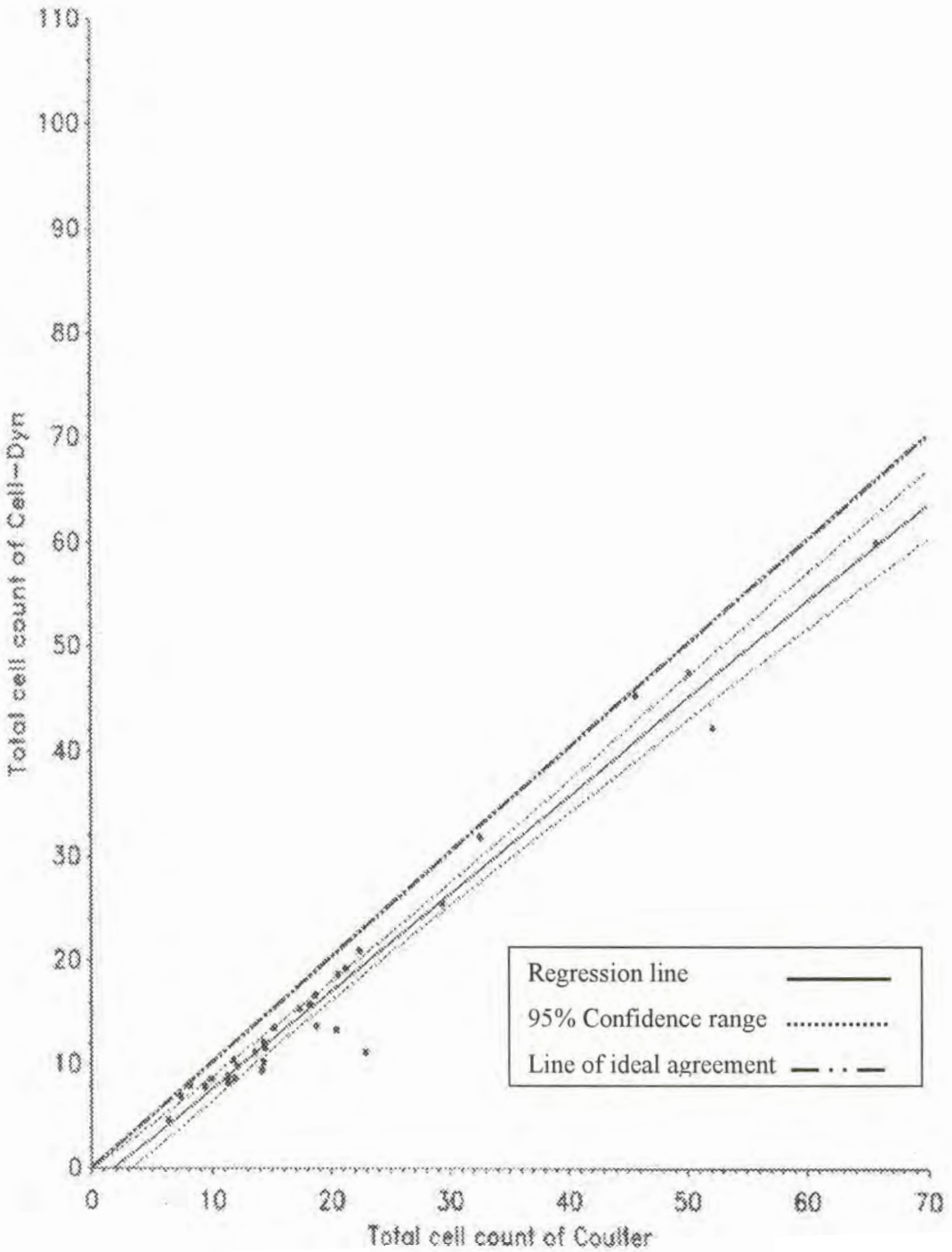


Table 5.2 Comparison of the Total White Blood Cell Counts measured on the Cell-Dyn 3500, the Baker System 9000 and the Coulter Model FN using the Friedman test.

Comparison	Z-statistic	p
Coulter/Cell-Dyn	6.48	< 0.05
Coulter/Baker	1.14	>0.10
Cell-Dyn/Baker	5.33	< 0.05

5.2 DIFFERENTIAL LEUKOCYTE COUNT COMPARISONS

5.2.1 Comparison of the Differential Leukocyte Counts of the Cell-Dyn 3500 against the 400 manual differential count

In the descriptions below the following abbreviations are used:
CDNeut: Cell-Dyn 3500 neutrophil count;
CDLymph: Cell-Dyn 3500 lymphocyte count;
CDMono: Cell-Dyn 3500 monocyte count;
CDEosin: Cell-Dyn 3500 eosinophil count;
CDBaso: Cell-Dyn 3500 basophil count;
MNeut: Manual neutrophil count;
MLymph: Manual lymphocyte count;
MMono: Manual monocyte count;
MEosin: Manual eosinophil count;
MBaso: Manual basophil count;

The results of the leukocyte subpopulation counts are summarized in Table 5.3

5.2.1.1 Neutrophil Count of the Cell-Dyn 3500 compared to the 400 manual cell count

5.2.1.1.1 Comparison of all the data

The data were compared by the t-test using the mean difference of paired data. The mean difference was 1.224 (t = 12.969). Therefore there is a statistically significant difference between the neutrophil counts of the Cell-Dyn 3500 and the 400 manual cell count (p = 0.0001). Figure 5.7 shows the relationship between the counts. The regression equation being CDNeut = 1.084MNeut + 0.239. The correlation coefficient was 0.981.

5.2.1.1.2 Comparison after samples with "WBC Diff Alert" and "WBC Count Alert" flags have been omitted

The data were compared by the t-test using the mean difference of paired data. The mean difference was 1.287 (t = 10.816). Therefore there is a statistically significant difference between the neutrophil counts of the Cell-Dyn 3500 and the 400 manual cell count (p = 0.0001). Figure 5.8 shows the relationship between the counts. The regression equation being CDNeut = 1.097MNeut - 0.024. The correlation coefficient was 0.984.

Table 5.3 Comparison of the Differential Leukocyte Counts Performed by the Cell-Dyn 3500 and by the Examiners, compared by regression analysis and paired *t*-test, n = 361

Cell Type	Slope	Intercept	p model	r	Mean Difference	t	p (t)
Neut	1.084	0.239	0.0001	0.981	1.224	12.969	0.0001
Lymph	0.753	0.163	0.0001	0.782	-0.325	-7.993	0.0001
Mono	0.228	0.315	0.0001	0.097	-0.605	-9.868	0.0001
Eosin	0.344	0.011	0.0001	0.304	-0.407	-11.826	0.0001
Baso	0.017	0.142	0.9240	0.000	0.122	9.335	0.0001

Figure 5.7: Cell-Dyn 3500 Neutrophil Count Compared to the Manual Neutrophil Count.

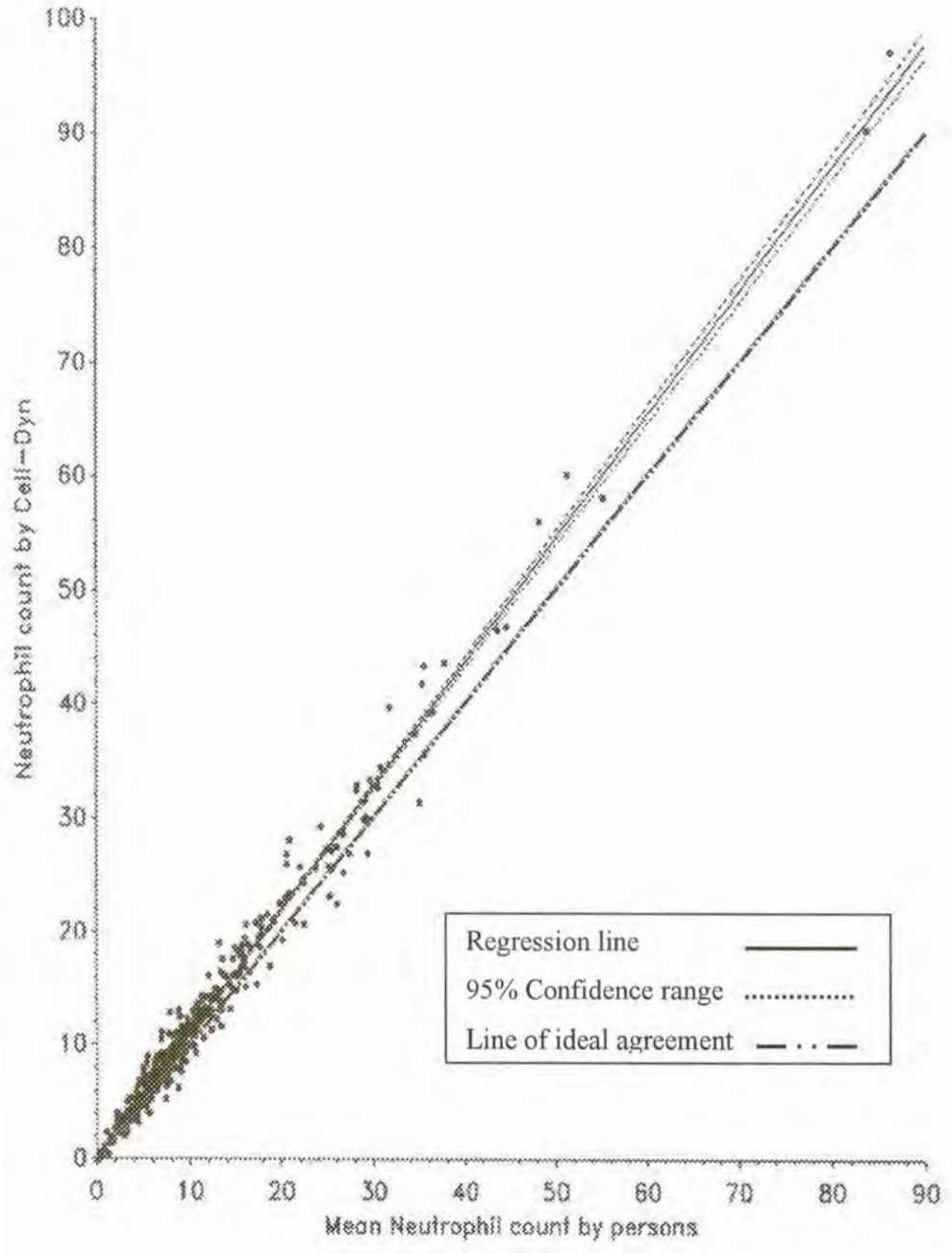
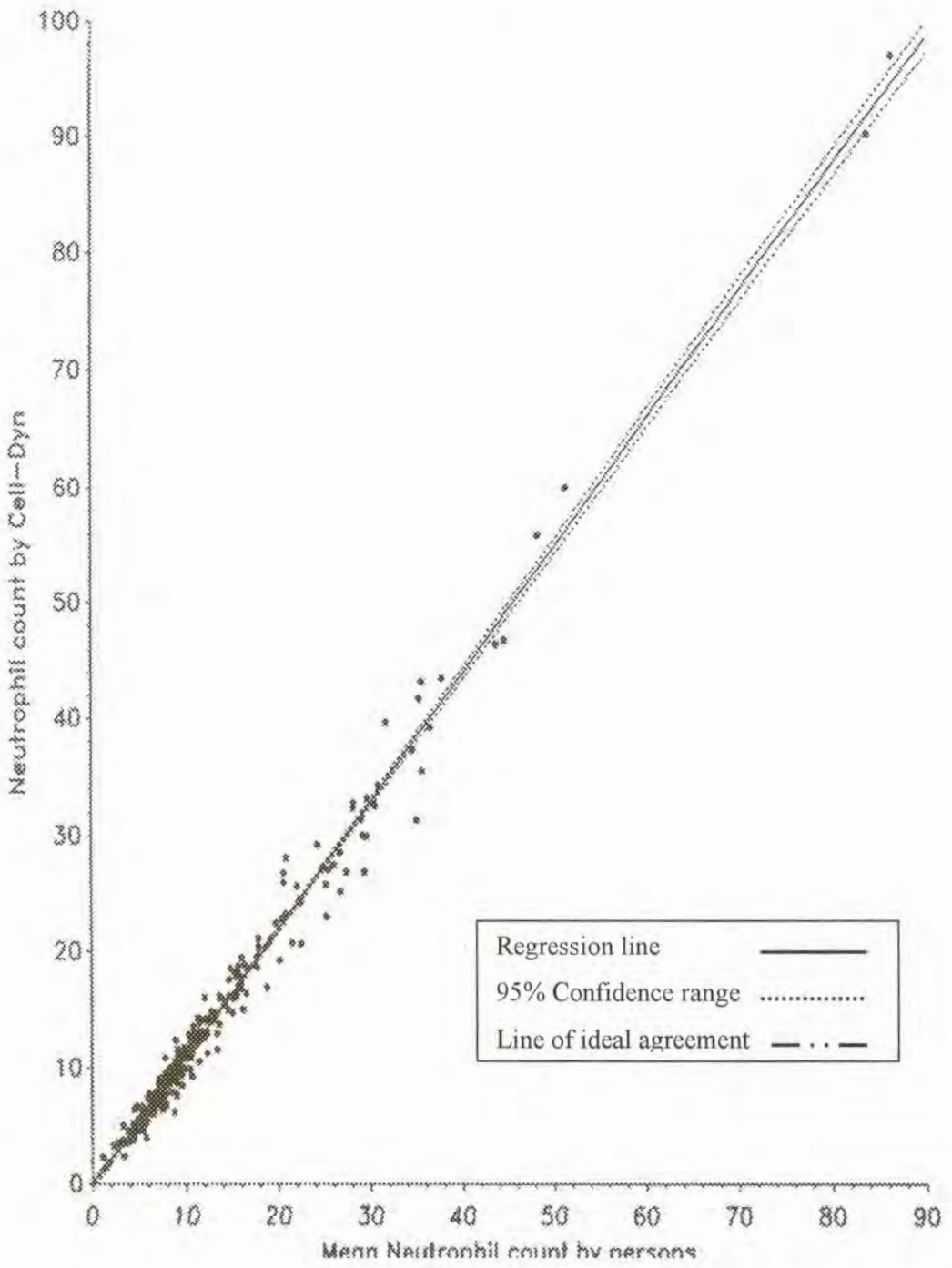


Figure 5.8: Cell-Dyn 3500 Neutrophil Count Compared to the Manual Neutrophil Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



5.2.1.2 Lymphocyte Count of the Cell-Dyn 3500 compared to the 400 manual cell count

5.2.1.2.1 Comparison of all the data

The data were compared by the t-test using the mean difference of paired data. The mean difference was -0.325 (t = -7.993). Therefore there is a statistically significant difference between the lymphocyte counts of the Cell-Dyn 3500 and the 400 manual cell count (p = 0.0001). Figure 5.9 reveals that the values are scattered and that a large component is attributable to a slope difference. The regression equation being CDLymph = 0.753MLymph + 0.163. The correlation coefficient was 0.782.

5.2.1.2.2 Comparison after samples with "WBC Diff Alert" and "WBC Count Alert" flags have been omitted

The data were compared by the t-test using the mean difference of paired data. The mean difference was -0.368 (t = - 6.976). Therefore there is a statistically significant difference between the lymphocyte counts of the Cell-Dyn 3500 and the 400 manual cell count (p = 0.0001). Figure 5.10 reveals that the values are scattered and a large component of this difference is attributable to a slope difference. The regression equation being CDLymph = 0.663MLymph + 0.298. The correlation coefficient was 0.710.

Figure 5.9: Cell-Dyn 3500 Lymphocyte Count Compared to the Manual Lymphocyte Count.

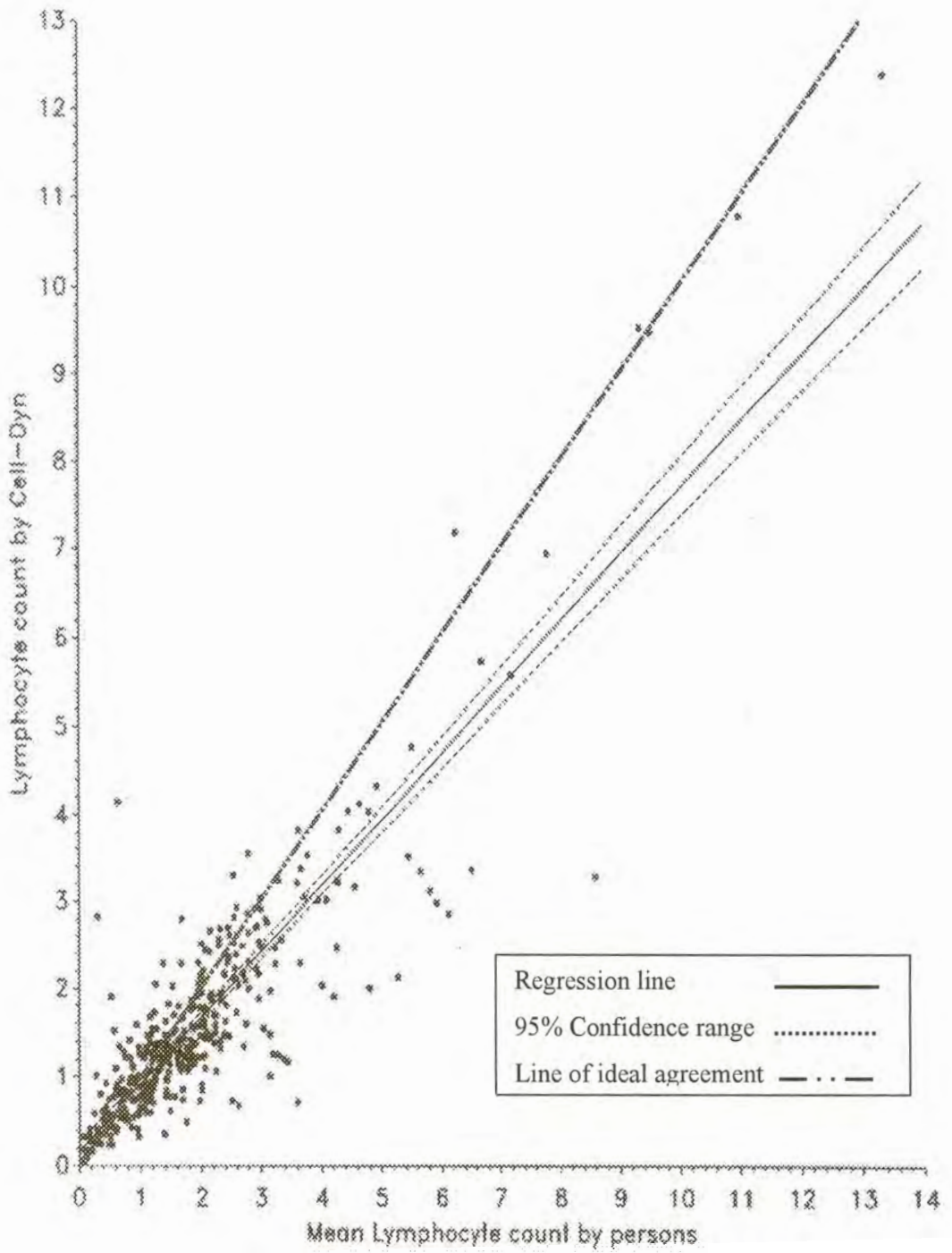
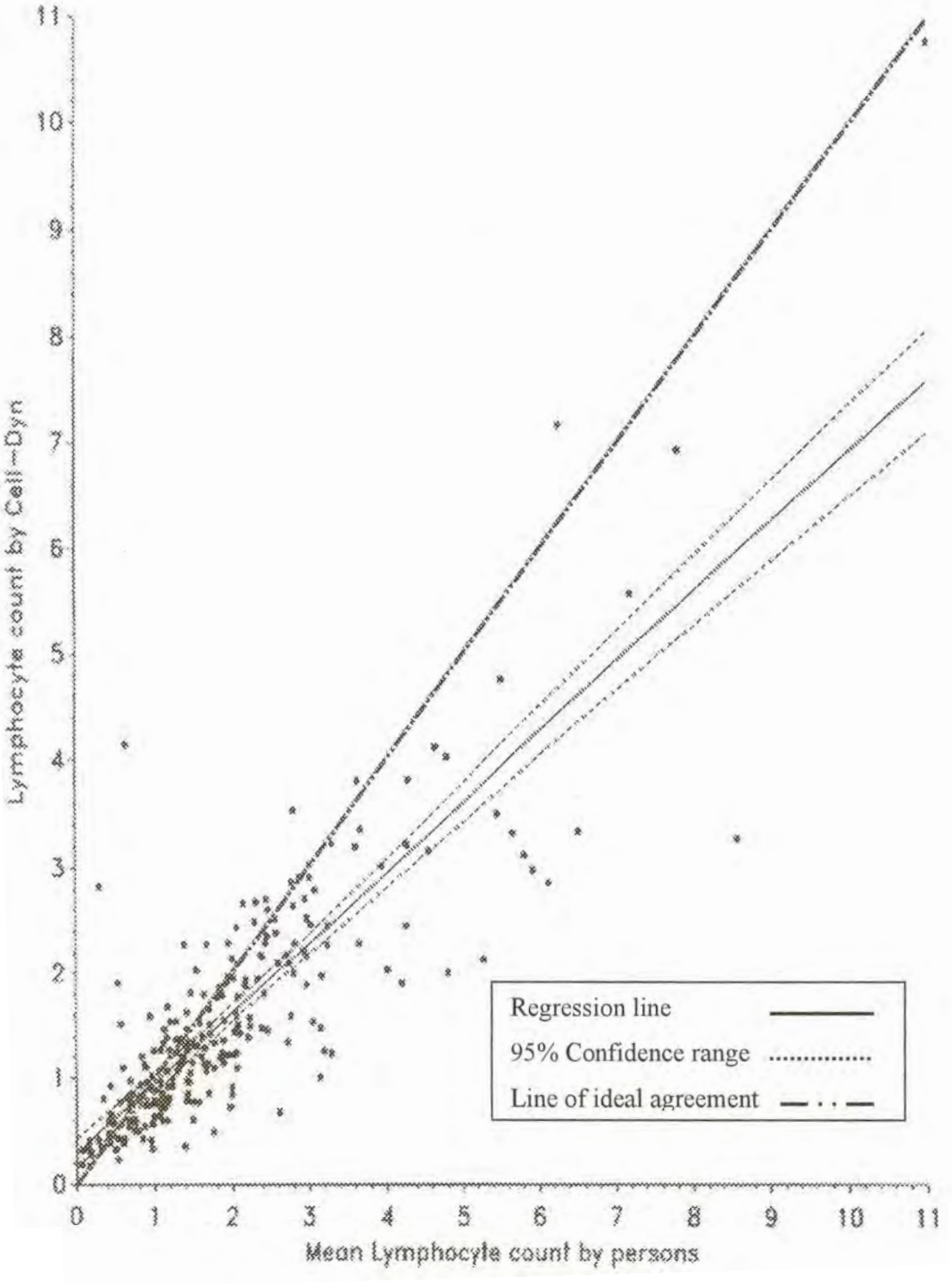


Figure 5.10: Cell-Dyn 3500 Lymphocyte Count Compared to the Manual Lymphocyte Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



5.2.1.3 Monocyte Count of the Cell-Dyn 3500 compared to the 400 manual cell count

5.2.1.3.1 Comparison of all the data

The data were compared by the t-test using the mean difference of paired data. The mean difference was -0.605 (t = -9.868). Therefore there is a statistically significant difference between the monocyte counts of the Cell-Dyn 3500 and the 400 manual cell count (p = 0.0001). Figure 5.11 reveals that there are two large clusters of values, and poor correlation. The one cluster approaches the regression line CDMono = 1,5MMono + 0. This cluster follows the line of ideal agreement to some extent. The other cluster, although poorly defined, approaches the regression line CDMono = 0.18MMono + 0. The regression equation is CDMono = 0.228MMono + 0.315. The correlation coefficient is 0.097.

5.2.1.3.2 Comparison after samples with "WBC Diff Alert" and "WBC Count Alert" flags have been omitted

The data were compared by the t-test using the mean difference of paired data. The mean difference was -0.817 (t = - 10.245). Therefore there is a statistically significant difference between the monocyte counts of the Cell-Dyn 3500 and the 400 manual cell count (p = 0.0001). Figure 5.12 reveals that left cluster follows the line of ideal agreement to a large extent. The regression equation being CDMono = 0.116MMono + 0.378. The correlation coefficient is 0.039.

Figure 5.11: Cell-Dyn 3500 Monocyte Count Compared to the Manual Monocyte Count.

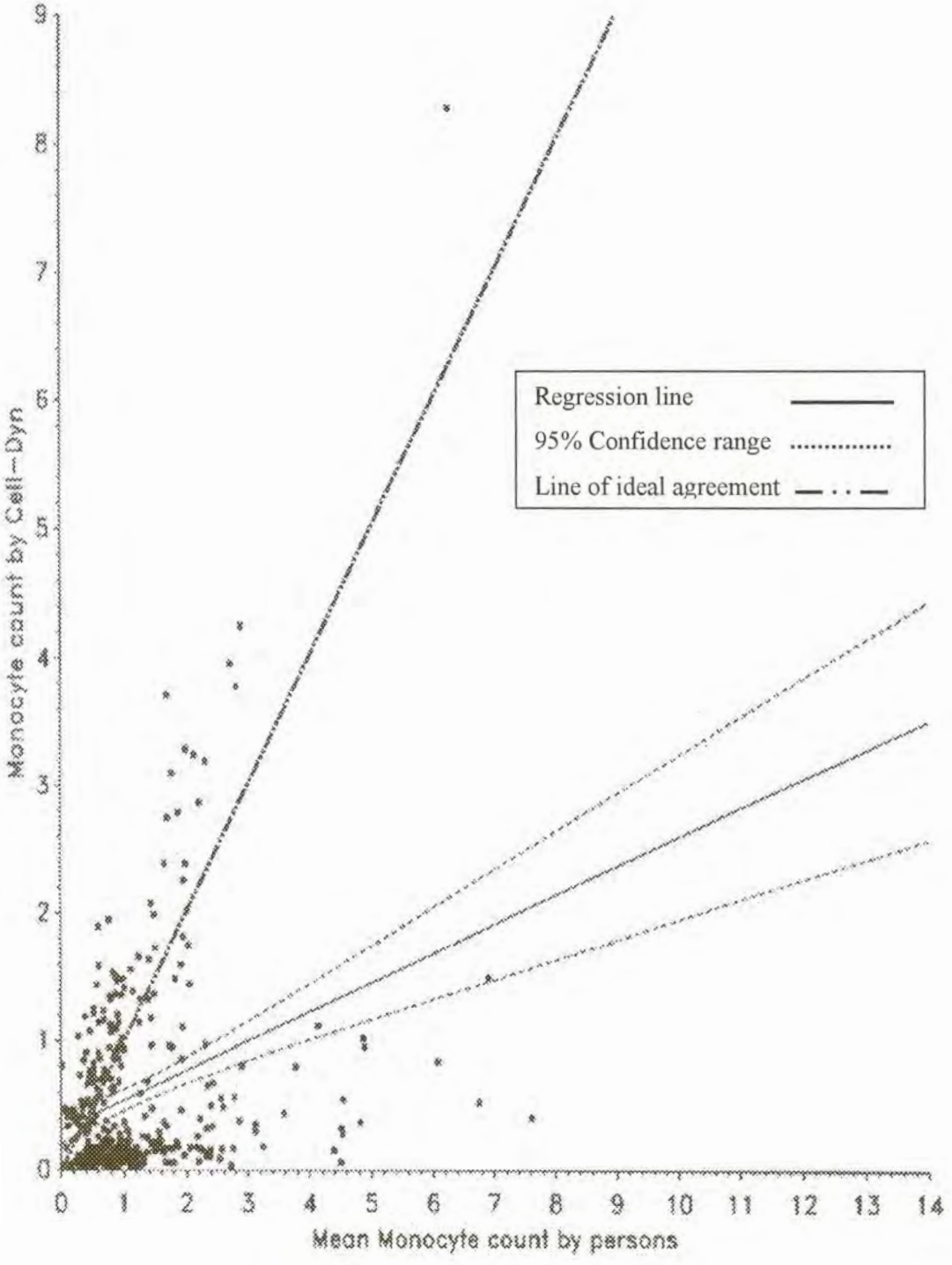
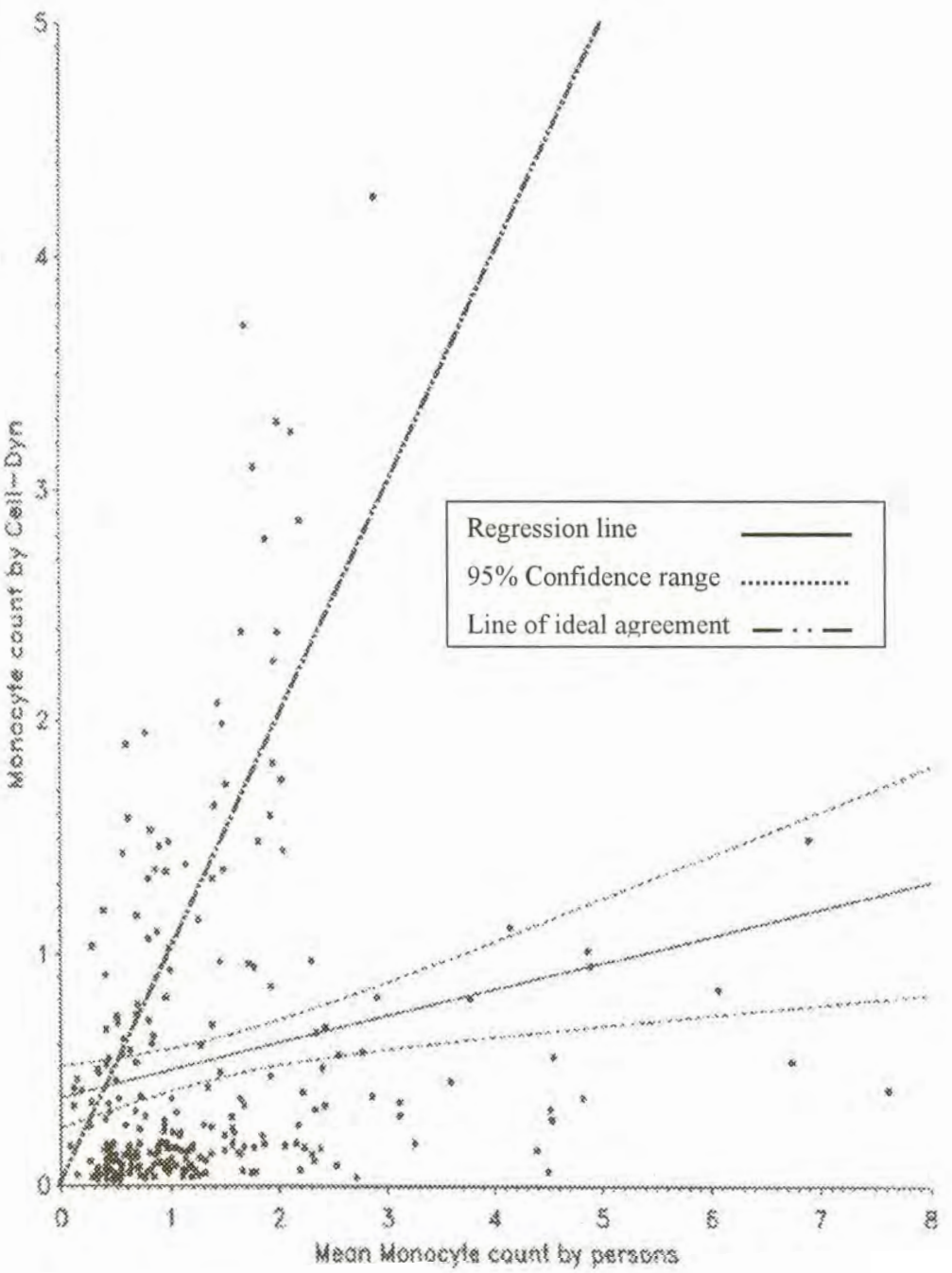


Figure 5.12: Cell-Dyn 3500 Monocyte Count Compared to the Manual Monocyte Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



5.2.1.4 Eosinophil Count of the Cell-Dyn 3500 compared to the 400 manual cell count

5.2.1.4.1 Comparison of all the data

The data were compared by the t-test using the mean difference of paired data. The mean difference was -0.407 (t = -11.826). Therefore there is a statistically significant difference between the eosinophil counts of the Cell-Dyn 3500 and the 400 manual cell count (p = 0.0001). Figure 5.13 reveals that there are two main clusters of values, with poor correlation. The upper, left cluster largely follows the line of ideal agreement. The regression equation being CDEosin = 0.344MEosin + 0.011. The correlation coefficient is 0.304.

5.2.1.4.2 Comparison after samples with "WBC Diff Alert" and "WBC Count Alert" flags have been omitted

The data were compared by the t-test using the mean difference of paired data. The mean difference was -0.228 (t = -9.876).Therefore there is a statistically significant difference between the eosinophil counts of the Cell-Dyn 3500 and the 400 manual cell count (p = 0.0001). Figure 5.14 reveals that there are two main clusters of values, with the upper left cluster following the line of ideal agreement closely. The regression equation being CDEosin = 0.674MEosin - 0.066. The correlation coefficient is 0.592.

Figure 5.13: Cell-Dyn 3500 Eosinophil Count Compared to the Manual Eosinophil Count.

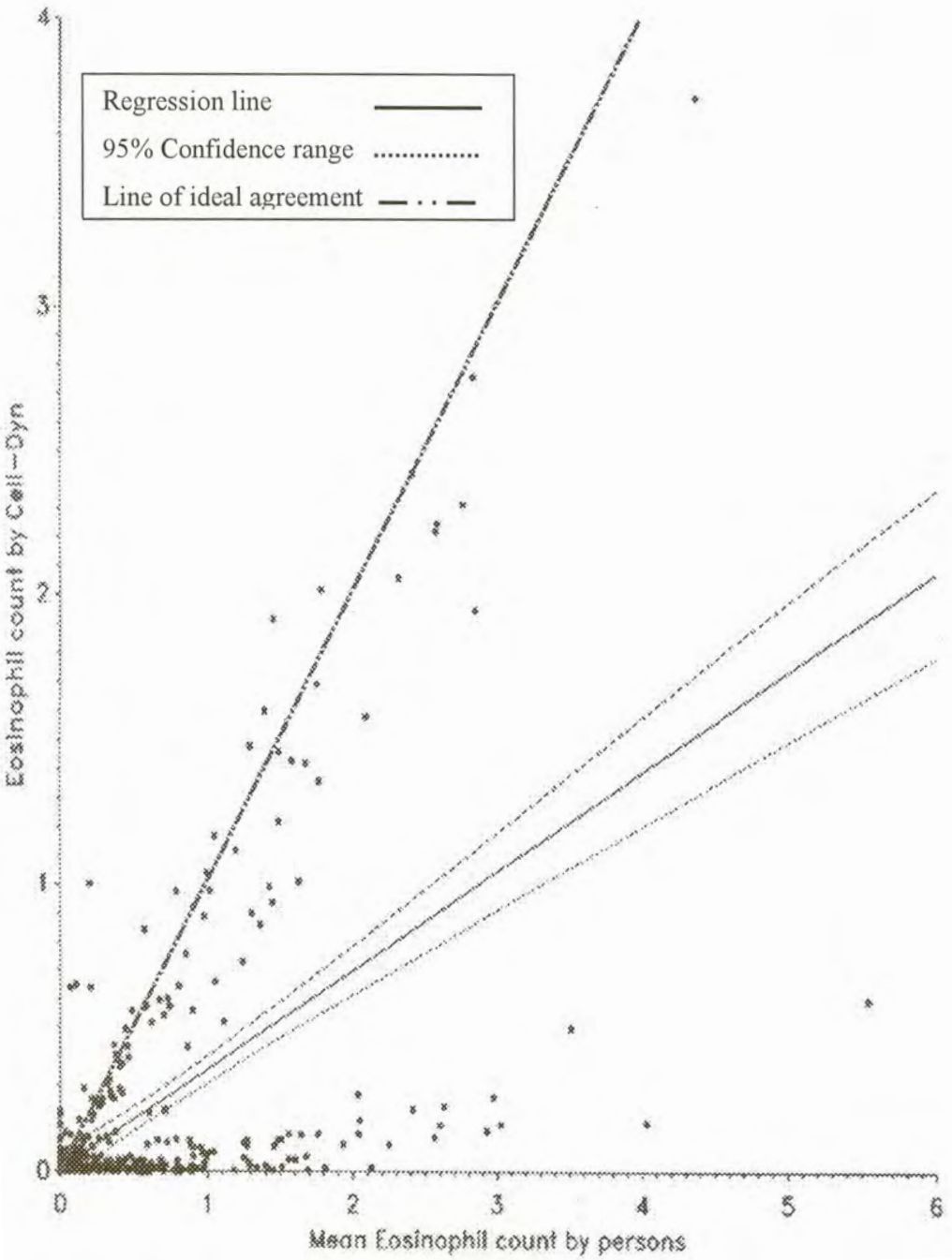
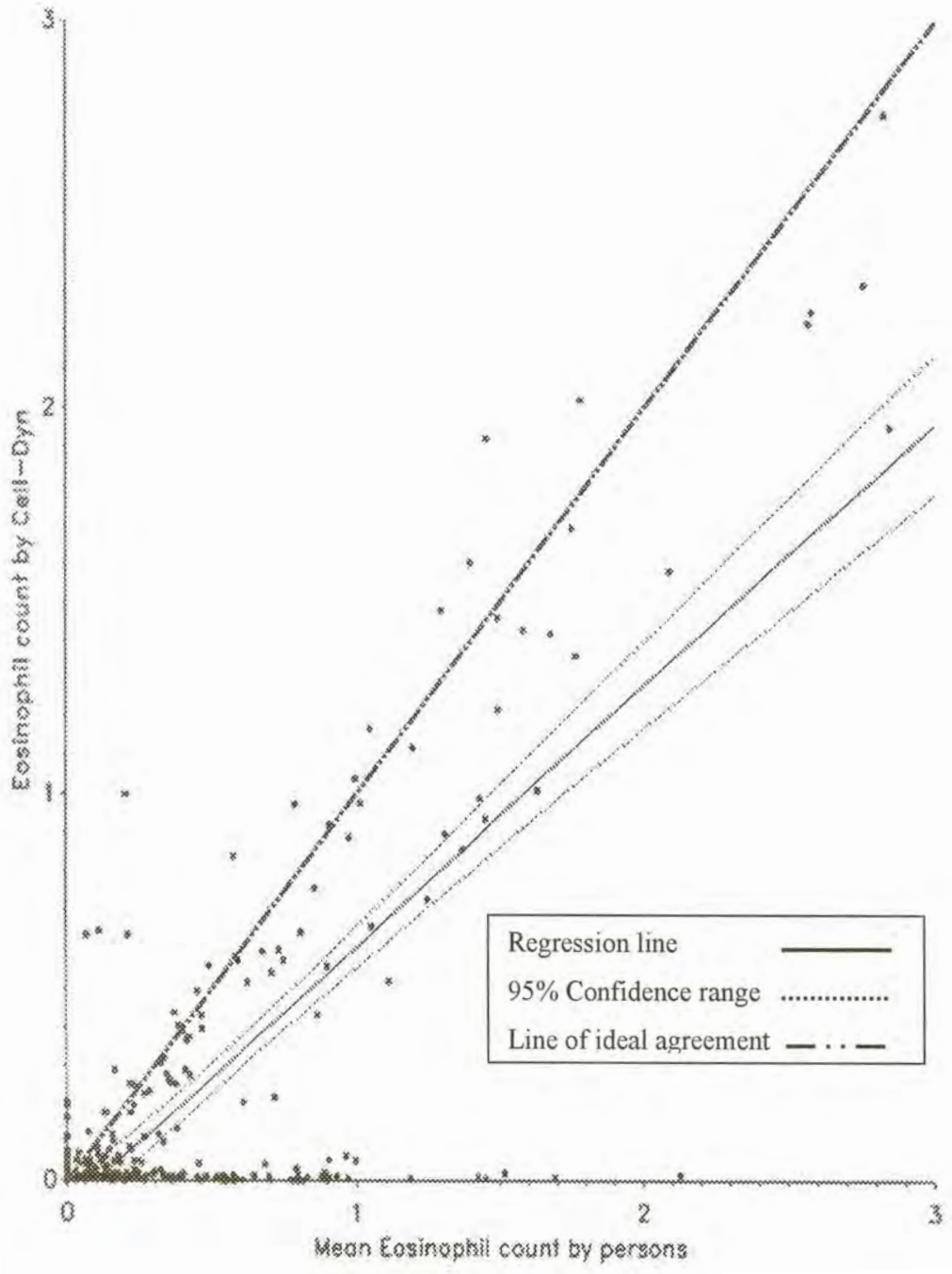


Figure 5.14: Cell-Dyn 3500 Eosinophil Count Compared to the Manual Eosinophil Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



5.2.1.5 Basophil Count of the Cell-Dyn 3500 compared to a 200 manual cell count

5.2.1.5.1 Comparison of all the data

The data were compared by the t-test using the mean difference of paired data. The mean difference was 0.122 (t = 9.335). Therefore there is a statistically significant difference between the basophil counts of the Cell-Dyn 3500 and the 200 manual cell count (p = 0.0001). Figure 5.15 reveals that there is no correlation at all. The regression equation being CDBaso = -0.017MBaso + 0.142. The correlation coefficient is 0.0000.

5.2.1.5.2 Comparison after samples with "WBC Diff Alert" and "WBC Count Alert" flags have been omitted

The data were compared by the t-test using the mean difference of paired data. The mean difference was 0.136 (t = 8.113). Therefore there is a statistically significant difference between the basophil counts of the Cell-Dyn 3500 and the 200 manual cell count (p = 0.0001). Figure 5.16 reveals that there is no correlation at all. The regression equation being CDBaso = 0.117MBaso + 0.150. The correlation coefficient is 0.0005.

Figure 5.15: Cell-Dyn 3500 Basophil Count Compared to the Manual Basophil Count.

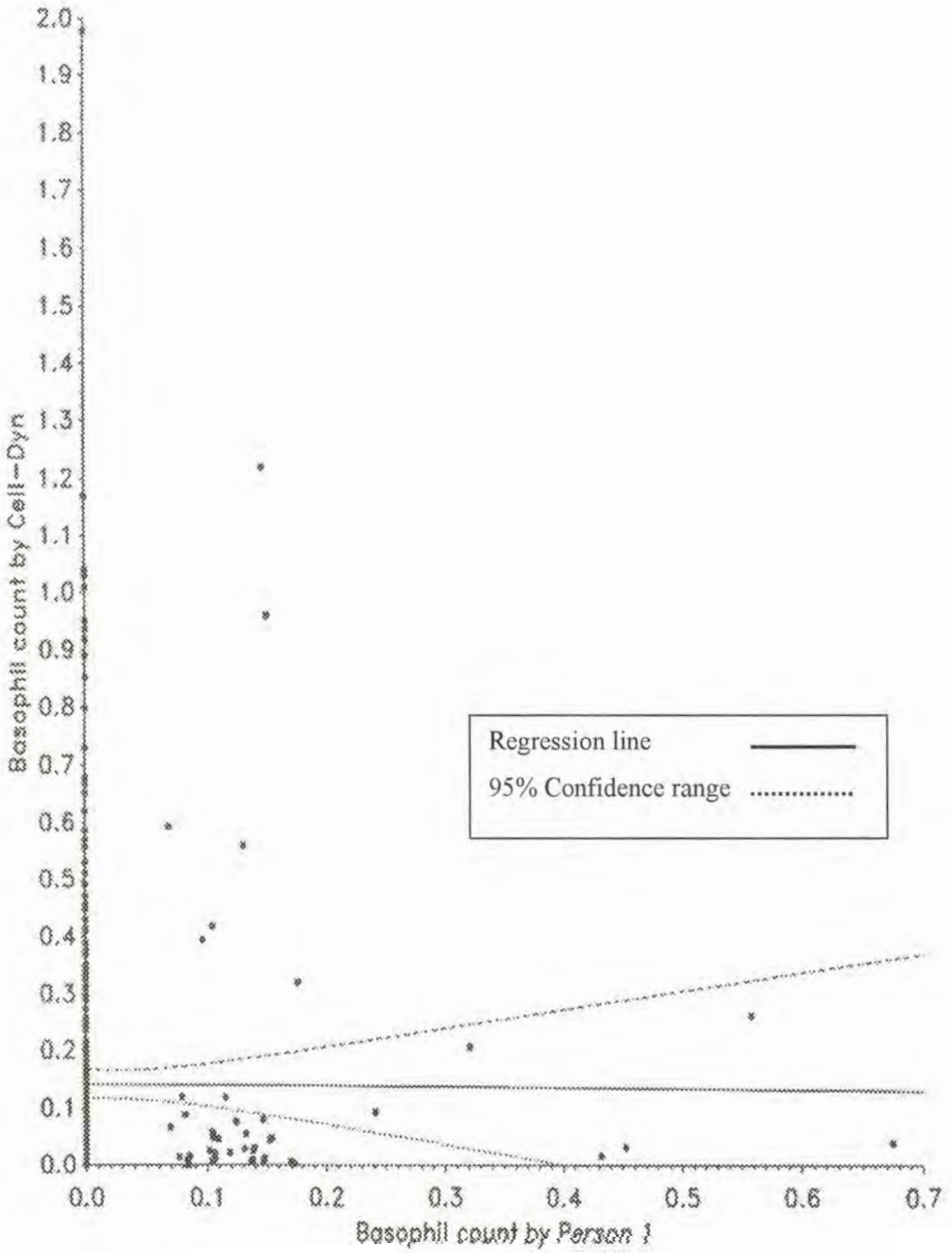
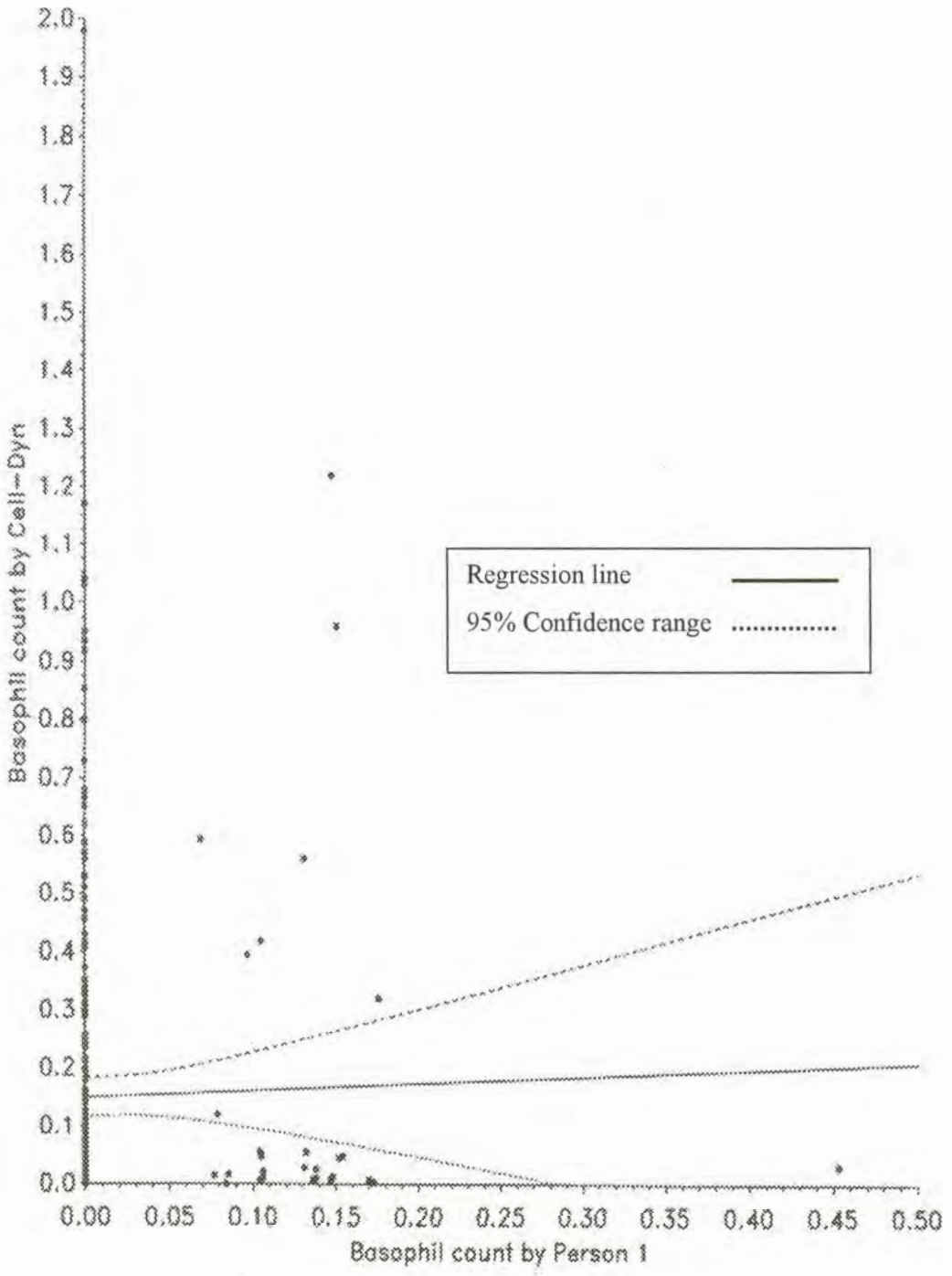


Figure 5.16: Cell-Dyn 3500 Basophil Count Compared to the Manual Basophil Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



5.2.2 Comparison of the Cell-Dyn 3500 flags with the Comments Made by the Examiners

5.2.2.1 Cell-Dyn 3500 flags for band cells and immature granulocytes compared to the presence of immature neutrophils reported by the Examiners

In 57.44% of the samples the Cell-Dyn and humans agreed about the classification of the band cells and immatures and in 42.56% of the samples there was a discrepancy. In 29.52% of the samples the Cell-Dyn 3500 reported on the presence of band or immature cells, whilst these were not noted by the examiners. In 13.04% of the samples the examiners noted band or immature cells, and the Cell-Dyn did not comment on the presence of these cells. The adjusted chi-square value is 13.131 and its p-value is 0.001, therefore there was a noticeable agreement visible in the pattern.

5.2.2.2 Cell-Dyn 3500 flags for variant lymphocytes and blasts compared to the comments of the Examiners for lymphocyte changes

In 44.64% of the samples the Cell-Dyn and humans agreed about the classification of the lymphocytes and in 55.4% of the samples there was a discrepancy. In 41.55% of the samples the examiners commented on the presence of variant lymphocytes or blasts, whilst the Cell-Dyn 3500 did not make similar comments. In 13.85% of the samples the Cell-Dyn commented on the presence of these cells, while the examiners had no such comments. The chi-square value is 1.559 and the p-value is 0.212. There is no agreement between the analyzer and the examiners in this data set.

5.2.2.3 Cell-Dyn 3500 flags for nucleated red blood cells compared to the comments of the Examiners for nucleated red blood cells

In 73.13% of the samples the Cell-Dyn and humans agreed about the presence or absence of normoblasts in the sample, in 26.87% of the samples either the Cell-Dyn 3500 reported normoblasts and the humans did not (9.70%), or the humans

reported normoblasts and the Cell-Dyn 3500 did not (17.17%). The chi-square value is 42.850 and its p-value is 0.001, therefore there was a clear agreement between the analyzer and the examiners.

5.3 LINEARITY STUDIES

5.3.1 Total Cell Count Linearity

5.3.1.1 Mean Total White Blood Cell Count

The regression equation is TCC = 0.229DF + 0.235. The correlation coefficient is 0.999 and the p-value is 0.0001. Figure 5.17 illustrates the good linear relationship for total white blood cell counts

5.3.1.2 Total Cell Counts of Individual Samples

In Figure 5.18 it is clear that the linearity of the total white blood cell counts of individual samples was not always as good as that of the mean of the total white blood cell counts. A similar pattern was observed with the neutrophil-, lymphocyte-, monocyte-, eosinophil- and basophil linearity studies amongst individual samples. However, the mean total white cell count of a number of samples is used to give an overall evaluation of the instrument.

Figure 5.17: Mean Total White Blood Cell Count of the Cell-Dyn 3500 against Dilution Factor.

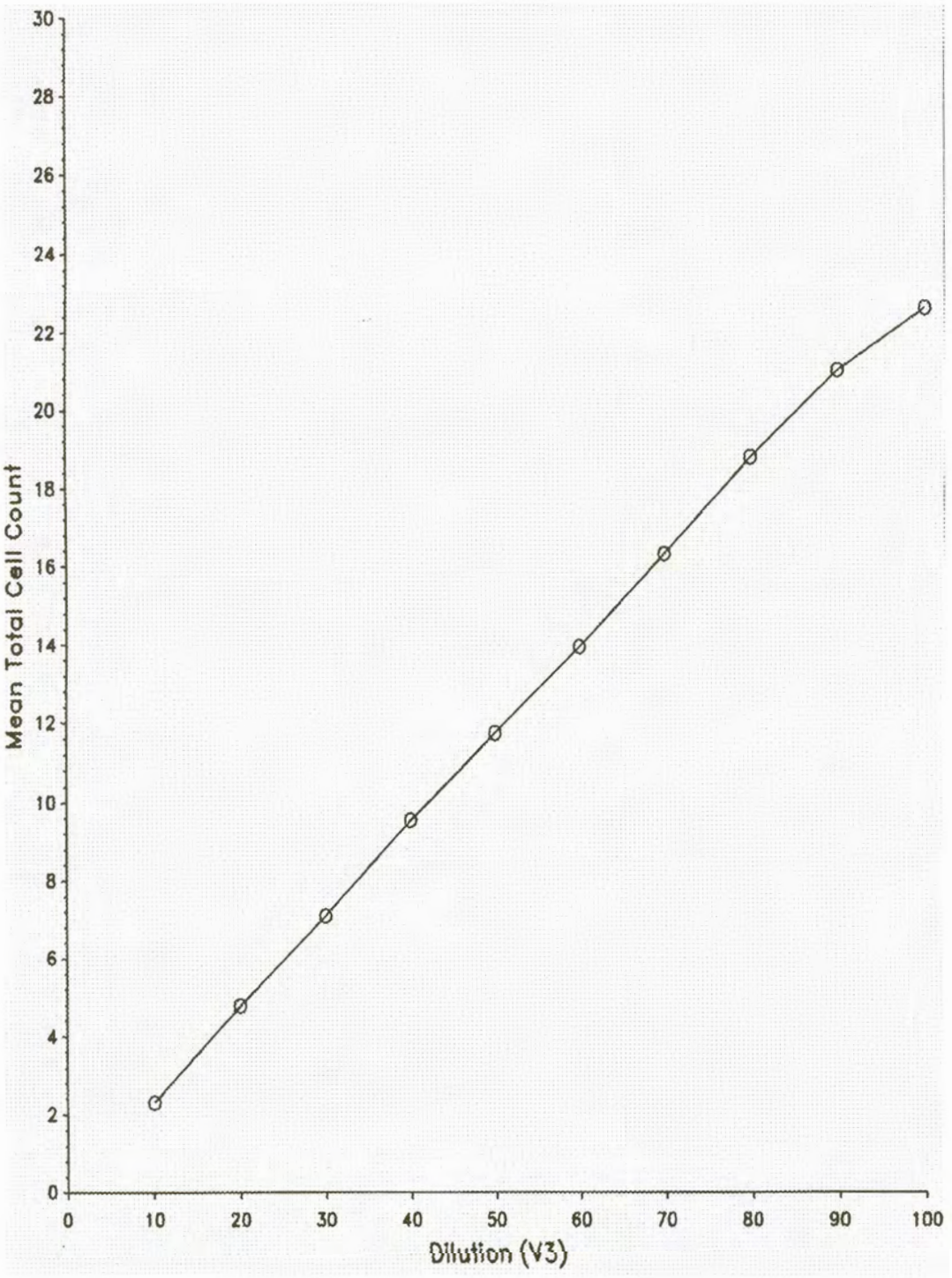
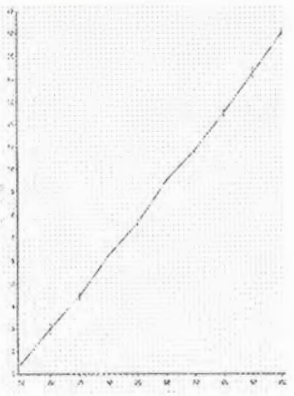
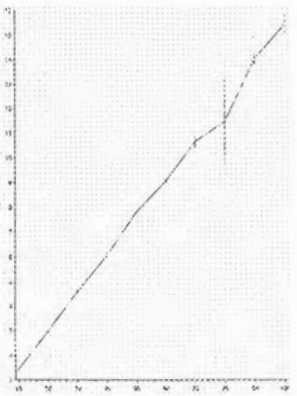


Figure 5.18: Individual Total White Blood Cell Counts of the Cell-Dyn 3500 on Ten Samples against Dilution Factor.

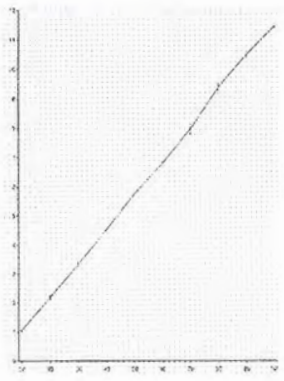
Sample 1



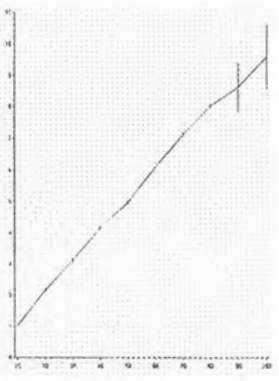
Sample 2



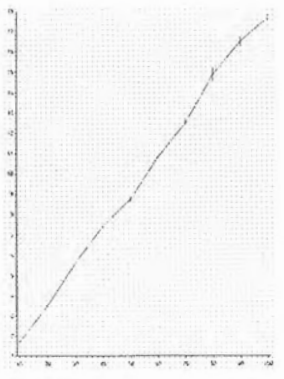
Sample 3



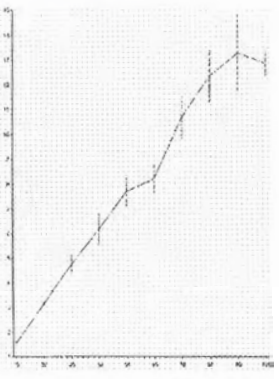
Sample 4



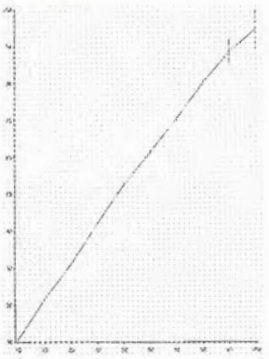
Sample 5



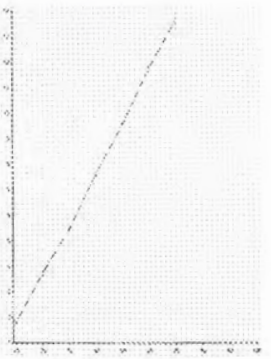
Sample 6



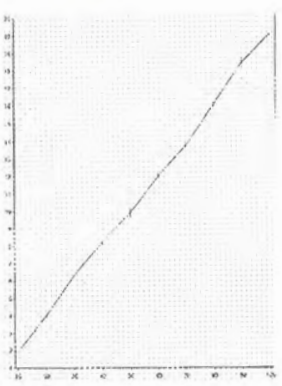
Sample 7



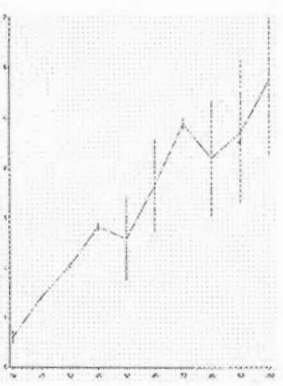
Sample 8



Sample 9



Sample 10



5.3.2 Neutrophil Count Linearity

5.3.2.1 Mean Neutrophil Count

The regression equation is Neutrophil count = 0.199DF + 0.362. The correlation coefficient is 0.994 and the p-value is 0.0001. Figure 5.19 illustrates that the linear relationship is good over the range of 10% to 90% dilution, but that there is a change in the slope between 90% and 100%.

5.3.3 Lymphocyte Count Linearity

5.3.3.1 Mean Lymphocyte Cell Count

The regression equation is Lymphocyte count = 0.016DF + 0.095. The correlation coefficient is 0.957 and the p-value is 0.0001. Figure 5.20 illustrates that although there is a good overall linear relationship, there is some distribution of points around the line.

5.3.4 Monocyte Count Linearity

5.3.4.1 Mean Monocyte Count

The regression equation is Monocyte count = 0.003DF + 0.025. The correlation coefficient is 0.863 and the p-value is 0.0001. Figure 5.21 illustrates a fairly good linear relationship for the lower dilution factors, but at 80% to 100% the values behave erratically.

5.3.5 Eosinophil Count Linearity

5.3.5.1 Mean Eosinophil Count

The regression equation is Eosinophil count = 0.002DF + 0.052. The correlation coefficient is 0.731 and the p-value is 0.0016. There is thus a poor linear relationship, which is demonstrated in Figure 5.22.

5.3.6 Basophil Count Linearity

5.3.6.1 Mean Basophil Count

The regression equation is Basophil count = 0.0003DF + 0.011. The correlation coefficient is 0.712 and the p-value is 0.0022. A very poor linearity is observed and this is illustrated in Figure 5.23.

Figure 5.19: Mean Neutrophil Count of the Cell-Dyn 3500 Vs Dilution Factor.

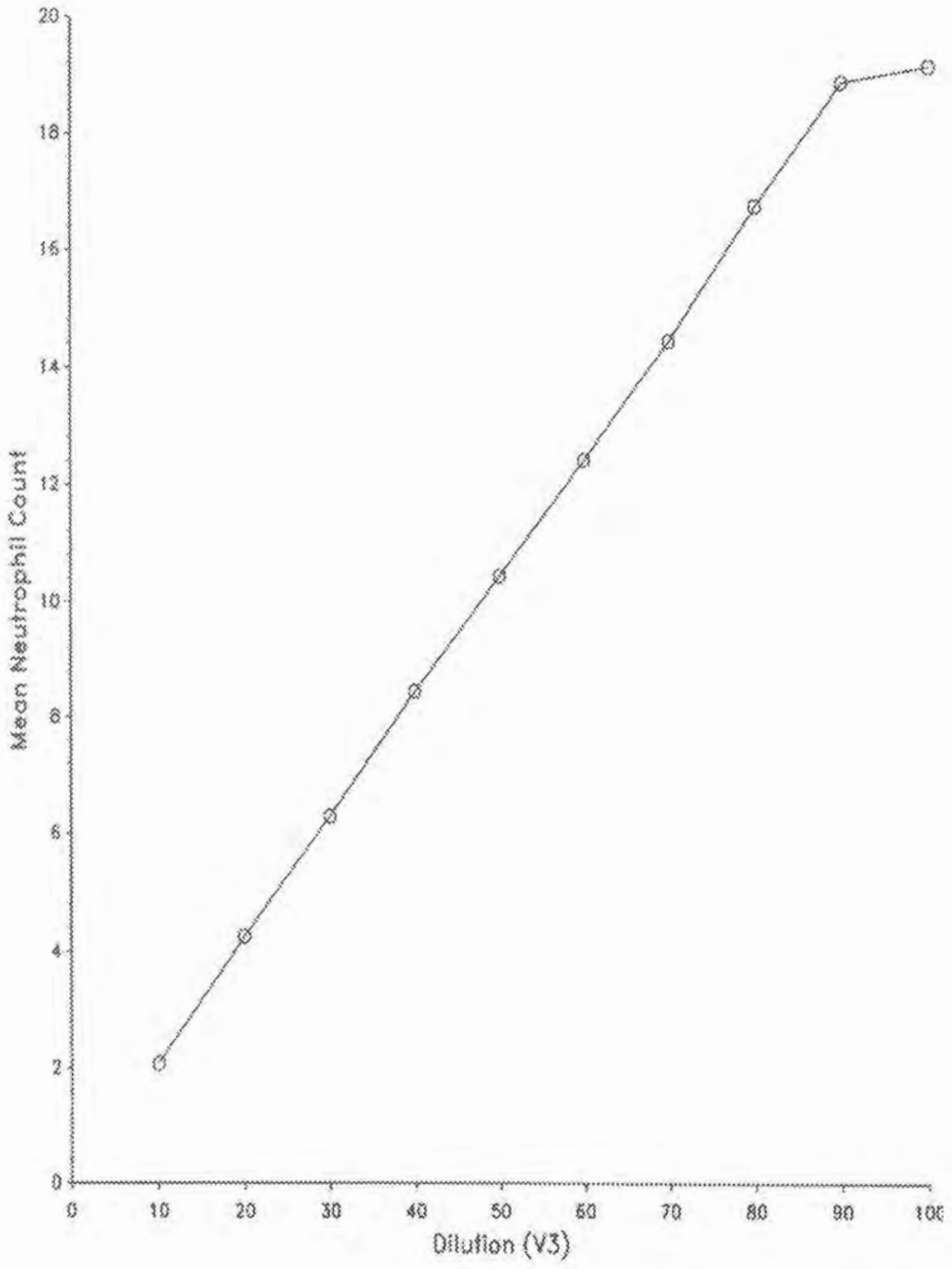


Figure 5.20: Mean Lymphocyte Count of the Cell-Dyn 3500 Vs Dilution Factor.

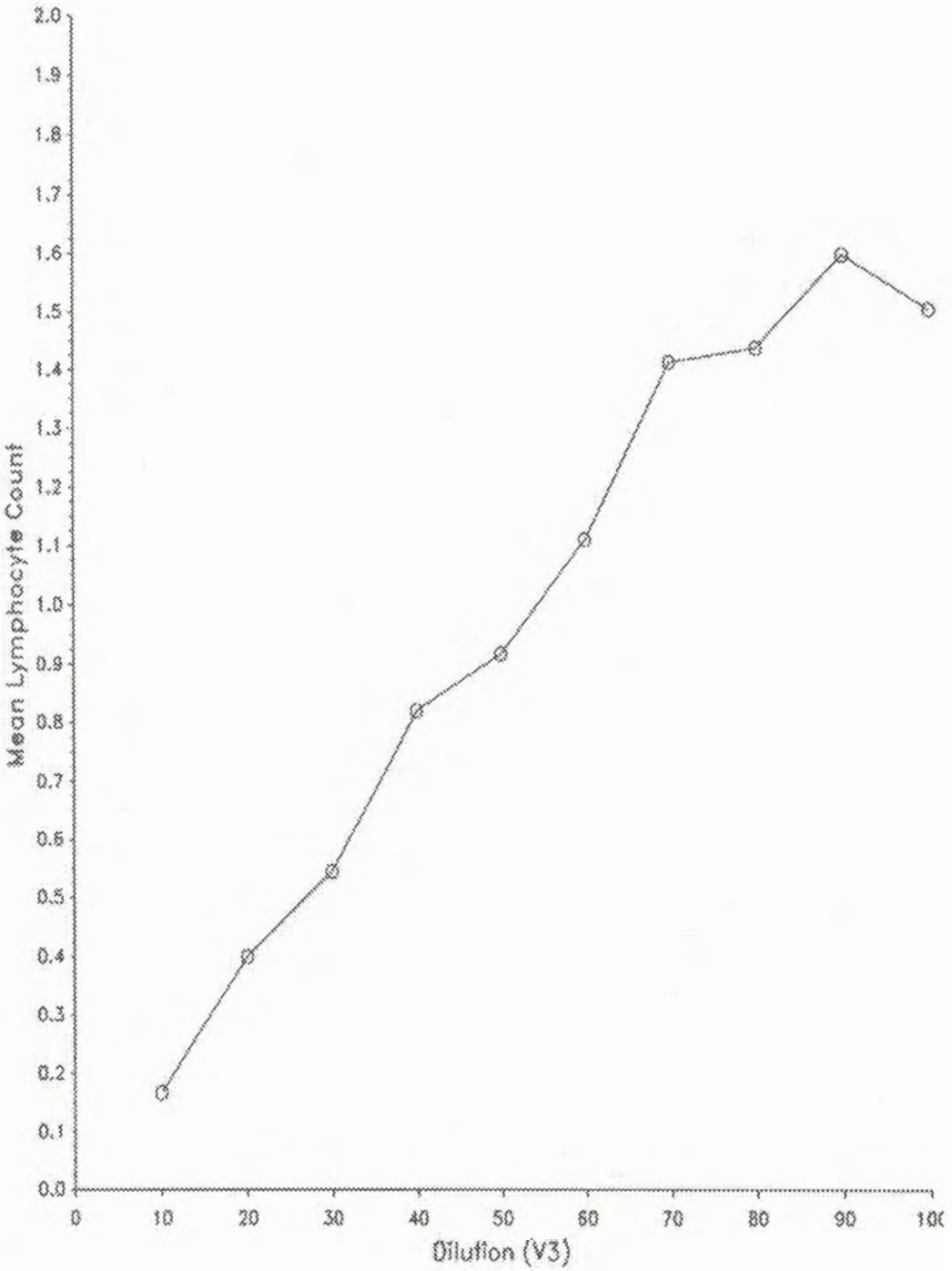


Figure 5.21: Mean Monocyte Count of the Cell-Dyn 3500 Vs Dilution Factor.

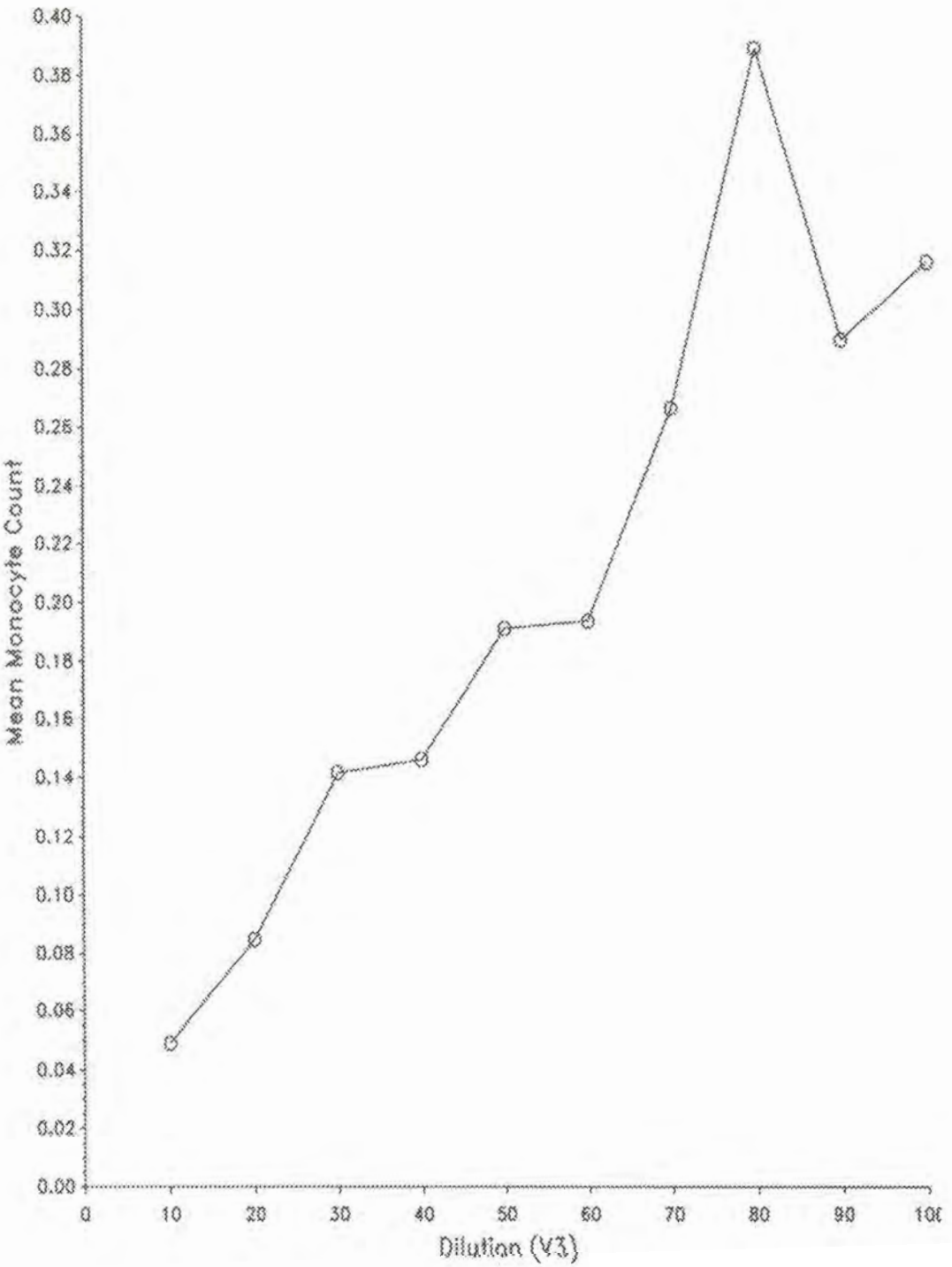


Figure 5.22: Mean Eosinophil Count of the Cell-Dyn 3500 Vs Dilution Factor.

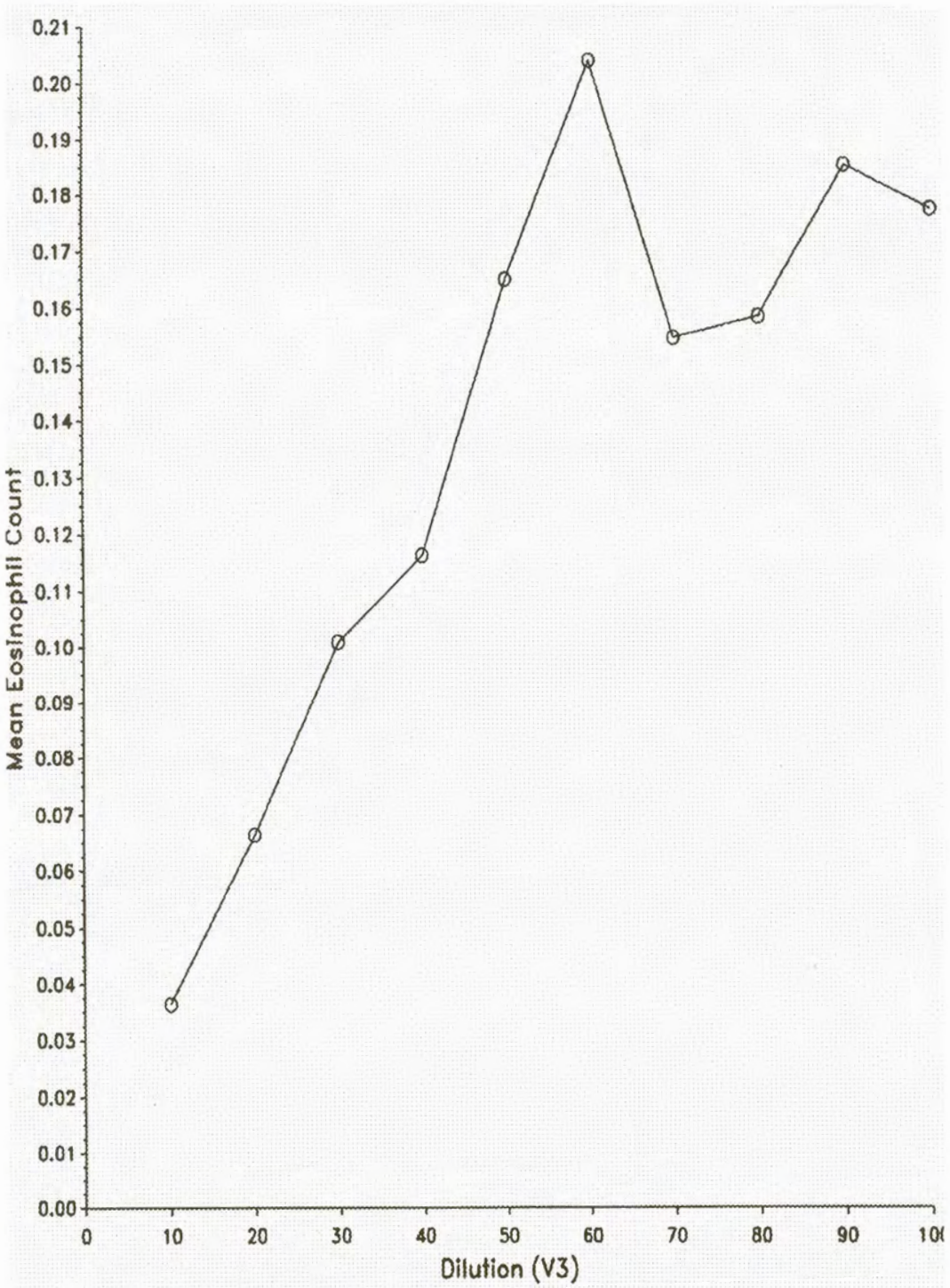
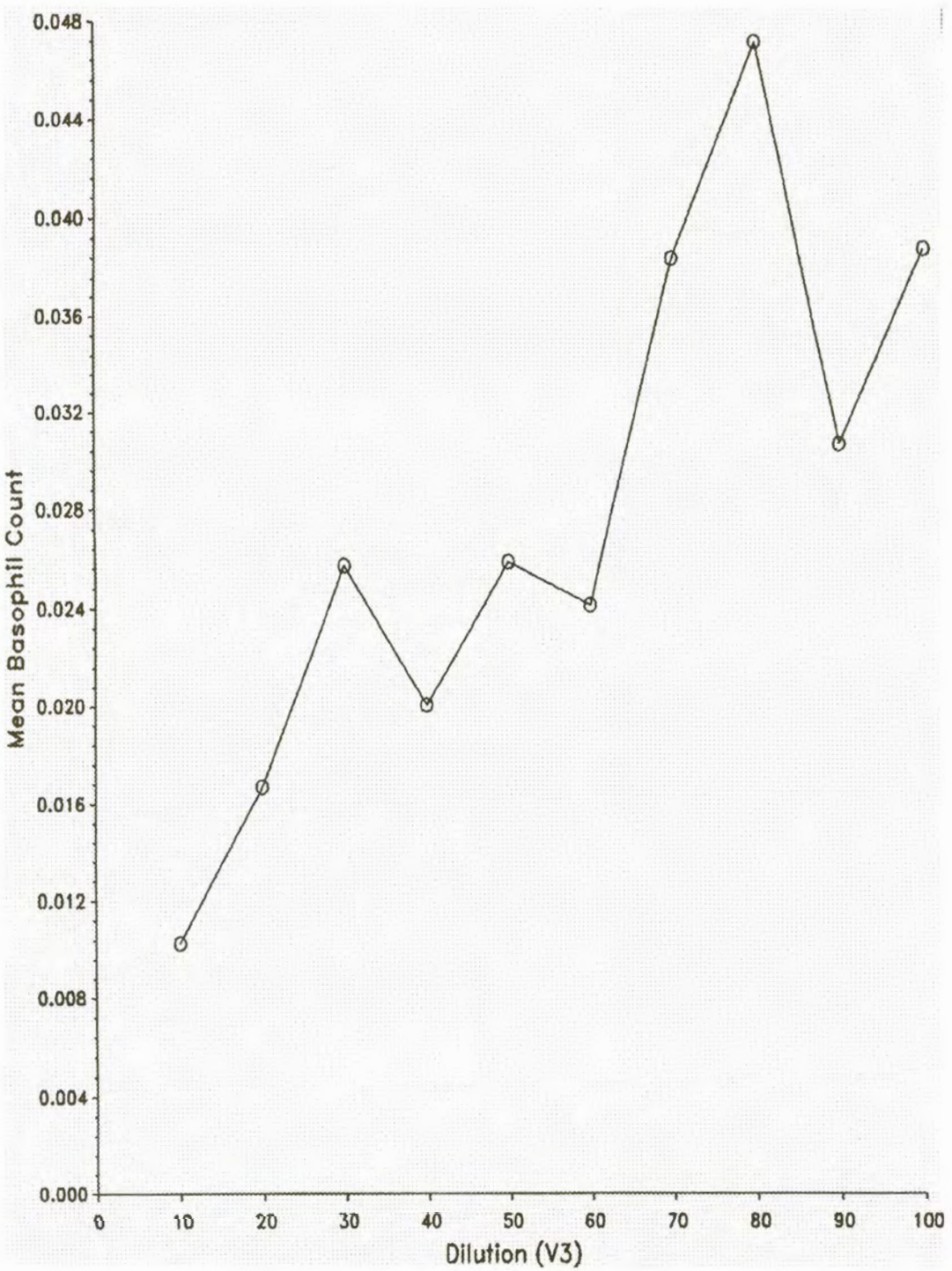


Figure 5.23: Mean Basophil Count of the Cell-Dyn 3500 Vs Dilution Factor.



5.4 CARRY-OVER ASSESSMENT

The calculated K-values were significantly less than 5%, 2% and 1% (see Table 5.4), the p-values being 0.0001. Therefore there is no significant carry-over observed in the Cell-Dyn 3500.

Table 5.4 Calculated K-values for Carry-Over Assessment

Sample	*K-value*
1	-0.004833
2	-0.000119
3	0.004463
4	0.002844
5	0.003654
6	0.003745
7	-0.001683
8	-0.041667
9	0.002212
10	-0.005730
11	0.006815
12	0.003813

5.5 PRECISION STUDIES

The statistical analysis of these data revealed that there might be a problem with the data. On further examination it became clear that there was a problem with the data entered into the computer. It was impossible to correct these data and repeat the analysis, without making certain assumption that could not be validated. Therefore it was decided to leave this section out, rather than to report dubious results.

CHAPTER 6: DISCUSSION AND CONCLUSION

6.1 COMPARISON OF THE TOTAL WHITE BLOOD CELL COUNTS

The total white blood cell count of the Cell-Dyn 3500 compared to the Baker System 9000 showed a statistically significant difference. However, this could possibly be explained by a calibration difference, since it is mainly due to a slope difference, which is sensitive to proportional error. Each instrument was calibrated using specific calibration materials, as prescribed by the respective manufacturers. Another factor that could have contributed to the slope difference is the von Behrens Plate used in the Cell-Dyn 3500. This plate prevents white blood cells in the impedance channel from swirling around and re-entering the sensing zone to be counted a second time and cause a false elevation in the white blood cell count. This technology is not used in the Baker System 9000, and could therefore explain the higher total white cell count of the Baker System 9000. Although the mean difference between the two instruments was statistically significant, there did not appear to be a clinically significant difference between the individual values overall. The mean difference was 2.70 and this was a 16.95% difference from the total white cell count of the Cell-Dyn 3500. The two instruments showed an excellent correlation, with the correlation coefficient being 0.989.

Calculation of the sensitivity ratio (SR) as described by Mandel[22] indicates that the two instruments are almost equally sensitive with respect to the accuracy of their total white blood cell counts. The SR for the total white blood cell counts, with the Baker System 9000 as the reference method is 0.982 and therefore very close to 1. Therefore, although only by a very small margin, this indicates that the Cell-Dyn 3500 is more accurate regarding the total white cell count.

Re-evaluation of the data after samples with "WBC Diff Alert", "WBC Count Alert" and "WBC Data Invalid" flags were omitted did not change the interpretation. The mean difference reduced slightly when samples with "WBC Count Alert" flags were omitted

from -2.703 to -2.355, and when samples with "WBC Data Invalid" flags were omitted from -2.703 to -2.382.

The total white blood cell count of the Cell-Dyn 3500 compared to the Coulter Model FN also showed a statistically significant difference. This could be explained partially by a calibration difference, since there is a slope difference and each instrument was calibrated using specific calibration materials, as prescribed by the respective manufacturers. The von Behrens Plate used in the Cell-Dyn 3500 would also have contributed to the higher counts seen with the Coulter Model FN. However, the intercept is -1.616. This could be explained by the fact that the Cell-Dyn 3500 does not count nucleated red blood cells, unlysed red blood cells, giant platelets and platelet clumps as white blood cells due to the advanced technology it uses. The Coulter Model FN is a very old haematology analyzer that uses impedance counting only and these cells and particles will be counted as part of the white blood cell count. Both the Cell-Dyn 3500 and the Coulter Model FN make use of co-incidence correction. The Coulter Model FN does not use digital technology, and may thus overcorrect. Although the mean difference between the two instruments was statistically significant, there did not appear to be a clinically significant difference between the individual values overall. The mean difference was 3.03 and this was a 19.02% difference from the total white cell count of the Cell-Dyn 3500. The two instruments showed a very good correlation, with the correlation coefficient being 0.968.

The SR for the two instruments, using the Coulter Model FN as the reference method is 0.962. This indicates that the two methods are almost equally sensitive, but the Cell-Dyn 3500 is slightly more sensitive than the Coulter Model FN with respect to the total white blood cell count.

These findings correspond well with the manufacturer's claims for the Cell-Dyn 3500, who reported a correlation coefficient of >0.96 for canine white blood cell counts when compared to the Coulter S+IV®[h] [1].

[h] Coulter Electronics, Inc

6.2 DIFFERENTIAL LEUKOCYTE COUNT COMPARISONS

6.2.1 Total Neutrophil Count of the Cell-Dyn 3500 compared to the 400 manual cell count

There is a statistically significant difference between the neutrophil counts of the Cell-Dyn 3500 and the 400 manual cell count, the mean difference being 1.224, with the Cell-Dyn 3500 obtaining the marginally higher count. However, there is a very small slope difference and the intercept is close to zero. The two methods correlate very well, the correlation coefficient being 0.981. This is much better than the manufacturer's claim for canine neutrophil counts compared to manual counts, which is >0.76. The reason for this improved correlation might be due to the 400 manual cell count that was used in this study, compared to the 100 manual cell count used for the manufacturer's claims, as the 100 manual cell count has been shown to be an imprecise method[3, 42, 43, 47]. The SR for the two methods is 0.993, indicating that they are essentially equally sensitive for neutrophil counts.

This also compares well to findings in similar studies were the Cell-Dyn 3500 was evaluated for its usefulness in the human medical field. The correlation coefficient for neutrophils using the Cell-Dyn 3500 and a manual counting method was 0.974 in a study by Fournier *et al*[31] on adult and pediatric patients, a correlation coefficient of 0.936 was reported in a similar study by Vives-Corrons *et al*[77] and in a study by Chow and Leung[17] a correlation coefficient of 0.994 was found. In a study by Sanzari *et al*[63] the correlation coefficient was 0.933. It also compares well to the Technicon H*1 in its ability to do neutrophil counts in dogs, where the correlation coefficient for neutrophils using the Technicon H*1 and a 400 manual cell count was 0.949[22].

One should however bear in mind that neutrophils constitute approximately 75% of the total white cell count in dogs. This would cause even fairly serious errors in the white cells with low normal counts (such as the basophils and eosinophils) to have very little

effect on the neutrophil data. In other species, such as cattle and horses, where the neutrophils represents a smaller percentage of the total white cell population, errors in other cell types will have a far more significant effect on the neutrophil data.

The omission of samples with "WBC Diff Alert" and "WBC Count Alert" flags did not appear to have much influence on the comparison of the Cell-Dyn 3500 with the manual neutrophil count. The only improvement was in the intercept, which changed from 0.239 to 0.024. This suggests that there were some individual outliers far from the mean value. These values tend to have a "fulcrum-like" effect on the intercept.

6.2.2 Total Lymphocyte Count of the Cell-Dyn 3500 compared to the the 400 manual cell count

There is a statistically significant difference between the lymphocyte counts of the Cell-Dyn 3500 and the 400 manual cell count, the mean difference being -0.325. The correlation between the two methods is fair, the correlation coefficient being 0.782. This is slightly better than the manufacturer's claim for canine lymphocyte counts compared to manual counts, which is >0.70. The reason for this improved correlation might be due to the 400 manual cell count that was used in this study, compared to the 100 manual cell count used for the manufacturer's claims (refer to 6.2.1). The SR for the two methods is 0.876, indicating that the Cell-Dyn 3500 is slightly more accurate for lymphocyte counts. The reason for this is most likely the larger number of cells counted by the Cell-Dyn 3500.

This does not compare well to findings in similar studies were the Cell-Dyn 3500 was evaluated for its usefulness in the human medical field. The correlation coefficient for lymphocytes using the Cell-Dyn 3500 and a manual counting method was 0.967 in a study by Fournier *et al*[31] on adult and pediatric patients, a correlation coefficient of 0.916 was reported in a similar study by Vives-Corrons *et al*[77] and in a study by Chow and Leung[17] a correlation coefficient of 0.885 was found. In a study by Sanzari *et al*[63] the

correlation coefficient was 0.890. It also does not compare well to the Technicon H*1 in its ability to do lymphocyte counts in dogs, where the correlation coefficient for lymphocytes using the Technicon H*1 and a 400 manual cell count was 0.903[22]. However in another study done by Tvedten[73] a correlation coefficient of 0.77 was found for canine lymphocytes evaluated on the Technicon H*1, which corresponds well with the finding in this study (correlation coefficient was 0.782). Lymphocytes constitute a relatively low percentage of the total white cell count in dogs, while in humans, lymphocytes are usually quite high. Because of the low number of lymphocytes in dogs, the count can easily be affected by errors in other cell types.

In a study by Wood *et al*[82] on human blood samples, lymphocyte counts increased in samples stored at room temperature over a 24 hour period. However, storage at 4°C caused a slight decrease in lymphocyte counts on the Cell-Dyn 3500[82]. Although the samples in this study were not kept for more than 4 hours at room temperature before analysis, transport conditions of samples, not collected at the Veterinary Academic Hospital, could have affected samples and have influenced lymphocyte counts in this way. However, in the study by Wood *et al*[82] the manual differential count showed similar changes when blood was stored at room temperature.

The omission of samples with "WBC Diff Alert" and "WBC Count Alert" flags did not improve the comparison of the Cell-Dyn 3500 with the manual lymphocyte count. The correlation coefficient is in fact better before correction (0.782) than after correction (0.710). Both the intercept and slope also gave slightly poorer results, changing from 0.163 to 0.298 and from 0.753 to 0.663 respectively.

6.2.3 Total Monocyte Count of the Cell-Dyn 3500 compared to the 400 manual cell count

There is a statistically significant difference between the monocyte counts of the Cell-Dyn 3500 and the 400 manual cell count. The mean difference was -0.605 and this was a

103.24% difference from the monocyte count of the Cell-Dyn 3500. The correlation between the two methods is extremely poor, the correlation coefficient being 0.097. This is in agreement with the manufacturer's claim for canine monocyte counts compared to manual counts, where no claim for correlation is made. The reason for this poor correlation might be due to the fact that canine monocyte counts are often so low that a variation between 1% and 3% seems statistically great, and secondly monocytes are not evenly distributed on a blood smear which means that they might be under-represented with manual cell counts[73]. The one cluster of cells corresponds fairly well to the line of ideal agreement (Figure 5.11), which indicates that the instrument is probably identifying these cells correctly, but is also counting some other cells as monocytes, which are not monocytes. The SR for the two methods is 0.183, indicating that the Cell-Dyn 3500 is much more sensitive for monocyte counts than the manual cell count. The reason for this is probably the larger number of cells counted by the Cell-Dyn 3500.

This does not compare well to findings in similar studies were the Cell-Dyn 3500 was evaluated for its usefulness in the human medical field. The correlation coefficient for monocytes using the Cell-Dyn 3500 and a manual counting method was 0.628 in a study by Fournier *et al*[31] on adult and pediatric patients, a correlation coefficient of 0.799 was reported in a similar study by Vives-Corrons *et al*[77] and in a study by Chow and Leung[17] a correlation coefficient of 0.765 was found. In a study by Sanzari *et al*[63] the correlation coefficient was 0.362. In a study done by Goossens *et al*[32] comparing the human monocyte counts on 6 different instruments with an 800 manual cell count, the correlation coefficients varied from 0.871 to 0.203. It is suggested that a technique using fluorescent labelled monoclonal antibodies should be used as a reference method [17, 32, 63].

The Technicon H*1 compares well in its ability to do monocyte counts in dogs with these results obtained on the Cell-Dyn 3500. The correlation coefficient for monocytes using the Technicon H*1 and a 400 manual cell count was 0.050[7]. In another study done by Tvedten[73] a correlation coefficient of 0.180 was found for canine monocytes evaluated on the Technicon H*1 and this also corresponds fairly well to the finding in the current study.

The omission of samples with "WBC Diff Alert" and "WBC Count Alert" flags does not improve the comparison of the Cell-Dyn 3500 with the manual monocyte count. All the parameters used for the comparison were poorer after omission of these samples than before. The correlation coefficient changed from 0.097 to 0.039, the mean difference changed from -0.605 to -0.817, the slope changed from 0.228 to 0.116 and the intercept changed from 0.315 to 0.378.

6.2.4 Total Eosinophil Count of the Cell-Dyn 3500 compared to the 400 manual cell count

There is a statistically significant difference between the eosinophil counts of the Cell-Dyn 3500 and the 400 manual cell count, the mean difference being -0.407. This was a 176% from the mean eosinophil count of the Cell-Dyn 3500. The correlation between the two methods is poor, the correlation coefficient being 0.304. This is much worse than the manufacturer's claim for canine eosinophil counts compared to manual counts, which is >0.70. The SR for the two methods is 0.552, indicating that the Cell-Dyn 3500 is more sensitive for eosinophil counts. However, a closer look at Figure 5.13 reveals that there is a distinct population of cells not counted by the Cell-Dyn 3500 as eosinophils, which are recognized by the examiners as eosinophils. Therefore the Cell-Dyn 3500 is not more sensitive in counting eosinophils but underestimates them. However the one cluster of cells correspond well to the line of ideal agreement (Figure 5.13) and these cells represent the correctly identified cells. It is not clear to the author which cells were incorrectly identified as eosinophils by the Cell-Dyn 3500. Abbott Laboratories has recently released the Cell-Dyn 3700, with new veterinary software, which is claimed to have an improved ability to count canine eosinophils.

This does not compare well to findings in similar studies were the Cell-Dyn 3500 was evaluated for its usefulness in the human medical field. The correlation coefficient for eosinophils using the Cell-Dyn 3500 and a manual counting method was 0.880 in a study by Fournier *et al*[31] on adult and pediatric patients, a correlation coefficient of 0.967 was

reported in a similar study by Vives-Corrons *et al*[77] and in a study by Chow and Leung[17] a correlation coefficient of 0.877 was found. In a study by Sanzari *et al*[63] the correlation coefficient was 0.812. It also does not compare well to the Technicon H*1 in its ability to count eosinophils, where the correlation coefficient for eosinophils using the Technicon H*1 and a 400 manual cell count was 0.804[22]. In another study done by Tvedten[73] a correlation coefficient of 0.87 was found for canine eosinophils evaluated on the Technicon H*1.

The omission of samples with "WBC Diff Alert" and "WBC Count Alert" flags improved the comparison of the Cell-Dyn 3500 with the manual eosinophil count significantly. The correlation coefficient was increased to 0.592. However this still does not compare favourably with the Technicon H*1 or the correlation coefficient for eosinophils using human blood samples.

The difference in the Cell-Dyn 3500's ability to correctly identify human and dog eosinophils probably lies in the fact that the granules in these two eosinophil-types differ. The amount of 90° depolarized light scatter is used to identify the eosinophils[1]. Human eosinophils have small round granules that fill the cytoplasm of the cell. Dog eosinophils have large round granules and often there are only a few granules in each cell. This can explain the difference in the amount of light scattered and therefore the ability to recognize these cells.

The Technicon H*1 makes use of the staining reaction of the cells with myeloperoxidase[72]. Eosinophils show intense peroxidase activity[22]. The difference in the method used for the recognition of these cells can therefore explain the difference in the ability of the Cell-Dyn 3500 and the Technicon H*1 to identify these cells.

The inability of the Cell-Dyn 3500 to correctly identify eosinophils probably does not have great clinical significance, as there are very few cases in which the miscounting of eosinophils will lead to incorrect treatment of a patient. Conditions where eosinophilias

are of diagnostic importance are: parasitisms, hypersensitiwities and certain neoplastic conditions[30, 41].

6.2.5 Total Basophil Count of the Cell-Dyn 3500 compared to a 200 manual cell count

There is a statistically significant difference between the basophil counts of the Cell-Dyn 3500 and the 200 manual cell count. The mean difference was 0.122 and this was an 88% difference from the mean basophil count of the Cell-Dyn 3500. There is no correlation between the two methods, the correlation coefficient being 0.000.

This is in line with the manufacturer's claim for canine basophil counts compared to manual counts, where no claim for correlation is made. It is not clear to the author what causes this discrepancy. One factor that plays a role is the fact that there are so few basophils in canine blood samples that it would be very difficult to count enough cells in order to obtain statistically significant values. Another possibility is the fact that the recognition of basophils by humans is based on the recognition of the basophilic granules, while the Cell-Dyn 3500's recognition is not based on the presence of the granules at all, since they are water soluble and are dissolved in the sheath solution[1]. Basophilic granules in dogs are difficult to see, which is clearly illustrated by the fact that the one examiner missed all the basophils. This could indicate that human examiners underestimate basophil counts dramatically. On the other hand the Cell-Dyn 3500 could be overestimating the basophil counts, because other cell types may fall into the category identified by the Cell-Dyn 3500 as basophils.

Even in studies were the Cell-Dyn 3500 was evaluated for its usefulness in the human medical field, the basophil counts did not correlate well. The correlation coefficient for basophils using the Cell-Dyn 3500 and a manual counting method was 0.410 in a study by Fournier *et al*[31] on adult and pediatric patients, a correlation coefficient of 0.399 was reported in a similar study by Vives-Corrons *et al*[77] and in a study by Chow and Leung[17]

a correlation coefficient of 0.387 was found. However, in a study by Sanzari *et al*[63] the correlation coefficient was 0.656. In another study by Bentley *et al*[6] on four different automated haematology analyzers, it was found that all four the analyzers overestimated basophil counts by factors ranging from 73% to 150%. The correlation coefficient for basophil counts were not reported in the studies done on the evaluation of the Technicon H*1 for its ability to do accurate leukocyte differential counts in dogs. An interesting phenomenon was seen in a study by Wood *et al* in which they found that basophil counts increased with the storage of samples, both at room temperature and in refrigerated samples[82]. This could be a factor contributing to the inaccuracy of the basophil counts by the CELL-DYN 3500.

The omission of samples with "WBC Diff Alert" and "WBC Count Alert" flags did not improve the comparison of the Cell-Dyn 3500 with the manual basophil count. The correlation coefficient improved from 0.000 to 0.005 and the slope improved from -0.017 to 0.117. However, the intercept changed from 0.142 to 0.150 and the mean difference changed from 0.122 to 0.136.

The inability of the Cell-Dyn 3500 to correctly identify basophils does not have any clinical significance as there are very few, if any, cases in which the miscounting of basophils will lead to the incorrect diagnosis or treatment of a patient. Basophilias are associated with hypersensitiwities and lipaemic conditions and the presence of basophilia is not necessary for the diagnosis of these conditions. The only potential problem would be that clinicians could be misled into believing that a patient has a basophilia, while it in fact does not have one. However, if clinicians are made aware of this problem, there should be no misinterpretations.

6.2.6 Comparison of the Cell-Dyn 3500 flags with the Comments Made by the Examiners

The Cell-Dyn 3500 and examiners agreed about the presence or absence of band cells and immature granulocytes in about 57% of the samples, which is a very poor agreement. The sensitivity was 70.78% and the specificity was only 46.70%. The false positive rate was 29.52%, which correlate well with a study by Iles-mann and Henniker[36], where they found a false positive rate of 20.4% for the band flag in human patients. In a study done by Fournier *et al*[31] the sensitivity and specificity of the flags given by the Cell-Dyn 3500 for immature granulocytes in human patients were 72% and 76% respectively. Sanzari *et al*[63] found a false negative ratio of 37.5% in a study done on 259 blood samples from human origin, which is not as good as the false negative ratio found in this study, which was 13.04%.

The poor agreement is not too surprising, as the distinction between band cells and mature neutrophils is a problem area in haematology[5]. Although the band cell count is a useful indicator of acute inflammation if clearly defined uniform criteria are used, it remains one of the common leukocyte recognition errors in practice[5, 29, 43]. Therefore it is clear that the manual band cell count can hardly be regarded as a standard for the evaluation of machine recognition. Another possible reason for this discrepancy lies in the fact that the examiners and the Cell-Dyn 3500 used different methods to identify band cells. The examiners' identification is mainly based on the morphology of the nucleus. The Cell-Dyn 3500 bases its identification of immature granulocytes on the presence of cells in a predetermined area on the 10°/0° scatter plot. This positioning is based on size and complexity and not on nuclear shape.

There was a poor correlation between the flags generated by the Cell-Dyn 3500 and the examiners regarding the presence of variant lymphocytes and blasts. The sensitivity was 24.25% and the specificity 69.32%. Vives-Corrons *et al*[77] found in a study at a human hospital that the sensitivity of the Cell-Dyn 3500 for variant lymphocytes was 50% and

the specificity was 90.76%, while it was 67% and 92.39% respectively for blast cells. In a study by Sanzari *et al*[63] they found a efficiency of 87.3%, a false positive ratio of 12.24% and a false negative ratio of 21.43%. The false positive ratio in this study was 13.85%, thus in agreement with Sanzari *et al*'s study. However, the false negative ratio in this study was 41,55%, much worse than that seen in the study of Sanzari *et al*.

Bentley *et al* observed, in a study evaluating four automated differential cell counters, including the Cell-Dyn 3000, that qualitative flagging was an area in which all four instruments showed poor performance[6]. Bentley feels that there is considerable doubt about these analyzers' abilities to identify specimens with qualitative leukocyte abnormalities[6].

The presence of normoblasts reported by the Cell-Dyn 3500 correlated well with the comments made by the examiners. In about 73% of the samples they were in agreement about the presence or absence of normoblasts. In 17% of the samples the examiners reported the presence of normoblasts where the Cell-Dyn 3500 failed to do so. However, this never happened in samples where the examiners reported a normoblast count of 10 or more per 100 leukocytes. The sensitivity was only 36,08%, but the specificity was 86.79%. This compares very well to the study that Fournier *et al*[31] had done where they found the sensitivity of the Cell-Dyn 3500 to be 33% and the specificity 98%. It also compares well with the sensitivity of 30.0% and specificity of 96.2% found in a study by Sanzari *et al*[63].

The NRBC flag is given when there is a difference between the WIC and WOC count that exceeds the expected limit, or if the count in the area below the white cell threshold on the 10°/0° scatter plot exceeds 5% of the total white cell count[1]. The cells in this area could include nucleated red blood cells, platelet clumps, giant platelets and abnormally shaped red cells[1]. The examiners will only identify nucleated red blood cells, and therefore the technique used by the Cell-Dyn 3500 can explain the false positives (9.7%).

The lack of sensitivity can be attributed to the fact that the Cell-Dyn 3500 only gives this flag once the area on the 10°/0° scatter plot exceeds 5%. Therefore small numbers of normoblasts will not be reported. This is also confirmed by the fact that the Cell-Dyn 3500 always gave this flag when the examiners reported 10 or more normoblasts per 100 white blood cells.

6.3 LINEARITY STUDIES

The Cell-Dyn 3500 showed excellent linearity for the total white blood cell count, with a correlation coefficient of 0.999. It must be noted that the intercept and slope is not expected to be 0 and 1 respectively, as the absolute counts on the y-axis are reported against the dilution factor on the x-axis, therefore not using the same units. The manufacturer does not make any claims with regards to the linearity of the total white cell counts on canine samples.

The linearities for the neutrophil count and the lymphocyte count are also very good, with correlation coefficients of 0.994 and 0.957 respectively. The linearities for the monocyte-, eosinophil-, and basophil counts are fair, with correlation coefficients of 0.863, 0.731 and 0.712 respectively. In light of the fact that the linearity for the individual leukocytes are not reported in other similar studies and that the manufacturer makes no claims with regard to the linearity of white blood cells in canine blood samples, these results can only be interpreted as being satisfactory.

6.4 CARRY-OVER ASSESSMENT

Samples with total white cell counts ranging from 0.990 x $10^9/\ell$ to 105.000 x $10^9/\ell$ were used in this study. There was no statistically significant carry-over observed for the Cell-Dyn 3500, which corresponds to the manufacturer's claim that it is <1%. This is to be expected since the instrument is thoroughly rinsed between each analysis. The sample

probe is rinsed internally and externally with diluent, while the WIC counting chamber and metering tube are rinsed with detergent, and the WOC chamber with sheath reagent[1].

6.5 PRECISION STUDIES

This was not done due to a fault that occurred in the data.

6.6 CONCLUSION

The following objectives were identified for the purpose of this project:

- To determine the accuracy of the total white blood cell count of the Cell-Dyn 3500 by comparing it to established and commonly used instruments.
- To determine the acceptability of the automated leukocyte differential counts generated by the Cell-Dyn 3500 by comparing them to the reference method as described by the National Committee for Clinical Laboratory Standards.
- To determine if the Cell-Dyn 3500 gives a linear relationship over the physiological and, usually encountered, pathological range of white blood cell counts.
- To establish if the Cell-Dyn 3500 shows any carry-over.
- To determine if the Cell-Dyn 3500 operates with sufficient precision, by running the same sample a number of times.

The Cell-Dyn 3500 performed very well with regards to the determination of the total white blood cell count and neutrophil count. It also performed favourably with regards to the lymphocyte count. The Cell-Dyn did not perform well with regards to the determination of the monocyte and eosinophil counts, however there were clusters of cells visible that did correlate well to the line of ideal agreement in both these cell types. If the problem of the "incorrectly identified cells" can be solved, the Cell-Dyn 3500's

performance with these cell types would improve significantly. The performance with regards to basophil counts was very poor, but since these cells are not of much clinical importance in canine medicine, the Cell-Dyn 3500 should not be too strongly judged on this weakness.

Overall the Cell-Dyn 3500 did not perform well with regards to the flagging of abnormal samples, with the exception of the flagging of samples for the presence of normoblasts, which was good. However, if the different flags are used as a general alert and not a specific alert, most abnormal samples would be identified as such.

The Cell-Dyn 3500's performance regarding the total white cell count, neutrophil count and lymphocyte count linearity studies was satisfactorily. The performance regarding the rest of the linearity studies was not good, but considering the problems that the instrument experienced with the identification of these cells, this is not surprising.

The carry-over assessment gave excellent results with no carry-over observed.

It can thus be stated that the Cell-Dyn 3500 performed satisfactorily as a haematology analyser for canine total and differential white cell counting in general. It should be satisfactory as a screening tool for routine samples, but microscopic evaluation of abnormal samples will still be required. It must also be mentioned that changes and improvements to the software are continually being proposed and made, as was seen with the eosinophil count and the new veterinary software available with the Cell-Dyn 3700. This makes a final conclusion about the performance of the Cell-Dyn Systems as differential cell counters and haematology analysers in veterinary laboratory medicine impossible at this stage.