The evaluation of a new haematological cell counter, the CELL-DYN 3500, on canine leukocyte differential counts

by

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SAMEVATTING

Die evaluering van 'n nuwe hematologie selteller, die Cell-Dyn 3500, op differensiële witseltellings van honde

PRINSLOO, T. Universiteit van Pretoria, 2000

Hierdie studie is onderneem om die Cell-Dyn 3500 se vermoë om differensiële witseltellings op honde bloedmonsters te doen, te evalueer. Alhoewel differensiële seltellings as deel van die meeste roetiene hematologiese ondersoeke gedoen word, is dit 'n baie tydrowende proses. Dit is dikwels 'n beperkende faktor in die aantal monsters wat daagliks deur 'n laboratorium geprosesseer kan word. Verskeie pogings is in die verlede gemaak om hierdie proses te outomatiseer. Hematologie analiseerders word egter ontwerp vir gebruik in die mediese veld en is dus nie altyd akkuraat as dit in die veterinêre veld aangewend word nie.

Die Cell-Dyn 3500 maak gebruik van laserlig weerkaatsingstegnologie om die differensiële witseltelling te outomatiseer. Die tegnologie bied 'n unieke metode van sel identifikasie en hou daarom die belofte in dat dit 'n verskeie spesies aangewend kan word.

Bloedmonsters van normale en siek honde is in die studie geëvalueer. Die monsters is gekies om 'n wye versameling van patologiese monsters in te sluit. Die differensiële witseltellings van die Cell-Dyn 3500 is vergelyk met 'n handmetode soos deur die "National Committee for Clinical Laboratory Standards" voorgeskryf.

Gevolgtrekkings wat uit die studie gemaak kan word is as volg:

Die totale witseltellings van die Cell-Dyn 3500 en die Baker System 9000 vergelyk gunstig, met 'n korrelasie koëffisïent van 0.989.

Die neutrofiel tellings van die Cell-Dyn 3500 vergelyk gunstig met die neutrofiel tellings van die handmetode, met 'n korrelasie koëffisïent van 0.981.

Die limfosiet tellings van die Cell-Dyn 3500 het redelik, maar nie baie goed met die van die handmetode vergelyk nie (korrelasie koëffisiënt 0.782), alhoewel die resultate effens gunstiger is as waarop die vervaardigers aanspraak maak (korrelasie koëffisiënt >0.70).

Die monosiet telling van die Cell-Dyn 3500 het swak vergelyk met die monosiet tellings van die handmetode (korrelasie koëffisiënt 0.097), maar dit stem ooreen met die fiet dat die vervaardigers geen aanspraak maak op korrelasie nie.

Die eosinofiel tellings van die Cell-Dyn 3500 het baie swak vergelyk met die handmetode se tellings (korrelasie koëffisiënt 0.304), wat in teenstelling is met die aanspraak van die vervaardiger (korrelasie koëffisiënt >0.70).

Die basofieltelling van die Cell-Dyn 3500 het geen korrelasie met die van die handmetode getoon nie (korrelasie koëffisiënt 0.0000). Dit is in ooreenstemming met die vervaardiger se afwesigheid van 'n aanspraak op korrelasie.

Die waarskuwings gemaak deur die Cell-Dyn 3500 oor abnormale monsters het nie goed vergelyk met die opmerkings gemaak deur die ondersoekers nie, met die uitsondering van waarskuwings vir gekernde rooibloedselle, waar daar in 73.13% van gevalle ooreenstemming was. Die identifikasie van onvolwasse neutrofiele was ook redelik, met ooreenstemming in 57.44% van die gevalle.

Die Cell-Dyn 3500 het uitstekende liniariteit getoon vir die totale witseltelling, met 'n korrelasie koëffisiënt van 0.999.

Die Cell-Dyn 3500 het geen waarneembare oordrag tussen monsters getoon nie.

SUMMARY

The evaluation of a new haematological cell counter, the CELL-DYN 3500, on canine leukocyte differential counts

PRINSLOO, T. University of Pretoria, 2000

This study was undertaken to evaluate the Cell-Dyn 3500's ability to do differential white blood cell counts on canine blood samples. Although differential cell counts are done as part of most routine haematological evaluations, it is a very time consuming process, which is a limiting factor in the number of samples that a laboratory can process daily. Various attempts have been made in the past to automate this process. However, haematological analyzers are designed for use in the human medical field and are therefore not always accurate when applied in the veterinary field.

The Cell-Dyn 3500 makes use of laser light scatter technology to automate differential white cell counting. This technology provides a unique way of identifying cells and therefore has the promise that it can be applied to various species.

Blood samples from both normal and sick dogs were evaluated in this study. The samples were chosen to include as wide a range of pathological samples as possible. The differential white cell counts of the Cell-Dyn 3500 were compared to a manual differential white cell count method prescribed by the National Committee for Clinical Laboratory Standards.

Conclusions derived from the investigation are as follows:

The total white blood cell counts of the Cell-Dyn 3500 and that of the Baker System 9000 compared favourably, with a correlation coefficient of 0.989.

The neutrophil counts of the Cell-Dyn 3500 compared well to the manual neutrophil counts, with a correlation coefficient of 0.981.

The lymphocyte counts of the Cell-Dyn 3500 compared reasonably, but not very well to the manual lymphocyte counts (correlation coefficient 0.782), although the results in this study were slightly better than the manufacturer's claims for canine lymphocyte counts, which is >0.70.

The monocyte counts of the Cell-Dyn 3500 compared very poorly to the manual monocyte counts (correlation coefficient 0.097), which is in agreement with the manufacturer's absence of a claim for correlation.

The eosinophil counts of the Cell-Dyn 3500 compared very poorly to the manual eosinopil counts (correlation coefficient 0.304), which is in contradiction to the manufacturer's claim of >0.70.

The basophil counts of the Cell-Dyn 3500 showed no correlation with the manual basophil counts (correlation coefficient 0.0000), which is in line with the manufacturer's absence of a claim for correlation.

The flags given by the Cell-Dyn 3500 did not compare well with comments made by the examiners, with the exception of flags for nucleated red blood cells, where there was agreement in 73.13% of the cases. The identification of immature neutrophils was also reasonable, with a 57.44% agreement.

The Cell-Dyn 3500 showed excellent linearity for the total white cell count, with a correlation coefficient of 0.999.

The Cell-Dyn 3500 had no detectable carry-over between samples.

CHAPTER 1: INTRODUCTION

1.1 DIFFERENTIAL LEUKOCYTE COUNT

1.1.1 Introduction

The Leukocyte Differential Count is the determination of the proportion of, or absolute count per unit volume of defined classes or subsets of leukocytes in a blood sample[41, 42, 51]. The purpose of the differential count is to obtain a picture of the true distribution of the leukocytes in peripheral blood[3]. This is used by clinicians to determine the disease status of their patients. The leukocytes present in the peripheral blood are composed of five types of mature cells, i.e. neutrophils, lymphocytes, monocytes, eosinophils and basophils. Subdifferentiation of neutrophils into segmented or mature and band or immature forms and lymphocytes into normal, reactive or blast transformed forms is often done[43, 51]. A category of "other cells" is also sometimes included for all the other nucleated cells found in peripheral blood[51].

Definitions for "differential leukocyte count" vary according to the methods used to identify the leukocyte subclasses[51]. When cells are classified according to their morphological appearance on stained smears, there are different levels of discrimination. At the basic level, "polymorphonuclear" and "mononuclear" (or "round cells") are distinguished. At the intermediate level neutrophils, eosinophils, basophils, monocytes and lymphocytes are identified. At the highest level neutrophils are divided into segmented and band forms and lymphocytes into normal and blast transformed cells[43]. When the cells are identified by electronic counters, there again, are different levels of discrimination. Three-part automated differential analyzers identify major leukocyte groups; i.e. granulocytes, lymphocytes and monocytes[51, 52] or small cells, medium sized cells and large cells[15, 68]. The newer, more sophisticated haematology analyzers give five-

part differential counts (i.e. neutrophils, lymphocytes, monocytes, eosinophils and basophils) with comments on subclasses of neutrophils and lymphocytes as well as "other cells" in the sample[45, 51, 66, 75, 80]. Some of the most advanced analyzers will give a six or seven-part differential count (including band neutrophils and variant lymphocytes into their differentiation), such as the Technicon H-1/H-2/H-3 instruments[45] and the Cell-Dyn 3500 with the latest software package[1].

The techniques used to obtain a differential leukocyte count can be divided into two main groups: manual differential counting and automated differential counting. The manual differential count was introduced by Paul Ehrlich[8] in the early twentieth century and has since maintained a reputation as a valuable routine test amongst clinicians[28]. Attempts to automate differential leukocyte counts have been made since the early 1960's[45, 69] and automated leukocyte counts are currently widely used.

1.1.2 Manual Differential Leukocyte Counting

A manual differential leukocyte count is obtained through the morphological evaluation and identification of leukocytes on a blood film, stained with a Romanowsky stain and examined with a light microscope. The count is preferably conducted using the 1000 X magnification under oil immersion[41], but it can also be performed under lower magnifications (400 X and 500 X magnifications) by experienced personnel.

Various methods for the performance of a manual differential leukocyte count have been described. There are variations in the method used to collect the blood, the technique used to make the smear, the pattern used to count the cells and the number of cells counted. The mere fact that there is so much variation in the techniques, should indicate that results cannot be compared reliably unless a standardized method is used.

Factors that may influence the results of the leukocyte differential count when collecting the blood are:

- The physiological state of the patient at the time of collection[47]. Various studies have shown variation in differential counts following a change in climatic conditions. Some studies described differences in samples taken from patients in upright and recumbent positions (orthostatic effect), but others have found this not to be a significant difference[47]. It has also been shown that there is significant diurnal variation in white blood cell counts[8]. Sympathetic stimulation, especially in animals can also play an important role.
- The site of blood collection, i.e. peripheral or venous samples[47].

Blood smears can be made by either using two slides, where one slide is used as a spreader slide, or by using two cover-slides and pulling them apart. Various sizes of blood smears can also be used[47]. Different Romanowsky stains can be used for staining the smears. Vital stains and counting of the leukocytes by a haemocytometer can also be done, but is no longer in general use[47].

The most accurate counting method is to count all the leukocytes on a blood smear. This is obviously a very laborious method that leads to fatigue of the person performing the count. Some studies claimed that the most uniform results are obtained when every leukocyte on a very small film is counted[47]. The reason for the superior results obtained by counting the whole smear is because the white cells do not distribute evenly when a blood smear is made. The uneven distribution of the leukocytes was described and shown not to be accidental, but that the factors influencing the distribution could not be sufficiently controlled for rules to be laid down[47].

The different search patterns used to perform the counts can lead to great variation in the counts[47]. The count can be made by random selection of fields in different parts of the smear, or by a fixed pattern. The methods most commonly used are the straight edge method, the battlement method and the cross-sectional count[40, 47]. In a study done in 1940 these three methods in were compared and the battlement method was identified as the method giving the most accurate results[47]. It is also the method recommended by the National Committee for Clinical Laboratory Standards[51].

The battlement method was described as a "count made of three horizontal edge fields followed by two fields towards the center (so as to give three vertical fields), followed by two fields in a horizontal and then two fields in the vertical direction again"[47]. The straight edge count is performed by identifying and counting the cells in the field adjacent to the edge. Fields along both edges are examined[47]. The cross-sectional method starts at an edge field, moves across the body of the smear to the opposite edge and back across in a similar manner until the desired number of cells have been identified[47].

The number of cells counted when performing a differential leukocyte count varies greatly. The 100-cell differential count is the most common method, because it is the least labor-intensive method. However, it has been shown over and over to be an imprecise method[3, 42, 43, 47, 49]. The differential count does not appear to be sensitive enough to base decisions for the clinical care of patients on, except for the total neutrophil count[43].

One study conducted, indicated that a minimum number of 300 cells should be counted[47]. Various other investigators concurred[47].

The error due to chance is responsible for a large degree of inconsistency in differential leukocyte counts[3]. An error due entirely to chance occurs when our estimate of different types of units in a universe composed of a very large number of individual units is based on a study of an extremely small proportion of them[3]. If the white cell count is 10.0 X $10^9/\ell$, there will be 50 000 000 000 cells in 5 liters of blood, and we count only a few hundred of these![3] Thus even when there is no error in technique or interpretation, the chance error is unavoidable and will still be present[3]. In a similar statistical analysis of leukocyte differential counts, a similar conclusion was reached[42]. It was showed that in a perfect smear, containing exactly 20% lymphocytes, exactly 20% lymphocytes were only counted 9.93% of the time if 100-cell differential counts are done[42]. The error is inversely proportional to the square root of the number of cells counted[3]. Thus it was concluded that at least 400 cells must be counted to obtain reliable results[3]. The National

Committee for Clinical Laboratory Standards[51] also recommends a 400-cell differential count.

The manual differential count is also a very slow and labor-intensive procedure[8, 28, 47]. In a discussion, reported in a paper by Brecher *et al*[8], a Dr Bull comments that it takes 95 seconds to complete a differential count, but he goes on to say that it would be inappropriate to have a technologist do only white blood cell differentials for a whole day. In the experience of the Clinical Pathology Laboratory of the Onderstepoort Veterinary Academic Hospital, one technologist can do approximately thirty accurate manual 100-cell differential counts per day on diseased patients. This is because more thorough examinations of the blood smears have to be done for leukocyte abnormalities and parasites as well as for erythrocyte abnormalities and parasites.

1.1.3 Automated Methods for Differential Leukocyte Counting

Automated differential leukocyte counting methods have various advantages over manual methods. Firstly, most automated instruments count thousands of cells[1, 7, 50] compared to the 100 to 400 cells of the manual methods. This leads to considerable improvement in the precision of the method[50, 51] because the error due to chance is much smaller. Another advantage is the rapid screening of multiple samples, leading to improved efficiency[50]. Some of the modern automated analyzers can process up to 120 samples per hour[45]. A further advantage, is the improved safety that automated analyzers provide, because laboratory staff has less direct contact with the sample and very small sample volumes are required.

Various methods have been employed in the attempt to automate differential leukocyte counting and tremendous progress has been made in the past two decades. Some of the methods had fallen into disuse, only to be improved and introduced at a later stage again, such as quantitative buffy coat analysis and cell pattern recognition.

The methods used in automated leukocyte differential counting are electronic impedance counting and sizing, quantitative buffy coat analysis, cell pattern recognition, flow cytometry, electromagnetic conductivity and various combinations of these techniques.

1.1.3.1 Electronic Impedance Cell Counting and Sizing

Coulter Electronics initially introduced electronic impedance counting in the late 1950's in their Coulter haematology analyzers for cell counting[57]. The method was initially developed to replace manual cell counting, using a haemocytometer, with a faster and more accurate method[48, 57]. However, apart from the number of cells, the size distribution of the cells can also be determined using this method[48].

The blood sample is diluted and the cells are suspended in an electrically conductive medium. The diluted sample is now aspirated through an aperture, which is situated between two electrodes. As the cells pass through the aperture, they cause resistance in the electric circuit and a change in voltage results. This voltage change is then amplified and is shown on an oscilloscope. By using a threshold control circuit only pulses of a selected magnitude range are counted. The amplitude of the pulse is proportional to the cell size and the number of pulses is proportional to the number of cells passing through the aperture[35, 48, 57].

The impedance counters used for differential leukocyte counting generate a three-part differential count. Either the size of the cell or of the cell nucleus is used as the differentiating characteristic. The SYSMEX E-4000 and SYSMEX E-5000 classify the cells as small cells, intermediate sized cells and large[15, 28, 68]. The Coulter Counters, using impedance counting for white cell differentiation classify the cells as lymphocytes, granulocytes and mononuclear cells[28]. The Serono-Baker SYSTEM 9000 calls these subpopulations lymphocytes, midrange cells and granulocytes. Essentially, the small cells are lymphocytes, the intermediate sized cells are mononuclear cells (nuclear size is the discriminating factor) but can also include eosinophils and basophils and the large cells or granulocytes are mainly neutrophils, including band cells[64].

Various reports in the human literature claim that the three-part differentials are sufficient and acceptable for the majority of cases presented for differential counting[2, 15, 28, 68]. Duncan and Gottfried evaluated the usefulness of the three-part differential count in a hospital setting[28]. They found that combined with a qualitative slide review, the three-part differential was suitable for 84% of the samples submitted for a differential count.

1.1.3.2 Quantitative Buffy Coat Analysis

In 1840 Thomas Addison discovered that leukocytes collect above the packed cells when centrifuged. In 1890 Hedin and in the 1930's Wintrobe described the use of the thickness of the buffy coat as a rough estimate of the total white cell count[21]. In 1940 Bessis subdivided the leukocytes in the buffy coat layer from top to bottom as lymphocytes, monocytes and granulocytes[21]. This characteristic of the white blood cells based on their different specific gravities has since been used, together with their different staining reactions with supravital stains, in Quantitative Buffy Coat (QBC) systems to obtain a differential leukocyte count[46,60].

Blood is aspirated into a microtube coated with potassium oxalate and acridine orange[46, 60]. A precision molded cylindrical float is inserted into the microtube before centrifugation[60]. The cylindrical float has a specific gravity of 1.055[46], which is about halfway between that of plasma and the red blood cells. The tube is centrifuged for 5 minutes at 12 000 rpm[46], after which the float settles in the buffy coat layer. The buffy coat length is expanded vertically by a factor of ten[46, 60] because the float occupies 90% of the cross sectional area of the tube[46].

The various components of the buffy coat are further enhanced by the acridine orange[46, 60]. The cells fluoresce differently under excitation of blue violet light. The platelets appear pale yellow, the lymphocytes and monocytes are brilliant green and the granulocytes are orange-yellow[60]. Eosinophils are visible as an

orange-green band at the top of the granulocyte layer in canine and feline samples if the eosinophil count is in excess of 1.0 x $10^9/\ell$[46].

The potassium oxalate prevents the lighter erythrocytes from mixing with the granulocytes[46, 60]. Potassium oxalate removes water from the erythrocytes, causing them to become more dense and smaller and therefore less likely to be present in the granulocyte layer. Anti-red blood cell antibodies have also been used to coat the QBC tubes in order to improve the separation between the red blood cells and the granulocytes[11].

An electronic ocular micrometer using a blue-violet light source[11, 46, 60] measures the lengths of the different layers. Band lengths are given in electronic counts and are then converted to numerical counts[46]. Conversion factors for dogs, cats and horses are available for use in veterinary medicine[46].

In the literature on human haematology QBC analysis is reported to be comparable in clinical sensitivity to the reference method[60]. It is also reported as a rapid, economical haematological evaluation technique in veterinary literature[11, 46] and as a very sensitive method in detecting microfilaraemia[11]. In a recent evaluation of the QBC VetAutoread, it was found to be good for white blood cell counts in dogs, but it was only fair in it's identification of granulocytes and mononuclear cells[53]. This was mainly attributed to insufficient separation of red blood cells and granulocyte layers[53].

1.1.3.3 Cell Pattern Recognition

Digital image analysis as applied to haematology has a long and uncertain history. Red cell counting was automated in the 1950's using television microscopes, but was replaced by flow cell counters[56]. Automated microscopes for haematology were sold in the 1970's[56]. These pattern recognition systems included the Corning LARC Classifier, Geometric Data Hematrak, the Abbott ADC500 and the Coulter Diff3 System[20]. Unfortunately these instruments were not economically

successful and production of most of them has been stopped[56]. However, an improved Artificial Intelligent Diagnostic System has recently been developed by Beksaç *et al*[4], based on a better software algorithm and made possible by the massive improvement in computer processing power in recent years.

Computerized microscopes form the basis of Pattern Recognition Analyzers. Differential leukocyte counts are performed by processing the digital images and classifying them according to their characteristic morphological features[55]. Morphological features evaluated to classify cells include cell area, cell colour, ratio of cell area to circumference, homogeneity of the cell, nuclear area, nuclear colour, homogeneity of the nucleus, cytoplasmic colour etc[4, 20, 55]. The cell identification is thus done in much the same way as a technologist would do it. It is therefore not surprising that cell pattern recognition correlates well with the 100 cell manual differential count, if it also evaluates 100 cells[55, 58, 59]. The biggest advantage of these systems is that 200-, 400-, 600- and 800-cell differentials can easily be done on them because of their increased speed[58]. When the number of cells counted is increased, the chances of detecting abnormal cells will increase. It has been shown that this is indeed the case with the HEMATRAK® [58].

The biggest disadvantage of the pattern recognition systems is that a stained slide is evaluated with a limited number of cells on the slide, often not more than 600. Staining of slides may also provide problems, since the intensity of stains may differ[20].

1.1.3.4 Electromagnetic Conductivity

High-frequency electromagnetic conductivity is only used as part of a combination analysis in the Coulter STKS[70, 45]. The electromagnetic energy is used to determine nuclear size and density[70]. As far as the author is aware its usefulness in the veterinary field has not been evaluated as such.

1.1.3.5 Flow Cytometry

Flow cytometry is a process in which individual cells or other biological particles are made to flow in a single file in a fluid stream past a sensor or sensors that measure the physical or chemical characteristics of the cells or particles[65]. The two techniques used most commonly in combination with flow cytometry for cell counting and identification are flow cytochemistry and laser light scatter measurements. These techniques are often used in combination for the identification of leukocytes.

i) *Flow cytochemistry*

Cytochemistry makes use of the biochemical characteristics of cells[40] and more specifically their staining ability with different dyes. Flow cytochemistry makes use of light scattering and light absorption[33] of the stained cells as they pass the sensor. Various stains can be used, including peroxidase[40]. In flow cytochemistry fluorescent dyes (fluorochromes) are commonly used[33]. One of the most widely used automated leukocyte differential cell counters, the Technicon H*1makes use of flow cytometry with myeloperoxidase[72] as a stain in order to generate a differential leukocyte count. The cells are identified using a combination of their staining reaction and their size[22]. Neutrophils have a strong peroxidase activity; monocytes show a weak activity and lymphocytes have no activity, while eosinophils show intense peroxidase activity[22].

ii) *LASER light scatter*

In 1968 Wyatt[83] explored the possibility that differential light scattering can be used to identify living bacterial cells. His theory was that "the characteristic of each distinct microorganism that scatters light is an essentially unique scattering pattern"[83]. This is due to the unique structural and biochemical features of each type of microorganism. In 1972 Brunsting and Mullaney[13] described light scattering patterns from coated spheres as a model for light scattering from biological cells. They observed that the scattering pattern of spheres at larger

angles were structured and sensitive to the core sphere size. This suggested that it could be used as a possible method for differentiating between biological cells, which are similar in size, but different in internal structure[13]. Later the same year, they described a possible method of mammalian cell identification by differential light scattering[14]. They concluded that scattering measurements made in the forward angle is an indication of cell size, whereas measurements made outside the forward angle are influenced by nuclear size and internal structure[14].

In 1975 Salzman *et al*[61] described a technique using the light scatter at two different angles with respect to the laser beam to identify unstained white blood cells. They were able to differentiate between human lymphocytes, monocytes and granulocytes using this technique. Two benefits of this technique, pointed out by them, are that staining and fixation are not necessary and therefore the risk of introduction of artifacts is limited and secondly very little sample preparation is required[61].

In a further improvement of the technique, De Grooth *et al*[24] introduced the use of a polarized laser beam. Depolarized orthogonal light scatter is measured and it has been shown that the depolarization is caused by multiple scattering processes inside the cell[24]. By using a combination of normal orthogonal light scatter and depolarized orthogonal light scatter, two populations of granulocytes could be identified. Cell sorting was used to demonstrate that the cells with higher depolarization are eosinophils[24]. Terstappen, De Grooth and others then went on to develop a four-parameter leukocyte differential count[69]. They used four different measurements in order to achieve this, namely the intensity of forward angle (0° to 2.6°) scatter, narrow angle (3.0° to 11.0°) scatter and normal and depolarized orthogonal light scatter[69]. The forward angle and the narrow angle scatter are used to differentiate between granulocytes, lymphocytes and monocytes, while the normal and depolarized orthogonal light scatter is used to distinguish between eosinophils and neutrophils[69]. Breaks in the light are used to count the cells[70].

Some of the haematology analyzers using the laser light scatter to count and identify cells include the Ortho ELT-8/ds analyser[78], the Coulter STKS[45, 75] and the Cell-Dyn analyzer range[1, 52, 74].

1.1.3.6 Combinations

Modern haematology analyzers make use of combinations of these techniques to yield accurate and complex multi-parameter counts. The Coulter STKS, for example, makes use of the impedance principle, electromagnetic conductivity and laser light scatter[39, 45, 70]. The Sysmex NE-8000 makes use of a non-optical approach by combining high-energy radio frequency and direct current methodology[39, 45]. The analysis on the Cobas Argos 5-Diff is a combination of impedance-, cytochemistry- and optical absorbance technology[45]. The Cell-Dyn series makes use of the impedance principle and laser light scatter by regular and depolarized light[1, 26, 31, 45, 74].

1.1.3.7 Use of Automated Methods in the Veterinary Field

Haematology analyzers are developed for use in the human medical field. As with other modern technological developments in medical science, it is inevitable that it would be applied to veterinary science. This should be done with caution and only after the specific analyzer has been evaluated for its use in the veterinary field. It is only in recent years that some of the manufacturers of haematology analyzers have started to consider the veterinary side as a potential market and are now adapting their instruments for use in the veterinary field.

The evaluation of animal leukocytes on analyzers developed for the human medical market has a number of potential problem areas. The first problem is the leukocyte size variation between different species[79, 81] and between animal and human leukocytes. When using impedance counting methods, it is important to set the lower threshold carefully in order to minimize the error associated with the interference of debris[79].

The second problem is that the staining reactions of leukocytes differ between species. This creates problems in systems using flow cytochemistry, such as the Technicon H-1, H-2 and H-3 analyzers. The Technicon H-1 has been evaluated and found acceptable for the determination of differential leukocyte counts in dogs[22, 71, 73] since the staining reactions of canine leukocytes are similar to those of human leukocytes. However, it has been found that the equine leukocyte differential count on the H-1 was unacceptable and the reason given for this, is that equine leukocytes, and specifically neutrophils, stain more weakly with peroxidase than human leukocytes[72, 73].

Another potential problem includes the difference between human and animal granulocyte granules in systems making use of the characteristics of the granules to differentiate between cell types. The difference in the reaction of cells to lytic reagents[25, 26, 54, 79] in systems making use of the principle of lysis to either eliminate certain cells, such as erythrocytes, or to strip the leukocyte cytoplasm in order to evaluate the nuclei can also possibly lead to errors. It is thus very important to evaluate an instrument for the acceptable use in every species that would be analyzed on it.

A number of electronic cell counters have recently been evaluated for their use in the veterinary field. When the results of these studies are analyzed, the importance of prior evaluation are clearly illustrated. Green reported on the effect that platelets sometimes have on red cell indices when using the Cell-Dyn 3500[34]. However, he also mentions that it can be corrected with the correct instrument settings and that minor software adjustments could rectify the problem[34]. In a study by Pastor *et al*[54] on the usefulness of the Sysmex F-800 for canine and feline haematology evaluation, platelet counts also presented some difficulties. In a study evaluating the Coulter AcT for use in domestic animals, problems were encountered with MCV in canine samples and with platelet counts in equine and

feline samples[23]. Feline and bovine white blood cell counts and bovine haemoglobin values also presented difficulties[23].

1.2 THE CELL-DYN 3500[a]

The Cell-Dyn 3500 is a fully automated haematology analyser. It creates a total white cell count (WBC) and a five-part differential leukocyte count, i.e. neutrophils, lymphocytes, monocytes, eosinophils and basophils with additional information on bands, immature granulocytes, blasts and variant lymphocytes. It also measures all the red cell and platelet parameters. In order to measure all these parameters the analyser uses four measurements channels, which are[1]:

a) An impedance channel, for determining the WBC impedance count (WIC)
b) An optical channel for determining the WBC optical count (WOC) and differential cell count
c) An impedance channel for red blood cells and platelets analysis
d) A channel for haemoglobin determination.

The Cell-Dyn has two modes for sample aspiration, an open mode and a closed mode. When using the closed mode the samples are placed in a sample loader, the tube stopper is pierced and blood is aspirated. After the instrument has aspirated the sample, it is diluted, mixed and the parameters are measured in the different channels.

1.2.1 White Blood Cell Measurements

The Cell-Dyn gives three basic WBC measurements, i.e. the WIC, WOC and the reported white blood cell count. With each sample analysis, the WIC and WOC are measured and the values compared. A flag will be displayed if the difference between the two counts exceeds a predetermined value. The WOC is the primary value reported as the white blood cell count. Differences can be due to resistant red cells, nucleated red cells or fragile white blood cells. Nucleated red cells will be included in the total white cell count of the WIC,

while they will not be included in the WOC count. Fragile white cells will cause a false low white cell count in the WIC, as the lytic reagent can also damage them, while they will still be counted in the WOC. Lytic-resistant red cells will cause interference in the WOC, leading to a large percentage of the WOC count located in the stroma region. The comparison of the two methods allows the instrument to identify and report these abnormalities in an attempt to give an accurate white blood cell count.

1.2.1.1 White Cell Impedance Counting (WIC)

For determination of the WIC, a dilution of the sample is made with diluent (L/N 99231-01) and WIC/HGB Lyse (L/N 99431-01). The latter reagent lyses the red cells and strips the cytoplasm from the white blood cells, leaving only the white blood cell nuclei to be counted, using the aperture impedance method. In order to obtain an absolute cell count, the precise volume of blood that passes through the aperture during the count cycle is known. A volumetric metering process is used to ensure that a precise volume of sample is analysed.

Cells that exit the aperture tend to swirl around and can re-enter the sensing zone. To prevent this, and thus the cells from being counted twice, a von Behrens Plate is located in the WIC counting chamber. The WIC is also corrected for coincidence passage loss. Coincidence passage loss is a reduction in the count due to the fact that two or more cells can pass through the aperture simultaneously. This will lead to the generation of a single pulse with high amplitude and increased pulse area, giving the impression that only one large cell has passed through. The coincidence passage loss can be predicted statistically and can be corrected.

1.2.1.2 White Cell Optical Counting (WOC)

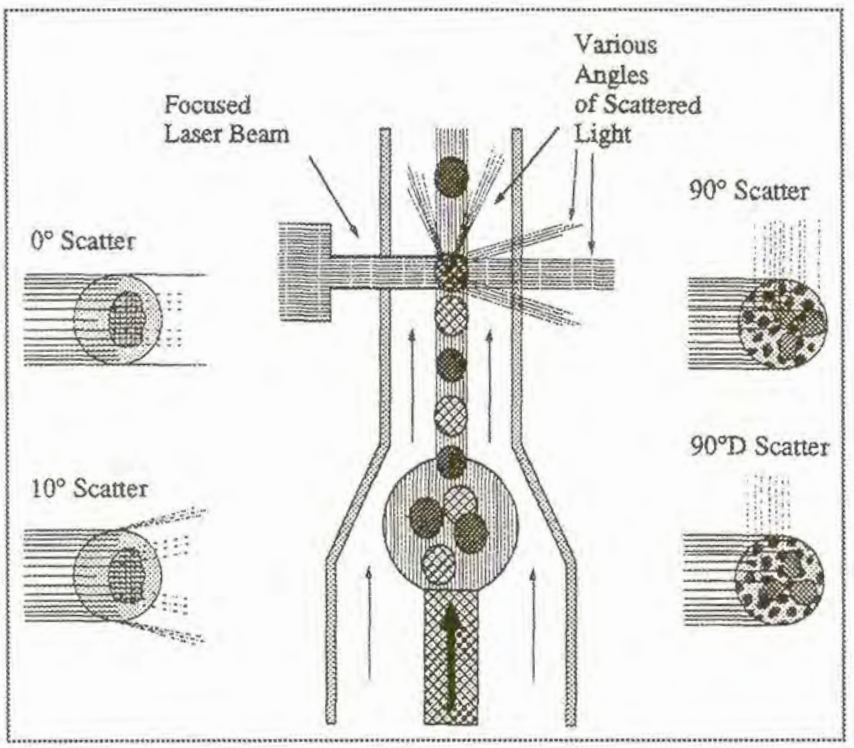
For determination of the WOC, a dilution of the sample is made with the sheath reagent (L/N 99321-01). The cellular integrity of the white cells, in the sheath fluid, is maintained, but the basophils change slightly due to their hygroscopic nature. A measured volume of this dilution is injected into the sheath stream. The cells are aligned in single file as they pass through the WOC flow cell. The WOC flow cell

is an optically clear quartz chamber and the light source is a vertically polarised Helium Neon laser.

The analyzer uses Multi-Angle Polarised Scatter Separation (MAPSS) technology. MAPSS measures the light intensity at four different angles (see Figure 1.1):

- forward angle light scatter (1-3°, referred to as 0°)
- orthogonal light scatter (70-110°, referred to as 90°)
- narrow angle light scatter (7-11°, referred to as 10°) and
- ninety-degree depolarised scatter (70-110°, referred to as 90°D).

Figure 1.1 Multi-Angle Polarised Scatter Separation technology on the Cell-Dyn 3500 (Reproduced with permission from Abbott Diagnostic Division)



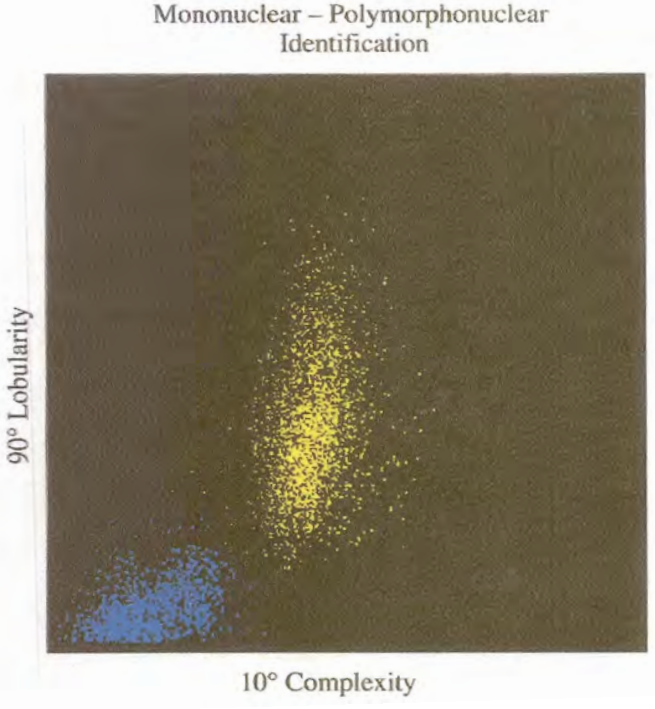
Combinations of these four measurements are used to classify the white cell subpopulations and to give morphological flagging. The number of occurrences above the threshold of the 0° channel is used to determine the white cell optical count. The white blood cell differential count is obtained from all four measurements, and five subpopulations are identified: neutrophils, lymphocytes, monocytes, eosinophils and basophils. The WOC data can be graphically presented as a scatterplot or as histograms.

Mononuclear-Polymorphonuclear Separation

The first step in the differential count of the Cell-Dyn is to classify the cells as mononuclear or polymorphonuclear. The classification is obtained by plotting the 90° (orthogonal) scatter, representing cellular lobularity, on the Y-axis of a

scatterplot against the 10° (forward angle) scatter, representing cellular complexity on the X-axis, see Figure 1.2. The mononuclear cells fall into the lower left part of the scatterplot and above and to their right, the cluster of polymorphonuclear cells is plotted. Once a cell has been classified in either of these two groups, it retains that classification, regardless of it's position in other scatterplots. The instrument determines the best separation between the two populations, using a dynamic threshold.

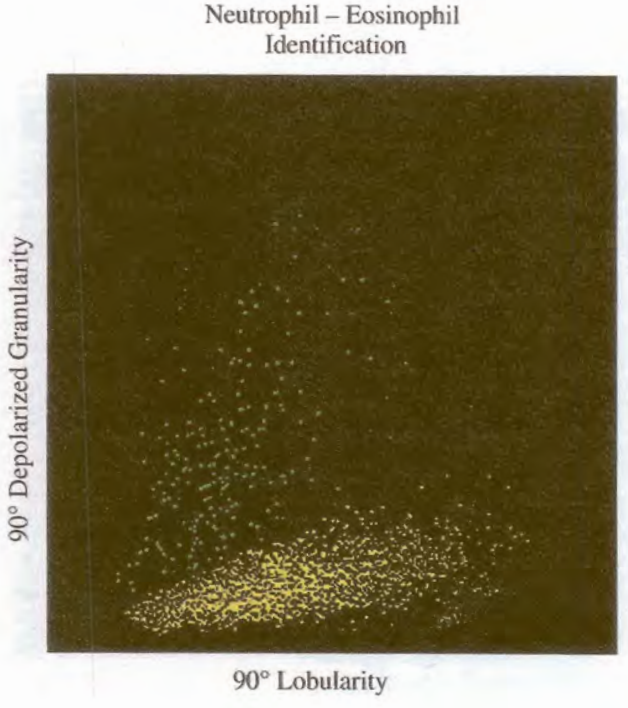
Figure 1.2 Mononuclear-Polymorphonuclear Separation (Reproduced with permission from Abbott Diagnostic Division)



Neutrophil-Eosinophil Separation

In order to distinguish between neutrophils and eosinophils the 90° depolarised light scatter is plotted on the Y-axis against orthogonal light scatter on the X-axis. Eosinophils scatter more 90° depolarised light that other cells due to the unique nature of their granules. Only the polymorphonuclear cells are plotted on this scattergram, the neutrophils are the cells in the lower of the two groups, see Figure 1.3.

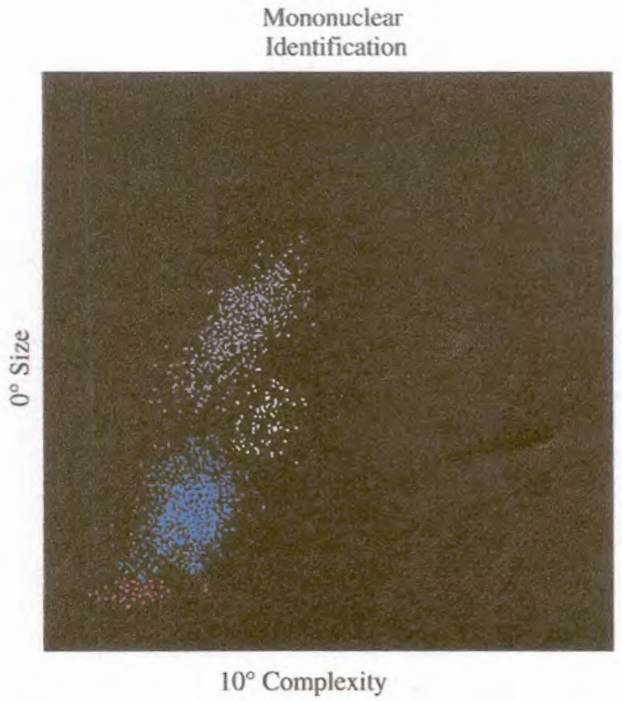
Figure 1.3 Neutrophil-Eosinophil Separation (Reproduced with permission from Abbott Diagnostic Division)



Mononuclear Separation

Lymphocytes, monocytes and basophils fall into this group. The 0° scatter, representing size, is plotted on the Y-axis and the 10° scatter is plotted on the X-axis, see Figure 1.4. The orientation of the neutrophil cluster is also used in the algorithm to help with the classification of the mononuclear cells.

Figure 1.4 Mononuclear Separation (Reproduced with permission from Abbott Diagnostic Division)



Basophils form part of the mononuclear group, because their granules are water soluble in the sheath fluid and in the process the cytoplasm becomes less complex. The lymphocytes are the cells in the lowest, large cluster on the scatterplot. The basophils are present in a small cluster that falls slightly to the right and above the

lymphocytes, whereas the monocytes lie in the big cluster at the top of the scatterplot.

The small cluster below the lymphocytes consists of cells, which are unlikely to be leukocytes. Cell types that can be present in this area are nucleated red blood cells, resistant red blood cells, giant platelets or platelet clumps. The Cell-Dyn uses information from the WIC channel to help with the identification of these particles. Cells in this area will not be included in the white cell count or the differential count.

1.2.2 Data Flagging and Messages

The operator is alerted of any instrument problems and data criteria that may affect the results. The instrument gives the following messages:

a) Instrument messages: Fault and status conditions are given when the instrument detects abnormal conditions during the processing of the sample.

b) Parameter flagging messages:

- Dispersional data flags are given when the numerical values of a given specimen exceed that of the pre-set limits.
- Suspect parameter flags are given when the instrument cannot measure a parameter due to sample abnormality. The flags generated when the white blood cell data cannot be measured are:
  - DLTA: the difference (delta) between the WIC and WOC exceeds the expected, pre-set limit
  - WBC: this flag is also generated when the difference between the WIC and WOC exceeds the limit, but specifically when there is a declining kinetic rate detected for the WOC, or more than 10% of the WOC count was in the stroma region. This usually indicates the presence of red blood cells resistant to the lyse reagent.
  - DIFF (NLMEB): An alert here will indicate a declining kinetic rate for the white cell count, or an abnormal cell population that the instrument cannot reliably classify.

b. 14451839
i. 14756420

- Suspect population flags are generated when the data indicate the presence of an abnormal subpopulation. Flags generated when there are abnormalities detected in the white cell population are:
  - BAND: This flag will be generated when the total number of cells in the band region is more than 12.5% of the total white cell count, or if the ratio of the suspected bands are more that 50% of the total neutrophil count or the neutrophil cluster on the 0° axis appears abnormal.
  - IG: The area where the immature granulocytes (metamyelocytes) are located exceeds 3% of the total white blood cell count.
  - BLAST: The area in the 90°/0° scatterplot where blast transformed cells are usually located exceeds 1% of the total white blood cell count; or the monocyte count is more than 20% of the total white cell count; or the monocyte count is more than 3% of the total white cell count and the monocyte data on the 0° axis is abnormal.
  - VARIANT LYM: The lymphocyte data on the 0°/10° scatterplot exceeds expected criteria.
  - NRBC: This flag is generated when the WIC is greater than the WOC or the area below the WBC threshold on the 0°/10° scatterplot exceeds 5% of the total white blood cell count. Cell types that may be present in this region are nucleated red cells, unlysed red blood cells, giant platelets and platelet clumps.
- Interpretative messages are only printed when the option for it is selected and it is generated when the pre-set numeric limits are exceeded. The white blood cell messages are: leukocytosis; leukopenia; neutrophilia; neutropenia; lymphocytosis; lymphopenia; monocytosis; eosinophilia and basophilia.

The Cell-Dyn 3500 has been evaluated in numerous studies for its use in differential leukocyte counting in human haematology and found to be very satisfactory[19, 25, 31, 52, 62, 77]. It has also been evaluated for its ability to handle and identify pathological samples[12, 36].

1.3 INSTRUMENT EVALUATION

1.3.1 General Instrument Evaluation

There are various levels of instrument evaluation, i.e. evaluation by the manufacturer, evaluation by consumer associations and evaluation by potential users[38, 67]. This paper will deal with the evaluation procedure for use by potential users of the instrument. The different stages of evaluation include a preliminary stage, planning stage, technical assessment and finally an efficiency assessment[9, 10, 37, 38, 67].

During the preliminary stage general information on the instrument must be gathered. This includes the instrument name, manufacturer, marketing company, price, maintenance costs and availability of consumables as well as spares[9, 38, 67]. It should also be determined what space and special requirements, such as water and electrical supply would be required [9, 10, 38, 67].

The planning stage has two legs, i.e. arrangements with the manufacturer and internal resource planning[38]. During the negotiations with the manufacturer, it is essential that a proper assessment of the time required to evaluate the analyzer must be made. An assessment of the reagents and control material must also be made to ensure that sufficient quantities will be available during the evaluation period. Arrangements regarding the service of the analyzer during the time of evaluation must also be made[38].

Internal resources planning revolves around the determination of staff requirements. Sufficient staff should be available for instrument evaluation, special sample collection, keeping daily records, performing the statistical analysis and for reporting on the performance of the instrument[38, 67]. Before technical evaluation starts, it is important that all necessary training of the staff is done[38, 67].

The technical assessment of new analyzers is done by the evaluation of a number of parameters. Evaluation standards for laboratory instruments in general[9] and automated

haematology analyzers in particular[37, 38, 51, 67, 76] have been published. The parameters included in the evaluation are linearity, precision, carry-over, comparability, accuracy and efficiency.

1.3.1.1 Linearity[10, 38, 67]:

The effects of dilution on samples should be assessed for variables whose magnitude should be proportional to dilution. An analytical method should give a linear relation at least over the physiological and commonly encountered pathological range. The linear relation should pass through the origin. Dilutions of a sample are made to give ten concentrations of the sample, i.e. 10%, 20%, 30%, . . . 90% and 100%.

1.3.1.2 Precision:

The precision of an instrument is evaluated by testing a sample on two or more occasions[37, 38, 67]. When a sample is analyzed a number of times, the precision is the degree to which the results vary from the mean of the results[9]. Precision studies should ideally be run over the whole pathological range that is encountered. It is preferable to assay more samples fewer times that to assay fewer samples more times[38, 67], in order to include as wide a range of samples as possible.

1.3.1.3 Carry-over:

The carry-over evaluation assesses the influence of the concentration of the test substance in one sample upon the results obtained for the following sample[9]. Broughton *et al*[9] described a method for the determination of total carry-over assessment. A sample with a high value is used for the first three specimens (h_1, h_2, h_3) and a sample with a low value for the next three (l_1, l_2, l_3). The carry-over between the specimens is then calculated using the following formula[10]:

Carry over = $(l_1 - l_3) \div (h_3 - l_3)$.

1.3.1.4 Comparability and accuracy:

The comparability of an instrument is its ability to produce results that agree satisfactorily with those obtained by accepted routine procedures[67]. The accuracy is defined as the agreement between the best estimate of a quantity and its true value[67]. A true value can only be obtained by a definitive or reference method and the only haematological parameters that can be determined accurately are haemoglobin and packed cell volume[38]. Therefore all other haematological parameters can only be compared to an acceptable routine procedure.

For comparability studies, as many samples as possible should be analyzed to include samples at the extremes of the pathological range. The full pathological range, as expected to be seen in practice, must be included in the analyis[38, 67]. Automated haematology analyzers usually have to be calibrated with a suitable material. This is a big limitation, since this can influence the evaluation. It may be difficult to decide whether an analyzer has been calibrated with a material of which the values were incorrect, or if the analyzer itself is not working correctly[38, 67]. This means that if Analyzer A always give values of 5% greater than Analyzer B, it is acceptable, as the difference could easily have been eliminated by proper calibration. On the other hand, it would not be acceptable if Analyzer A gives a result 10% higher than Analyzer B on some samples and 50% higher or lower on other samples[38, 67].

1.3.1.5 Efficiency:

An efficiency assessment is performed to determine the acceptability of an instrument in a specific working environment. A number of parameters are evaluated to assess this:

- The first factor to take into consideration is the operational time, this includes the throughput, start-up time, shut-down time and the number of samples that have to be repeated[38, 67].

- The next factor to consider is the reliability of the instrument and maintenance necessary. Records of all "down time" must be kept as well as records of the response time of the manufacturer for service[38].
- The format and style of data presentation must be commented on. The ease with which it can be interpreted and the possibility of it being linked to a central laboratory computer should be noted[38].
- Acceptability of the instrument by the staff should be determined. The level of expertise required must be noted[38, 67].
- The costs must be determined and consideration must be given to reagent costs, maintenance costs and costs of consumables. The best way to compare the cost between instruments is to look at the cost per sample evaluated[38, 67].
- The clinical usefulness of the instrument with respect to its screening ability, usefulness as a diagnostic tool and monitoring of patient therapy must be evaluated[38, 67].

1.3.2 Evaluation of Electronic Differential Leukocyte Counters

The difficulties in obtaining an accurate differential white cell count have already been discussed earlier in this chapter. For his reason the National Committee for Clinical Laboratory Standards (NCCLS) has published a standard for leukocyte differential counting that can be used in the evaluation of automated and semi-automated methods for leukocyte differential counting[7, 44, 51].

1.3.2.1 Reference Leukocyte Differential Count[51]

A total of 100 normal and 100 abnormal samples must be evaluated as a basis for the reference values. "Abnormal" is defined as the clinically significant alteration in distribution of mature cell types, or the presence of abnormal or immature cells in clinically significant concentrations.

Whole blood collected by venipucture in tripotassium ethylenediamine tetra-acetate (K_3EDTA) is the required specimen. The sample is rejected if there are any visible signs of clots. Abnormal conditions such as microscopically visible clots, haemolysis and lipaemia should be recorded.

Blood films from each sample must be prepared within 4 hours after blood collection. Samples should not be stored in a refrigerator and should be mixed thoroughly before blood film preparation. Three blood films are prepared on good quality microscope slides. The wedge-pull film technique is used. The films are marked "A", "B" and "spare". The blood films are stained with a Romanowsky stain (containing fixatives) within 1 hour after being made.

The blood films must be examined microscopically using the "battlement" method. Two hundred leukocytes must be counted on each blood film. The cells are classified as segmented neutrophils, band neutrophils, normal lymphocytes, variant lymphocytes, monocytes, eosinophils, basophils and other nucleated cells (excluding nucleated red blood cells). The results are expressed as a percentage of the total number of leukocytes counted. Any nucleated red blood cells are counted and expressed as the number per 100 leukocytes counted.

The examiners must be experienced in examining immature and abnormal cells. They should not perform more than 15 to 25 200-cell differential counts per day.

In 1990 Kohut proposed an abbreviated method for the assessment of electronic leukocyte differential counters[44]. According to his method 20 normal and 20 abnormal blood specimens are evaluated. Four wedge blood films are made and a 400-manual cell count is generated. It is suggested that 4 technologists be used to each scan one of the 4 films in order to generate a 400-manual count. According to Kohut, this reduces the slide-to-slide and technologist-to-technologist variation.

CHAPTER 2: PROBLEM

The absolute leukocyte differential count is used by clinicians as an indicator of their patient's disease status and response to therapy. From the literature it is clear that a 100 leukocyte manual differential count is not a very accurate way to establish the true distribution of leukocytes in blood.

Manual leukocyte counting is also a very slow and labour-intensive method. A laboratory technologist can only do a limited number of manual differential counts per day. With an ever increasing demand on laboratories to do more work more efficiently, automation has become an essential development in the clinical pathology laboratory in all possible areas.

The movement towards automation has led to the development of several methods for automated leukocyte differential counting. These methods are developed primarily for use in the field of human haematology. Some methods had been less successful and had fallen into disuse. Other methods, such as flow cytochemistry[7] and laser light scatter separation[12, 26, 31, 36] have been found to be very useful in human haematology.

Electronic impedance counting and sizing only reports a three-part differential leukocyte count, which has limitations due to the absence of reports on eosinophilias[73]. Quantitative buffy coat analysis does not give a five-part differential count, although it is reported to be an acceptable technique in veterinary haematology[11, 46].

Canine leukocyte differential counts have been reported to be accurate on the Technicon H*1[22], a technique which makes use of flow cytochemistry. However, this instrument requires various reagents and the differential leukocyte count presents problems in most of the other domestic species[72].

Differential leukocyte counting based on the method of multi-angle polarised scatter separation appears, on theoretical grounds, to be ideal for use in dogs. At the time that the investigator was approached, the Cell-Dyn 3500 had not been fully evaluated for this purpose. The manufacturer approached several veterinary schools all over the world to participate in the evaluation.

In order to evaluate the technology, the instrument incorporating the technology must also be evaluated in general, in order to determine if any discrepancies arising are due to the method employed or other technical problems inherent to the instrument rather than those due to working with non-human leukocytes.

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.

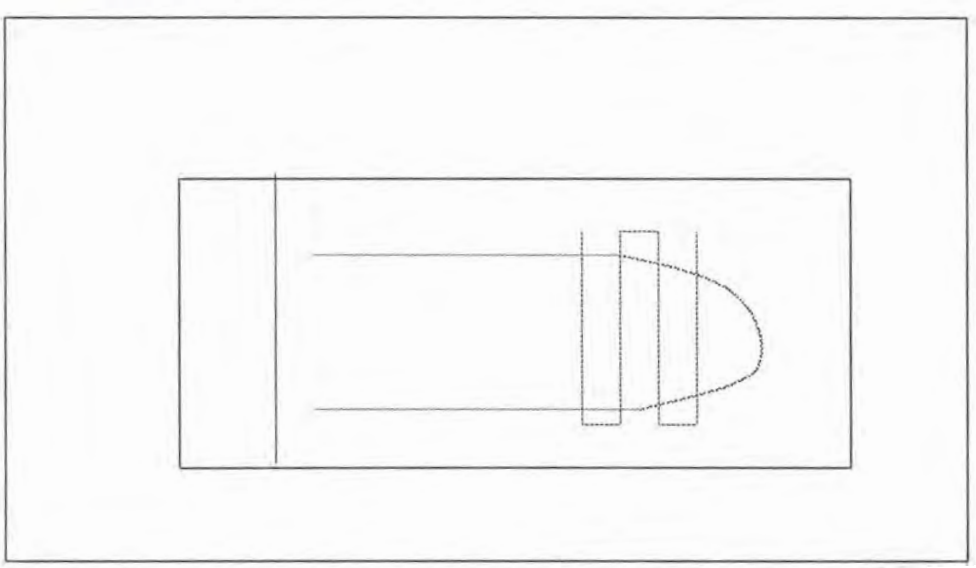
The qualitative comments which the examiners were asked to report were the presence of the following:

- 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.

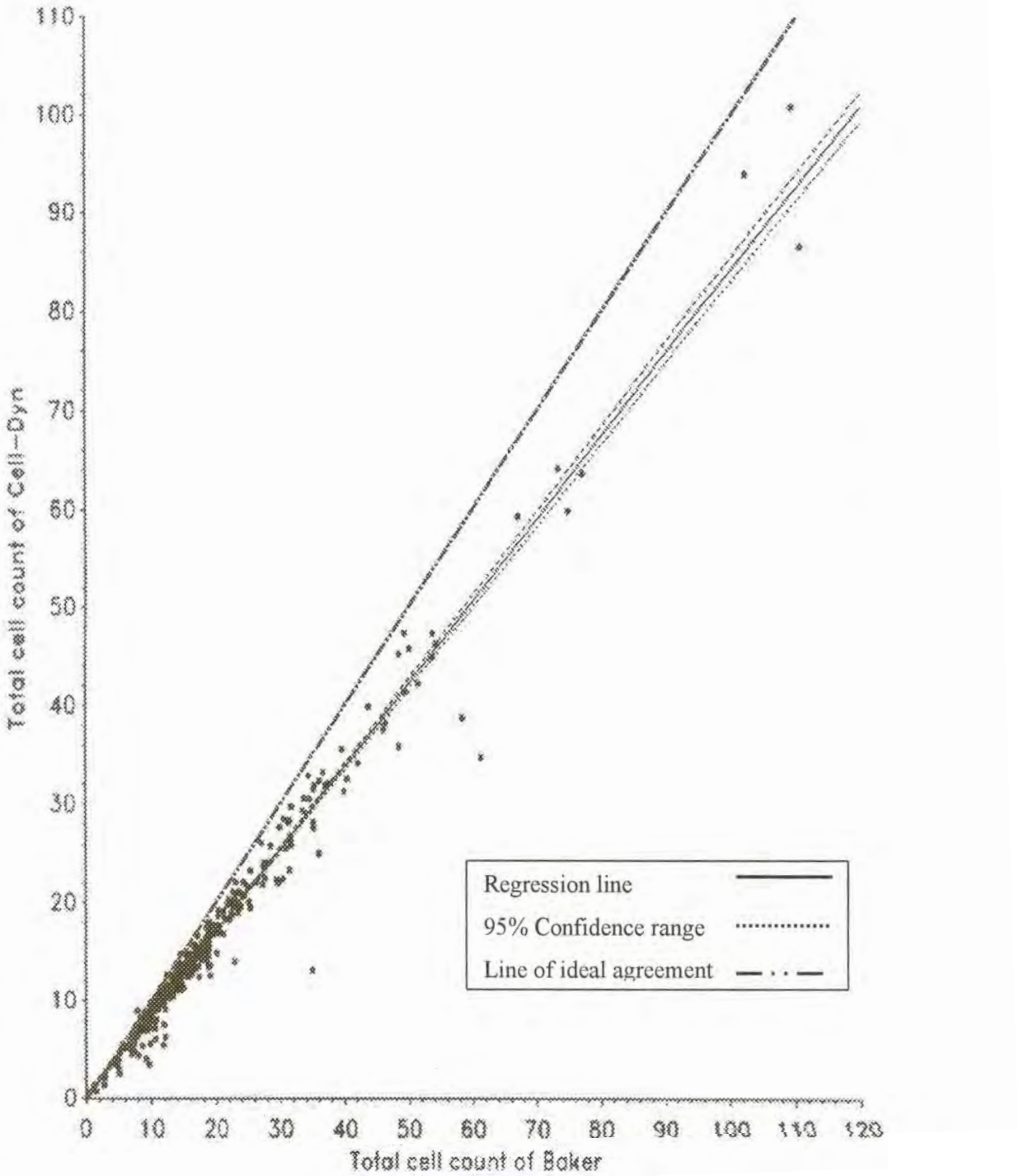


Figure 5.2: Total White Cell Count of the Cell-Dyn 3500 Compared with the Total White Cell Count of the Baker System 9000 after samples with "WBC Count Alert" and "WBC Diff Alert" Flags have been Omitted.

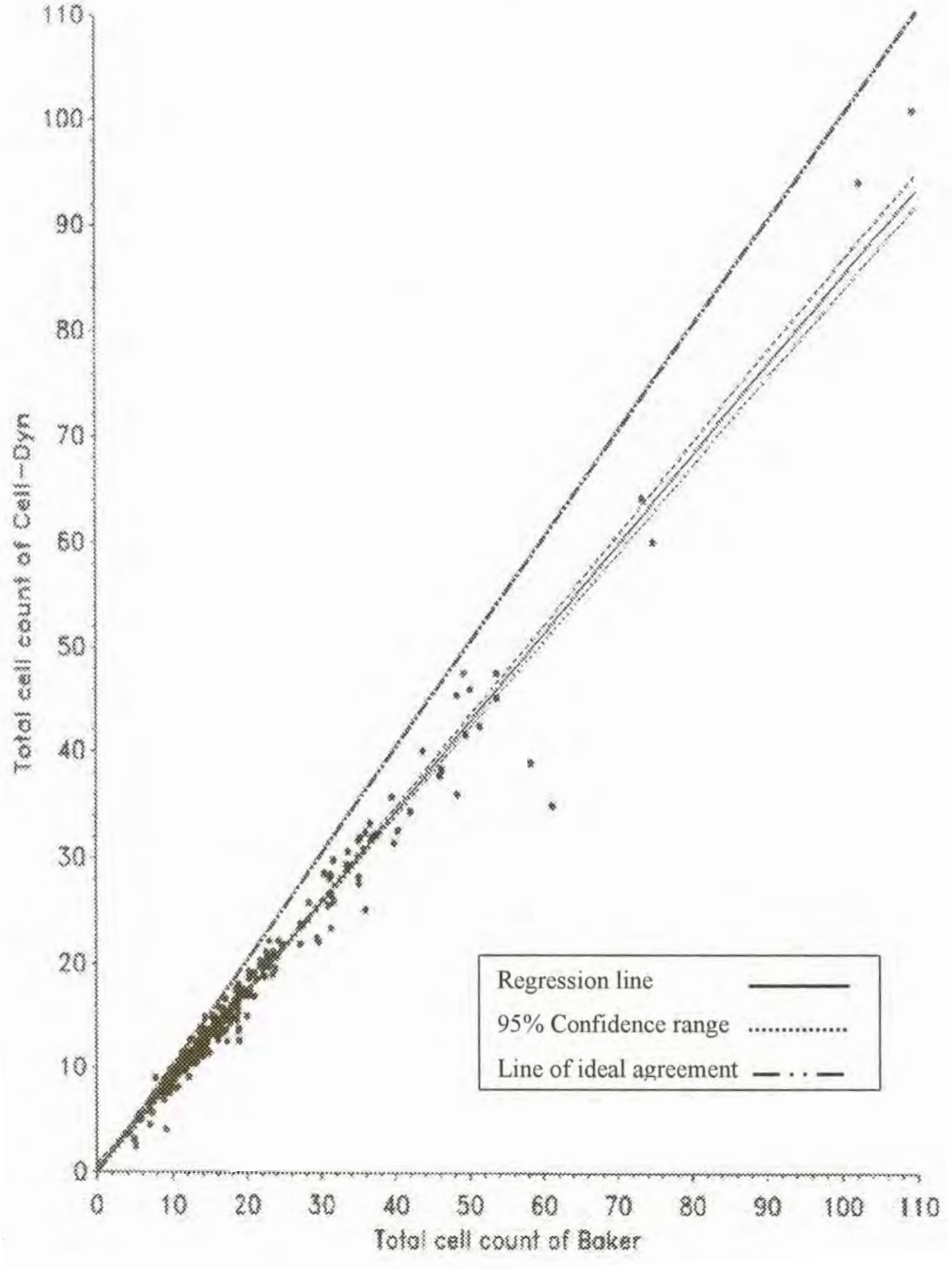


Figure 5.3: Total White Cell Count of the Cell-Dyn 3500 Compared with the Total White Cell Count of the Baker System 9000 after samples with "WBC Count Alert" Flags have been Omitted.

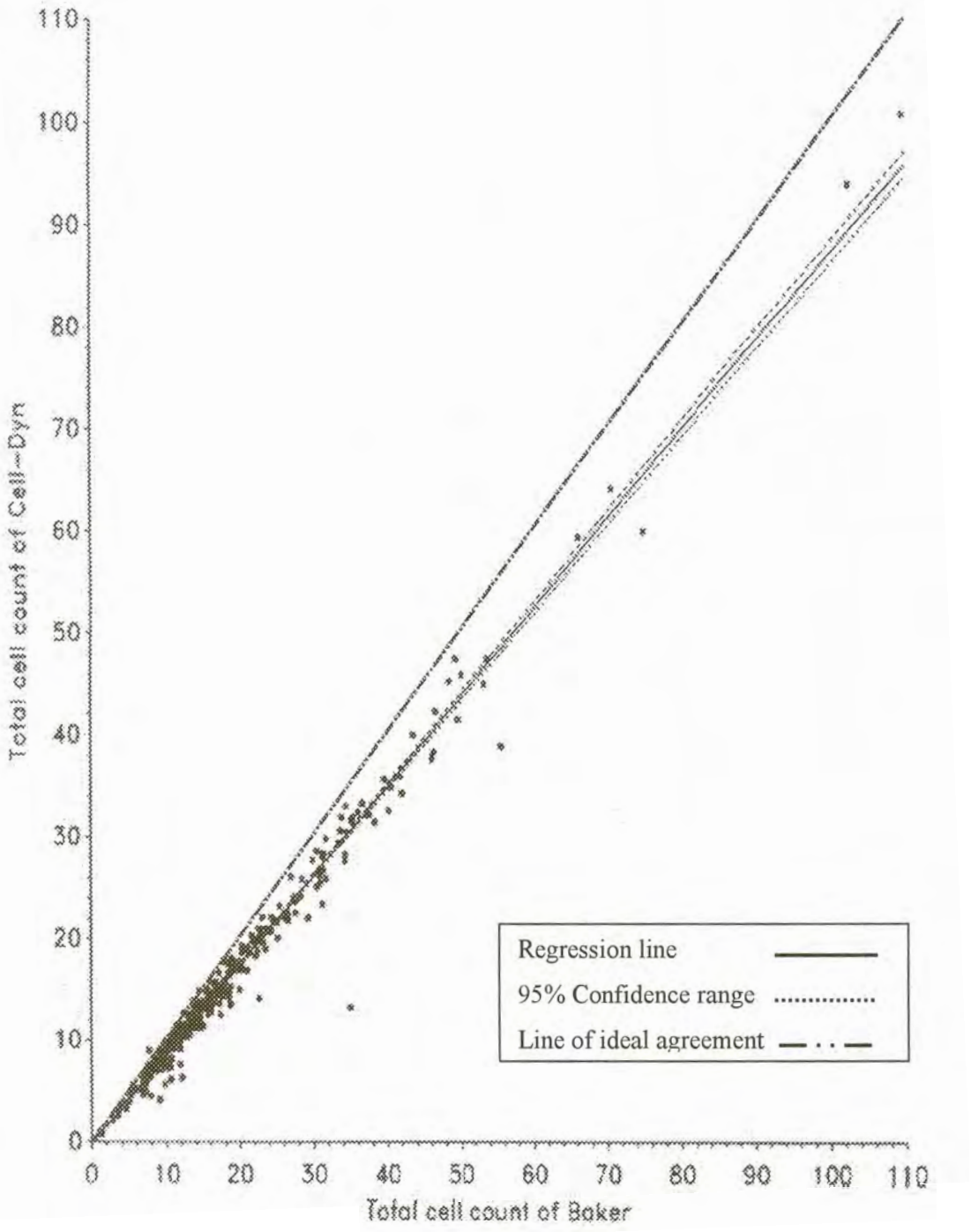


Figure 5.4: Total White Cell Count of the Cell-Dyn 3500 Compared with the Total White Cell Count of the Baker System 9000 after samples with "WBC Data Invalid" Flags have been Omitted.

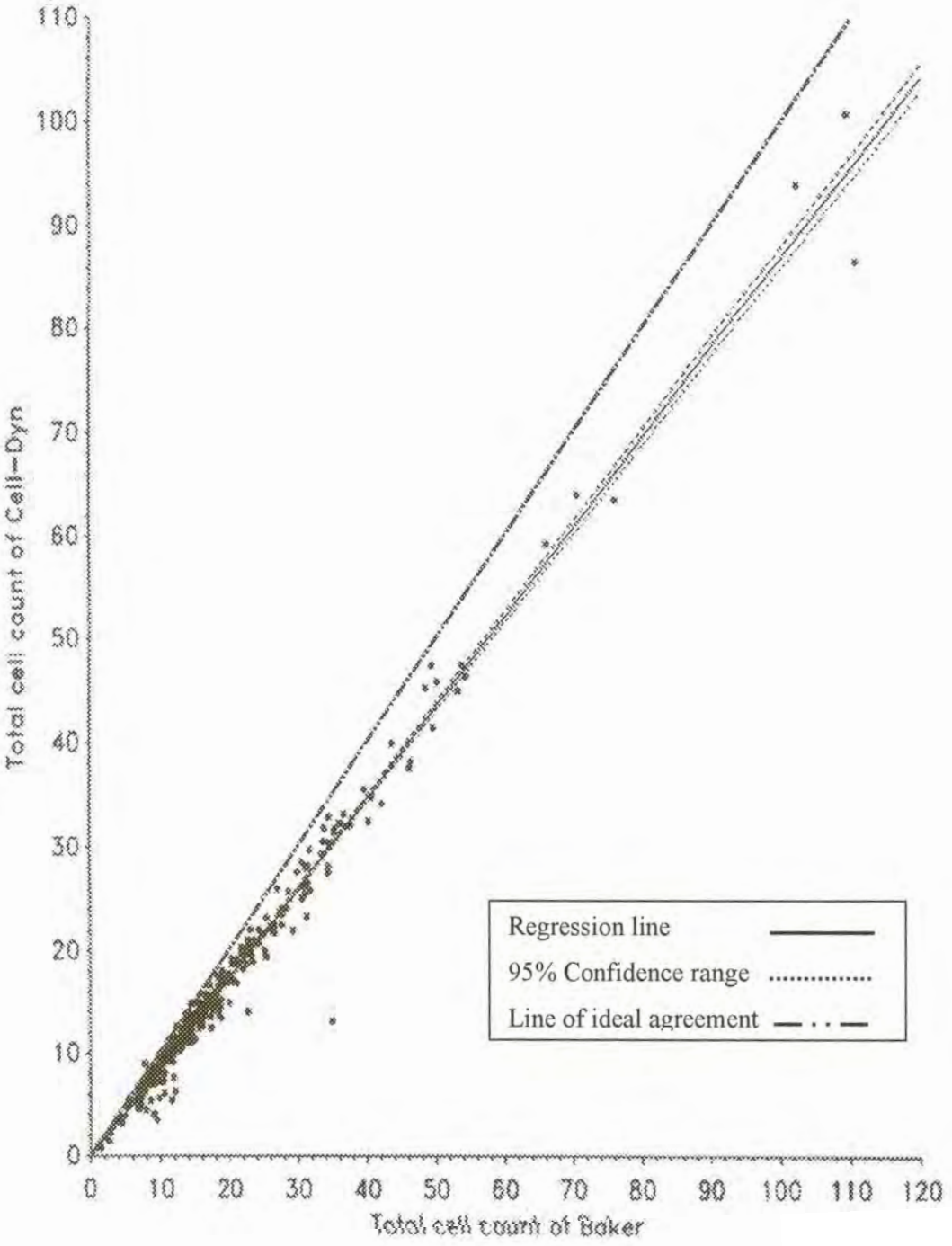
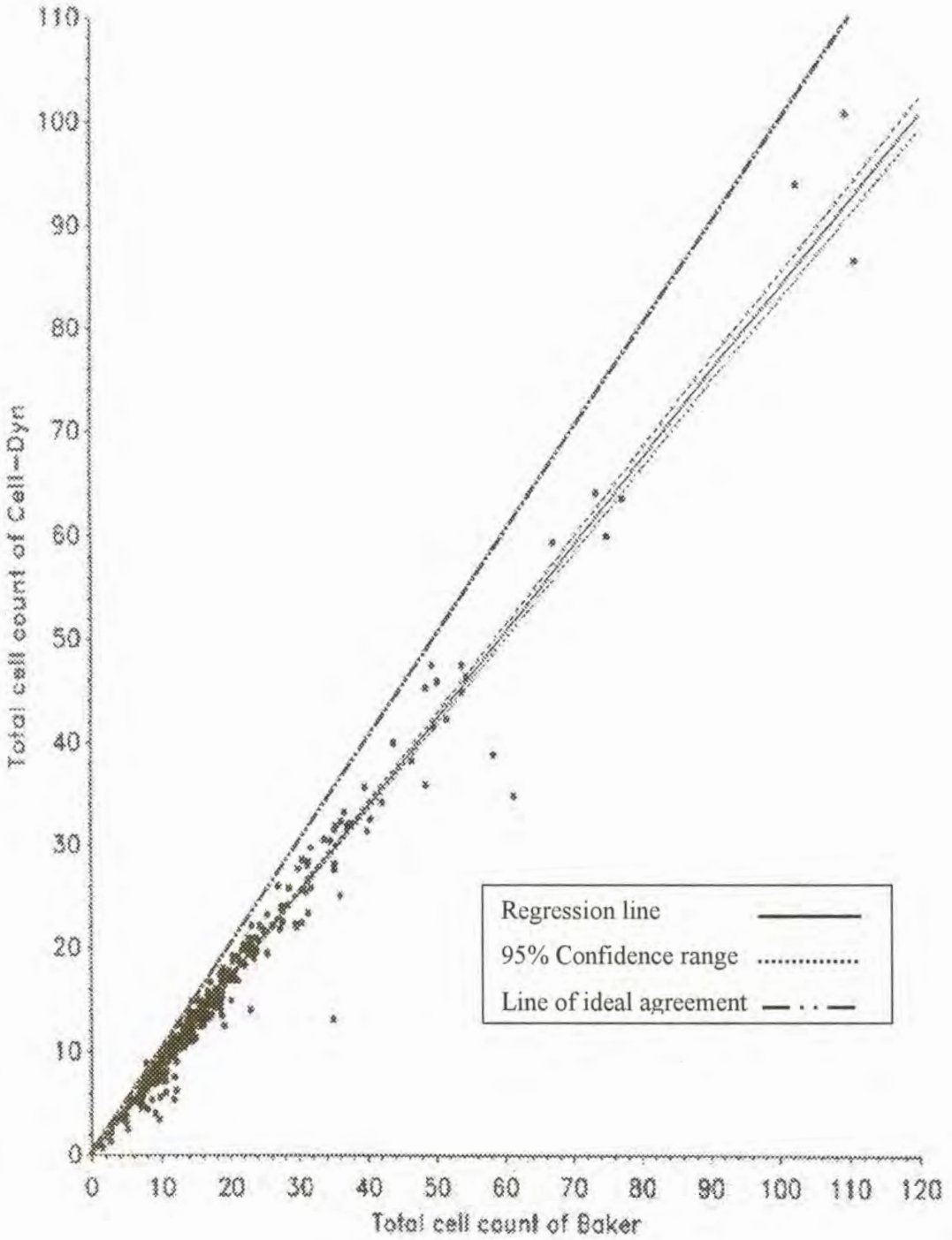


Figure 5.5: Total White Cell Count of the Cell-Dyn 3500 Compared with the Total White Cell Count of the Baker System 9000 after samples with a Monocytosis have been Omitted.



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.

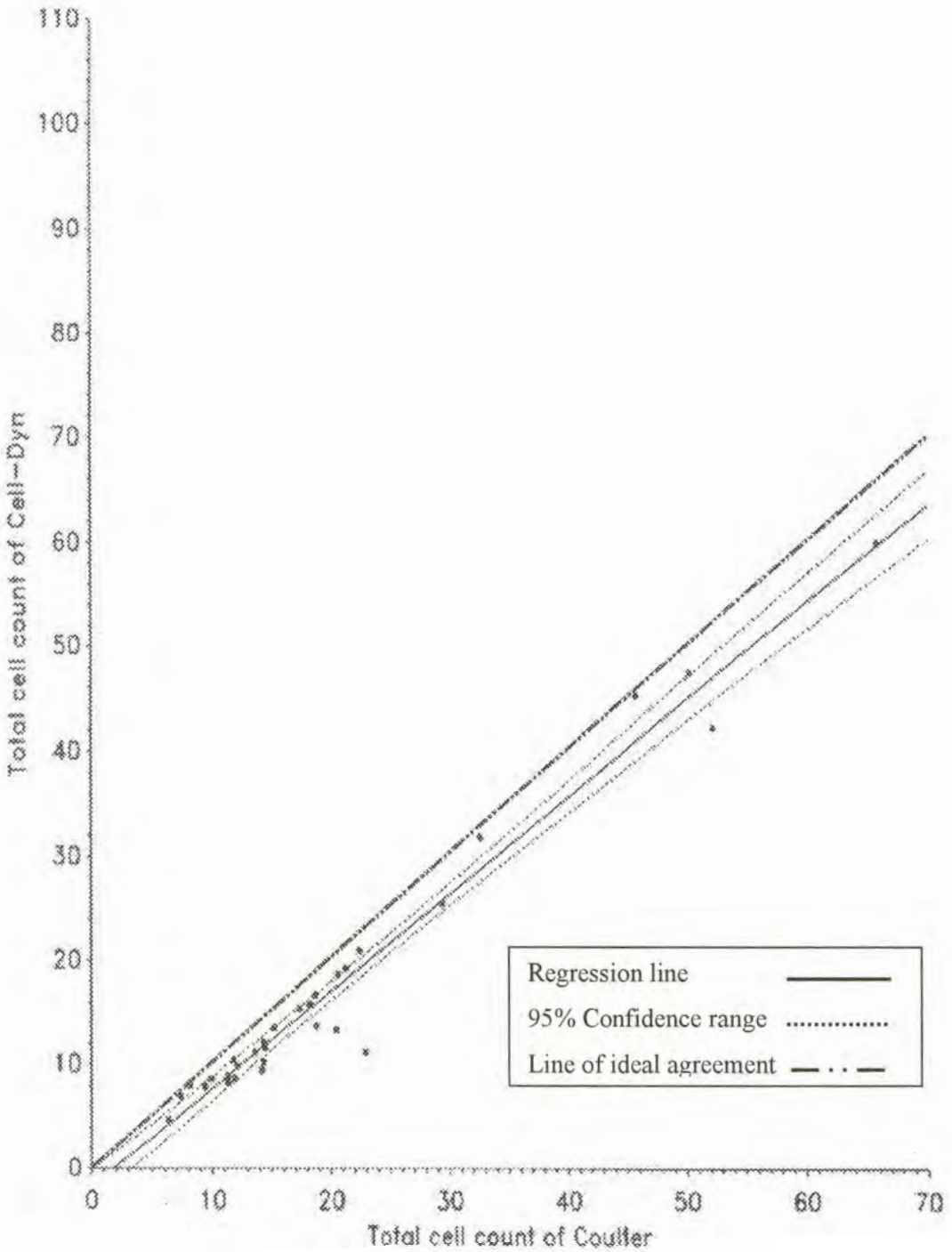


Table 5.2 Comparison of the Total White Blood Cell Counts measured on the Cell-Dyn 3500, the Baker System 9000 and the Coulter Model FN using the Friedman test.

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.

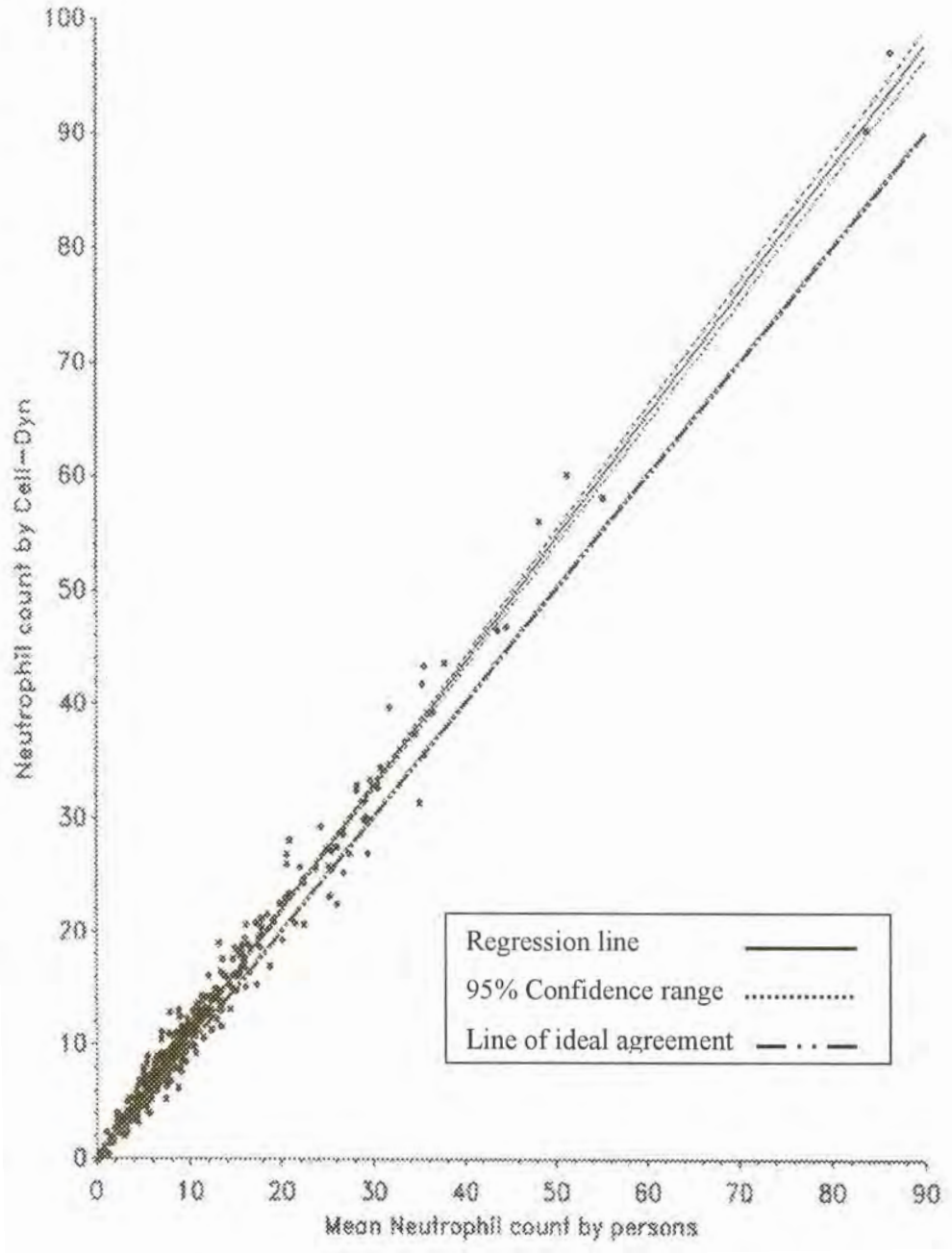
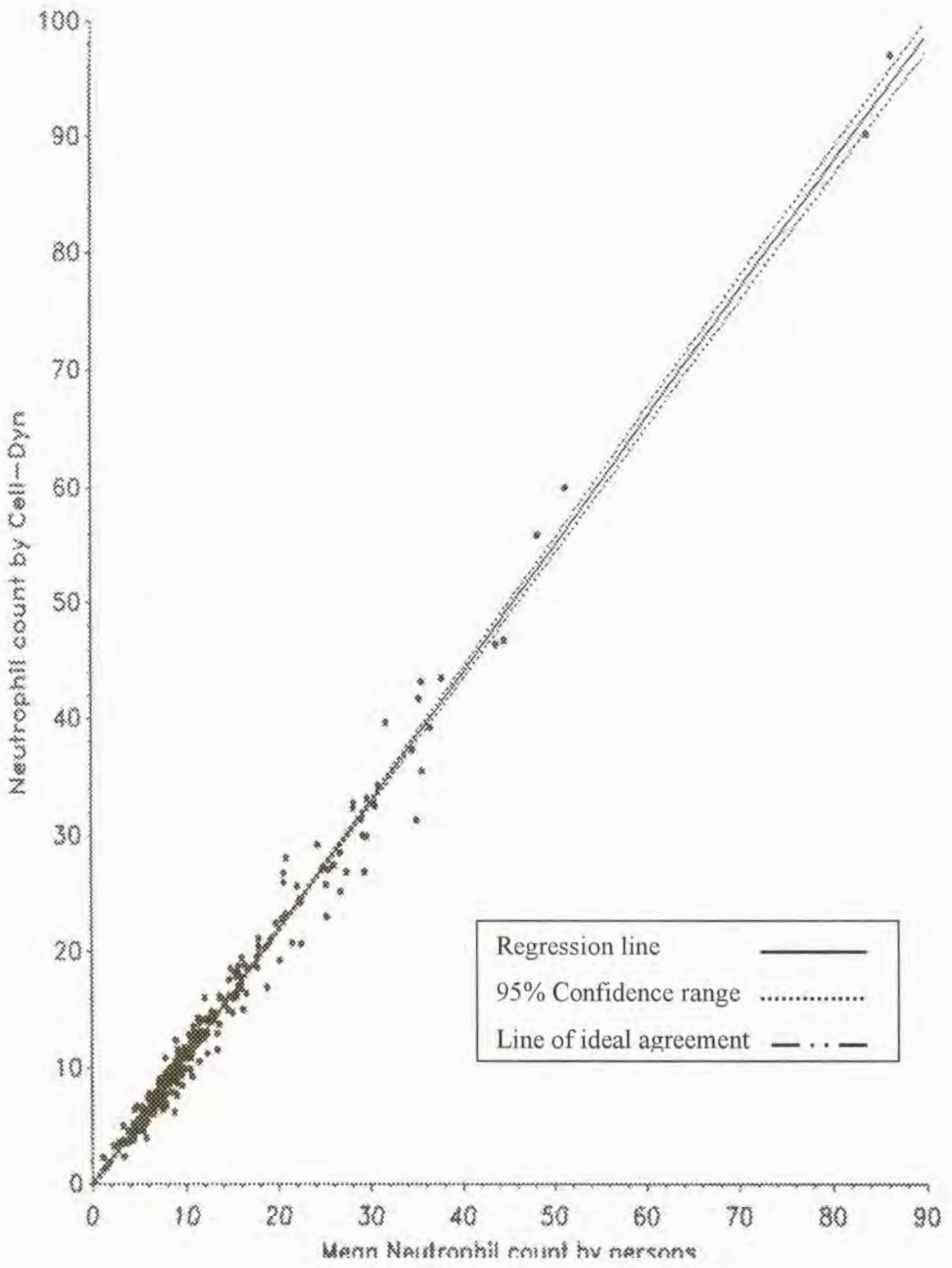


Figure 5.8: Cell-Dyn 3500 Neutrophil Count Compared to the Manual Neutrophil Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



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.

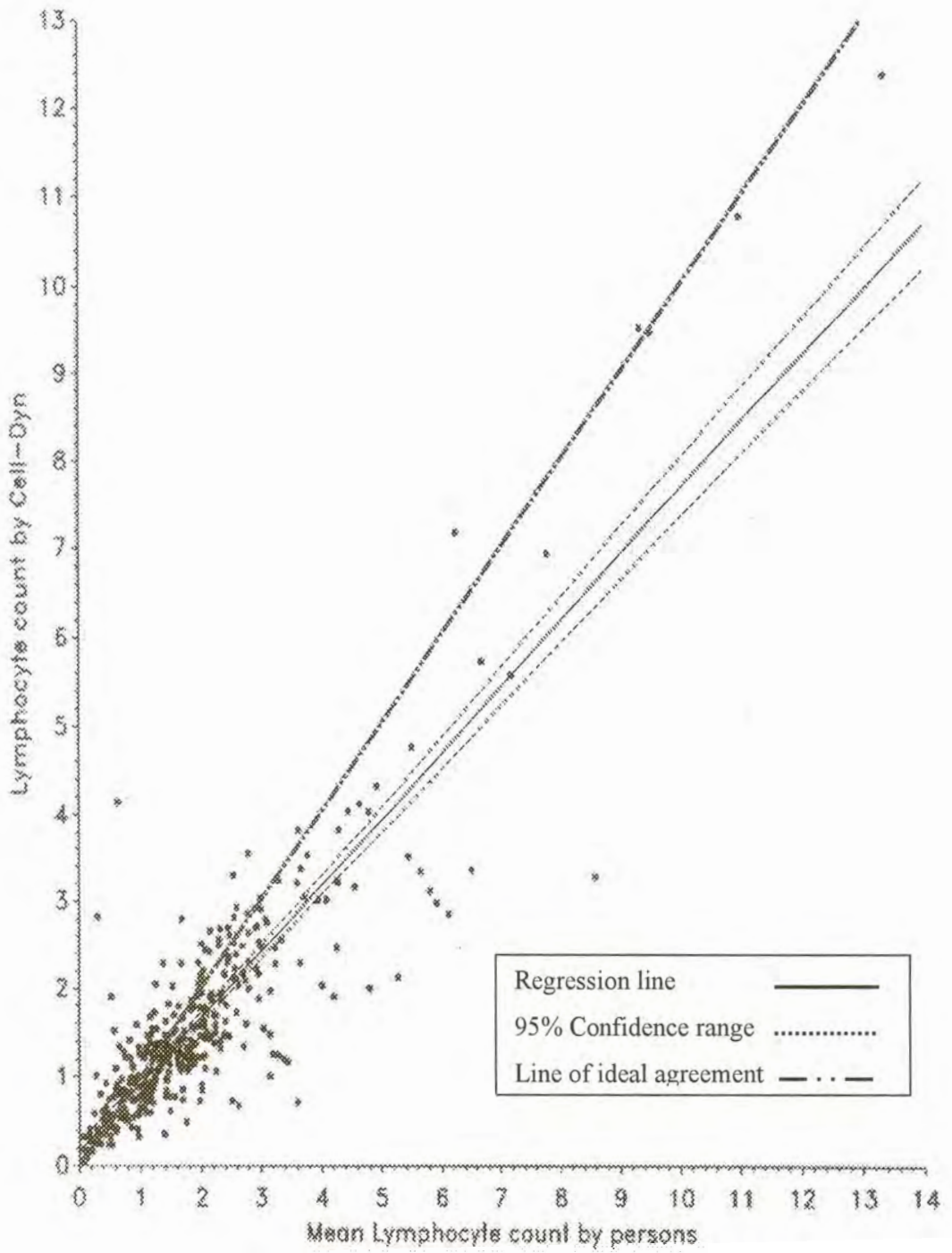
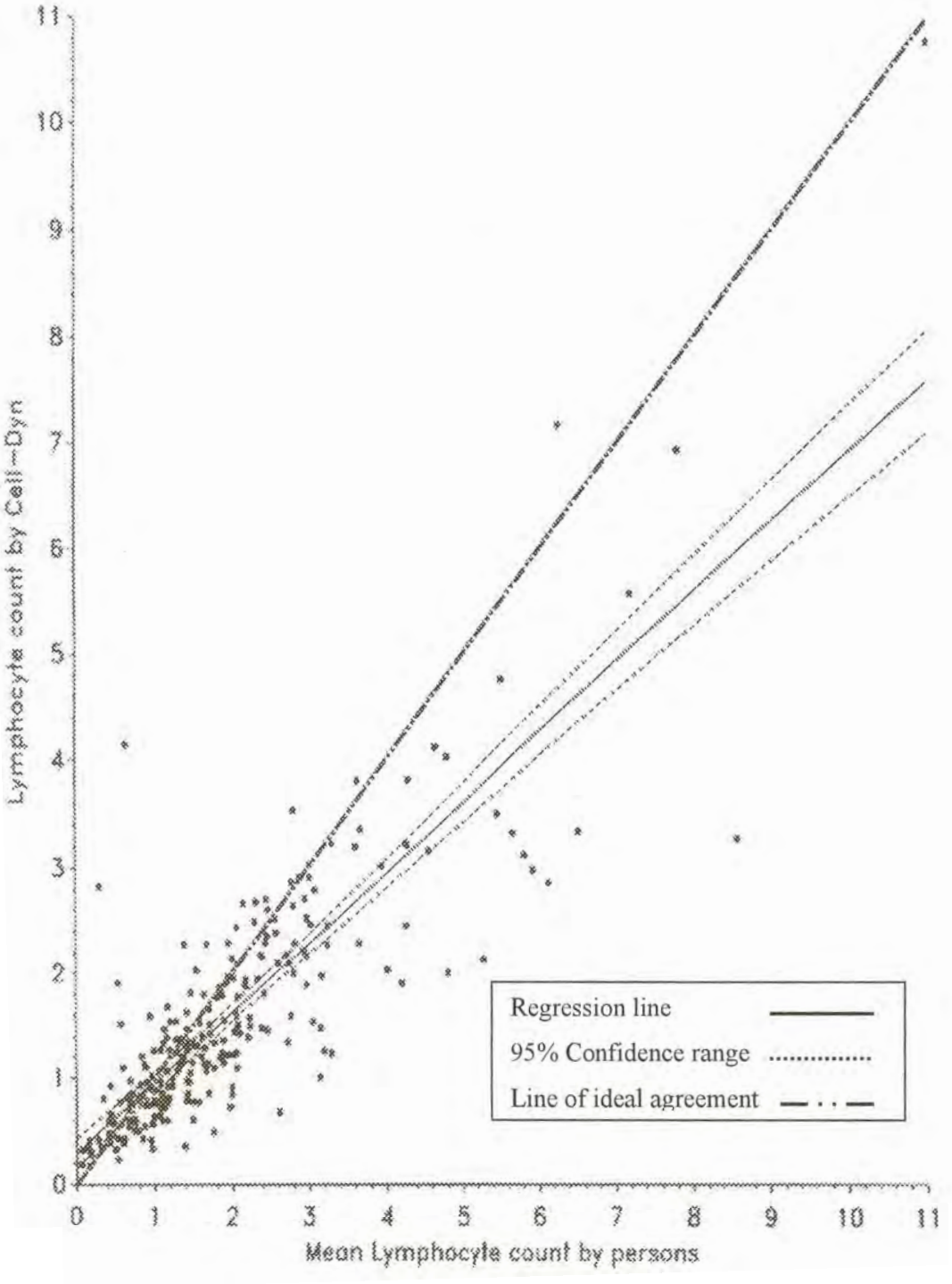


Figure 5.10: Cell-Dyn 3500 Lymphocyte Count Compared to the Manual Lymphocyte Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



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.

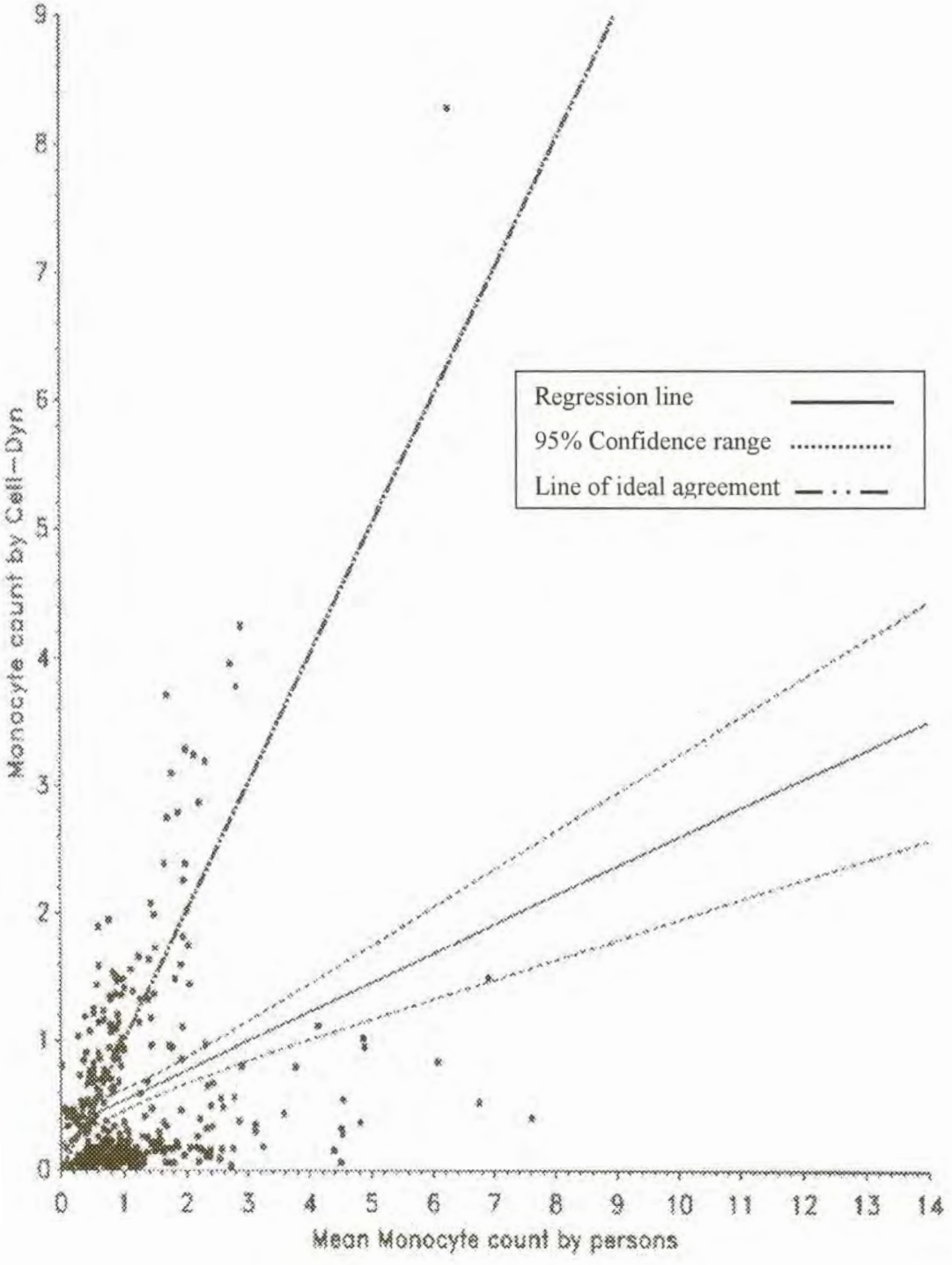
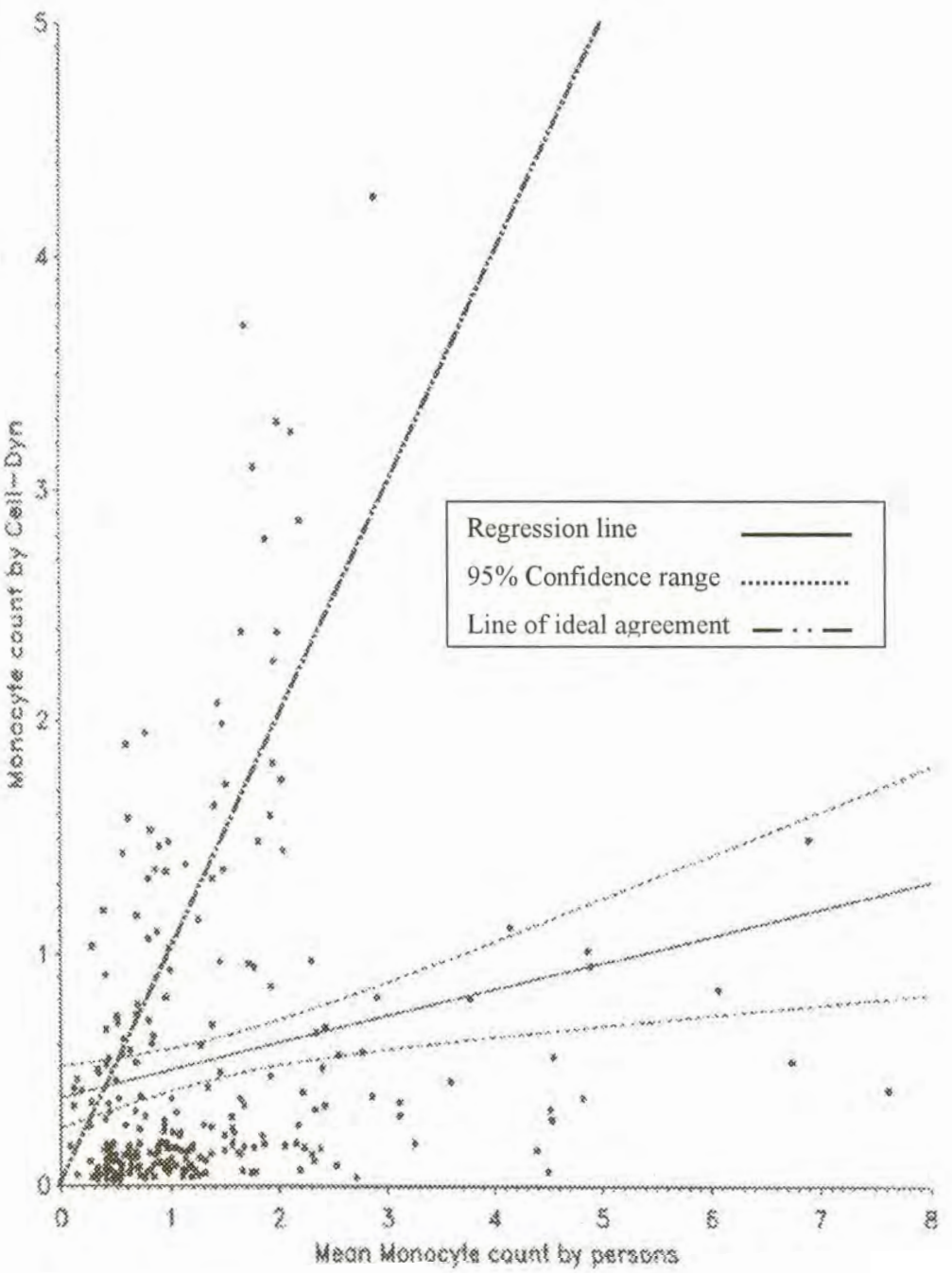


Figure 5.12: Cell-Dyn 3500 Monocyte Count Compared to the Manual Monocyte Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



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.

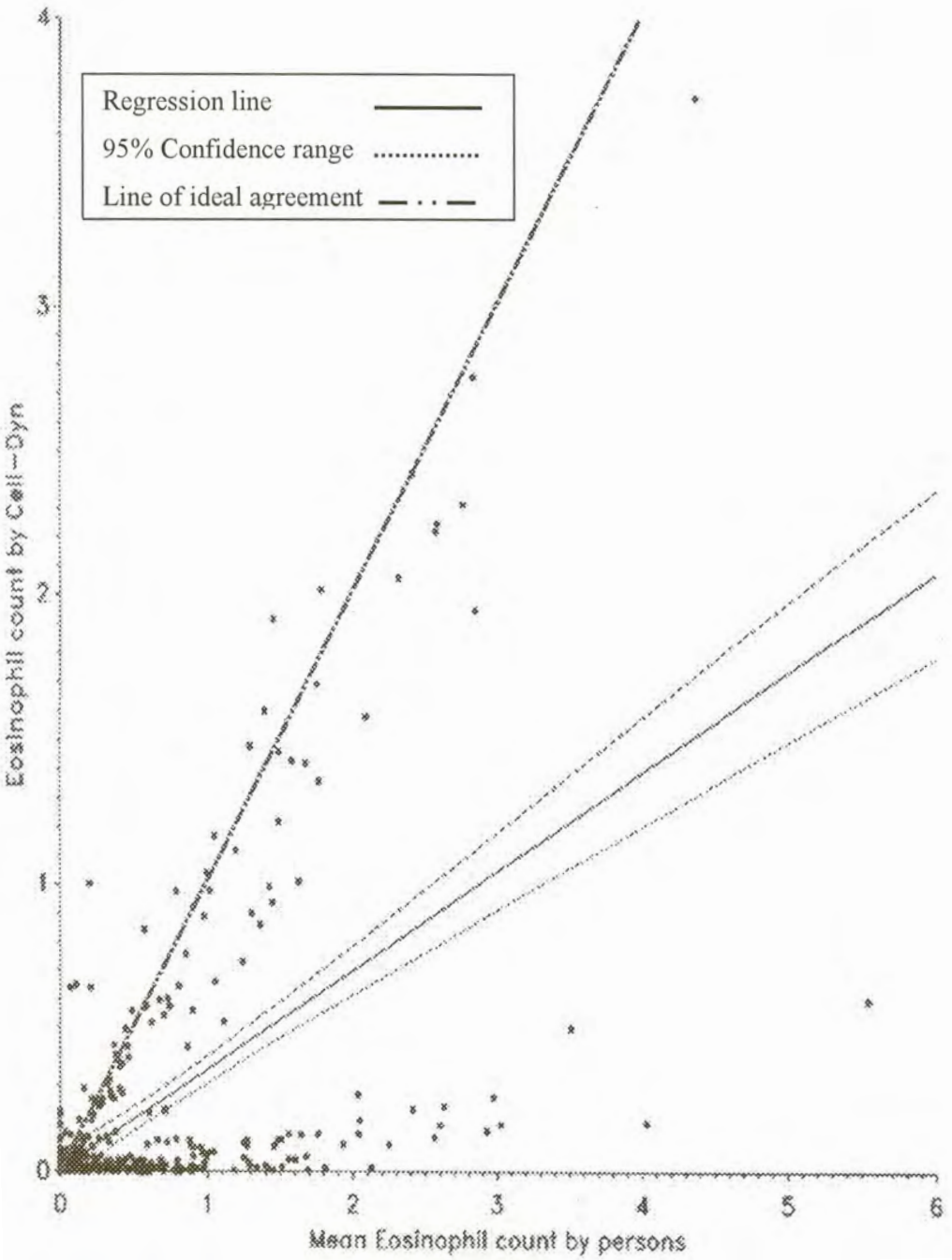
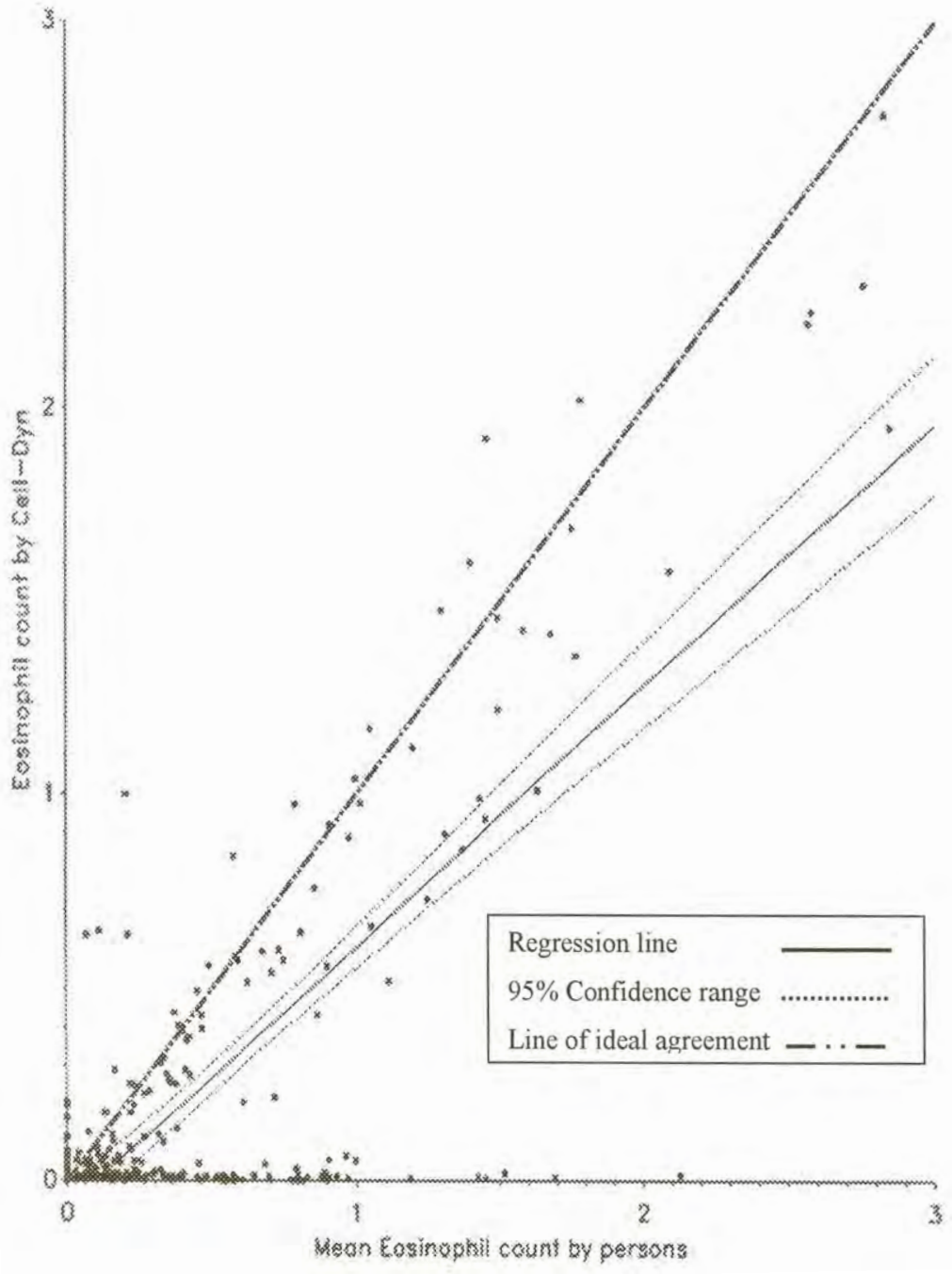


Figure 5.14: Cell-Dyn 3500 Eosinophil Count Compared to the Manual Eosinophil Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



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.

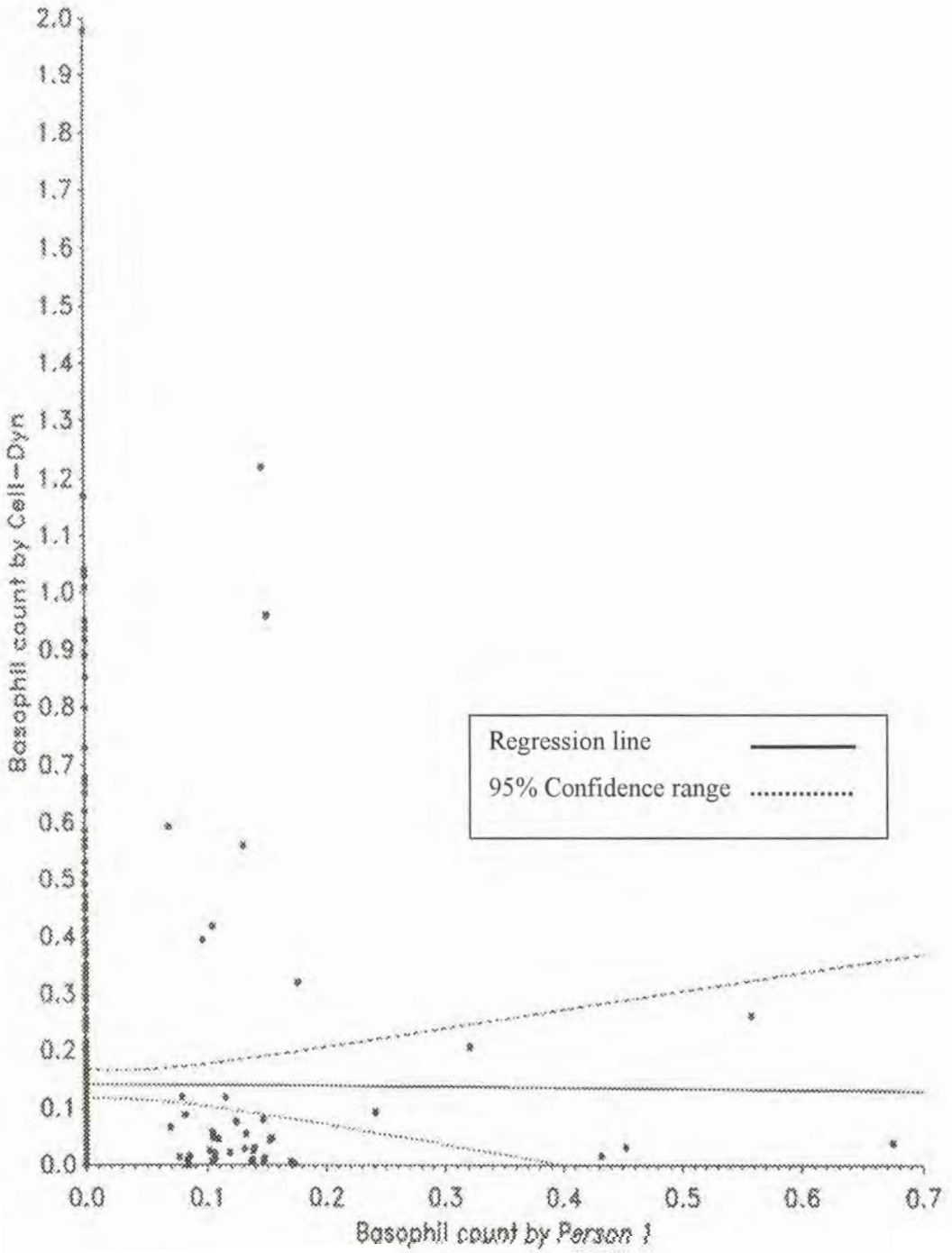
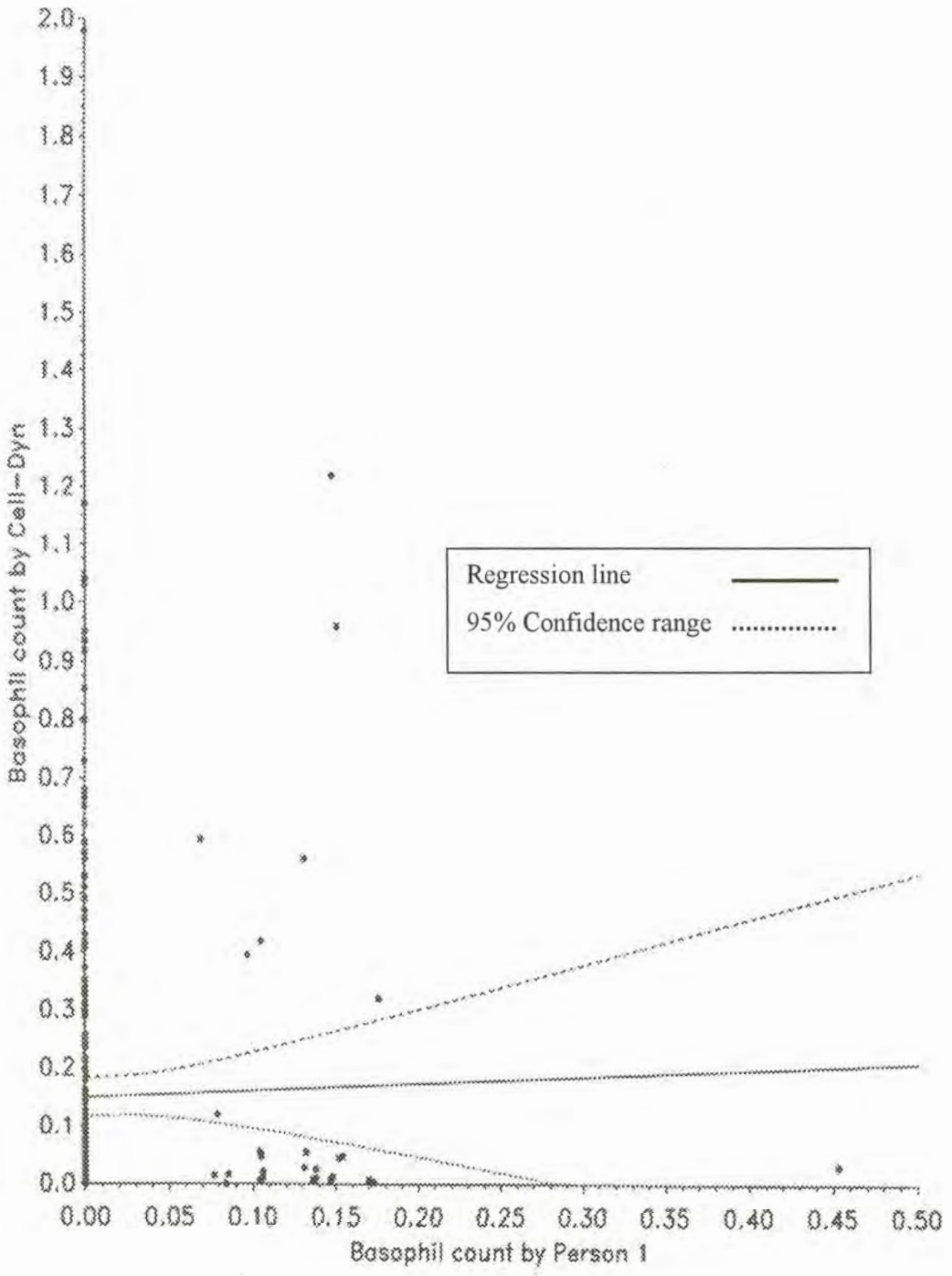


Figure 5.16: Cell-Dyn 3500 Basophil Count Compared to the Manual Basophil Count after the samples with "WBC Diff Alert" and "WBC Count Alert" Flags have been omitted.



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.

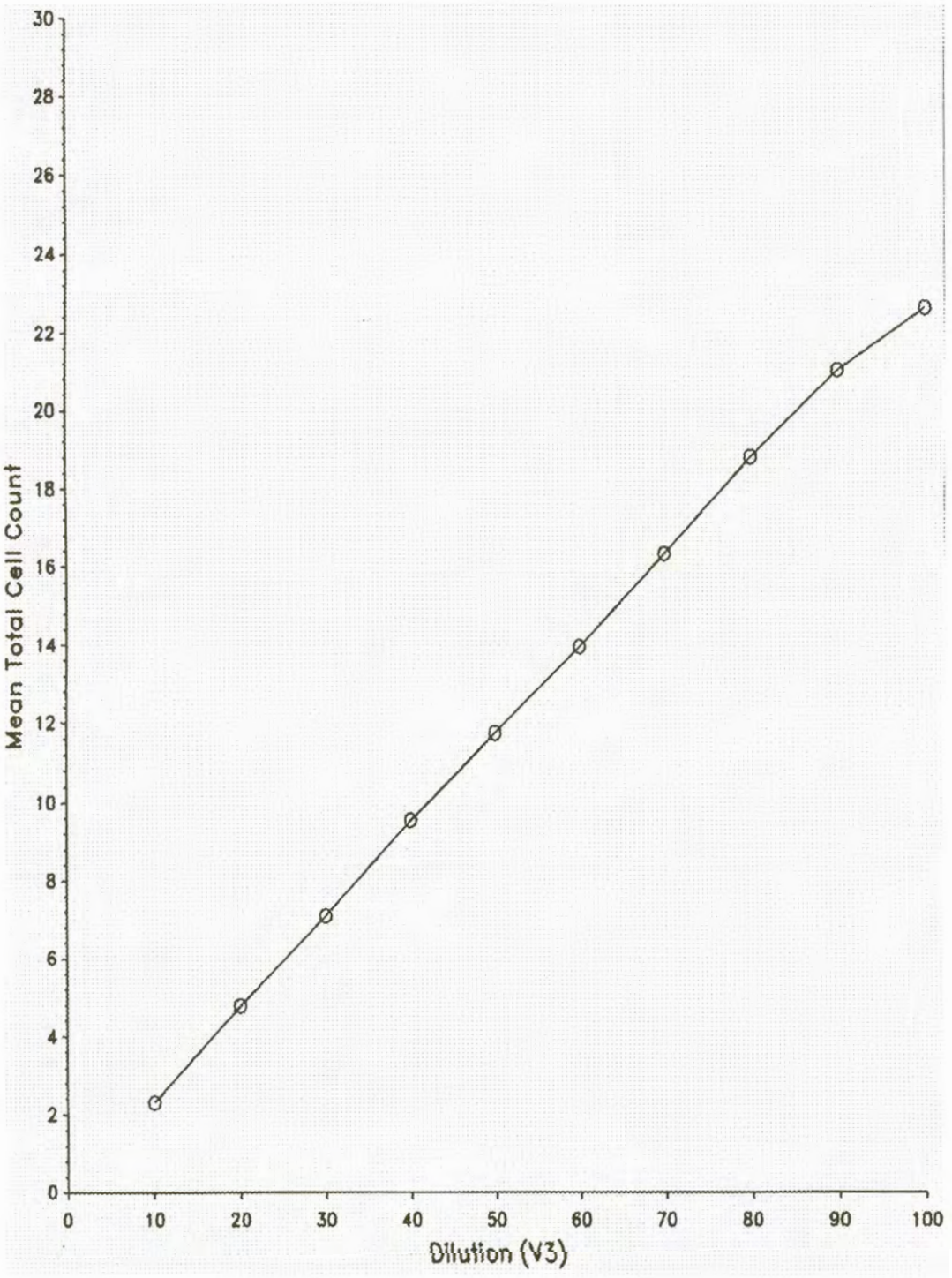
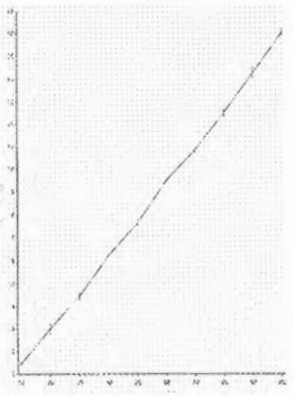
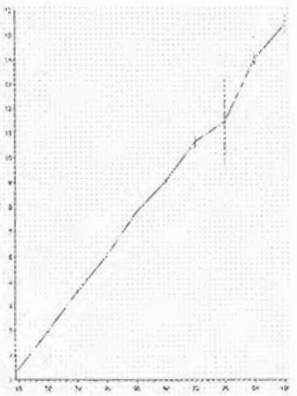


Figure 5.18: Individual Total White Blood Cell Counts of the Cell-Dyn 3500 on Ten Samples against Dilution Factor.

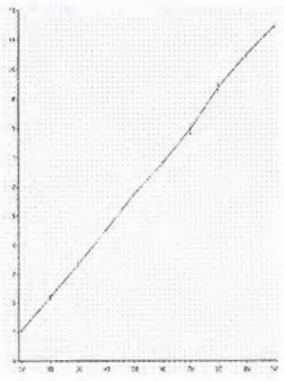
Sample 1



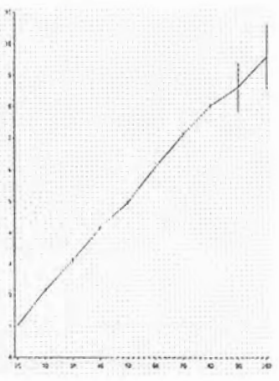
Sample 2



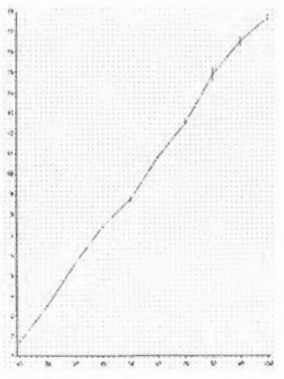
Sample 3



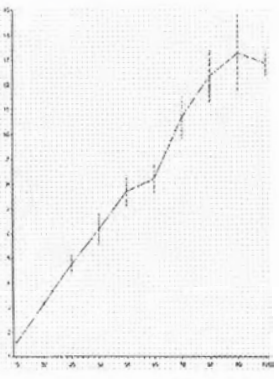
Sample 4



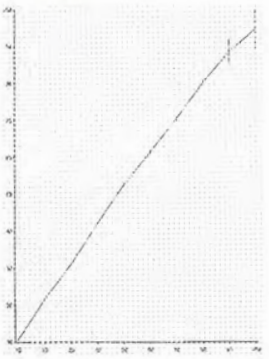
Sample 5



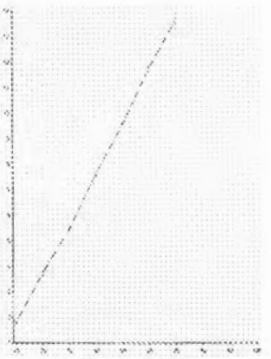
Sample 6



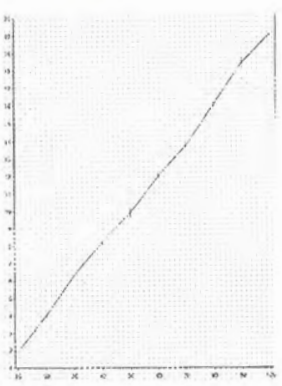
Sample 7



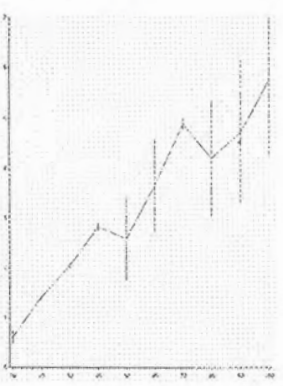
Sample 8



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.

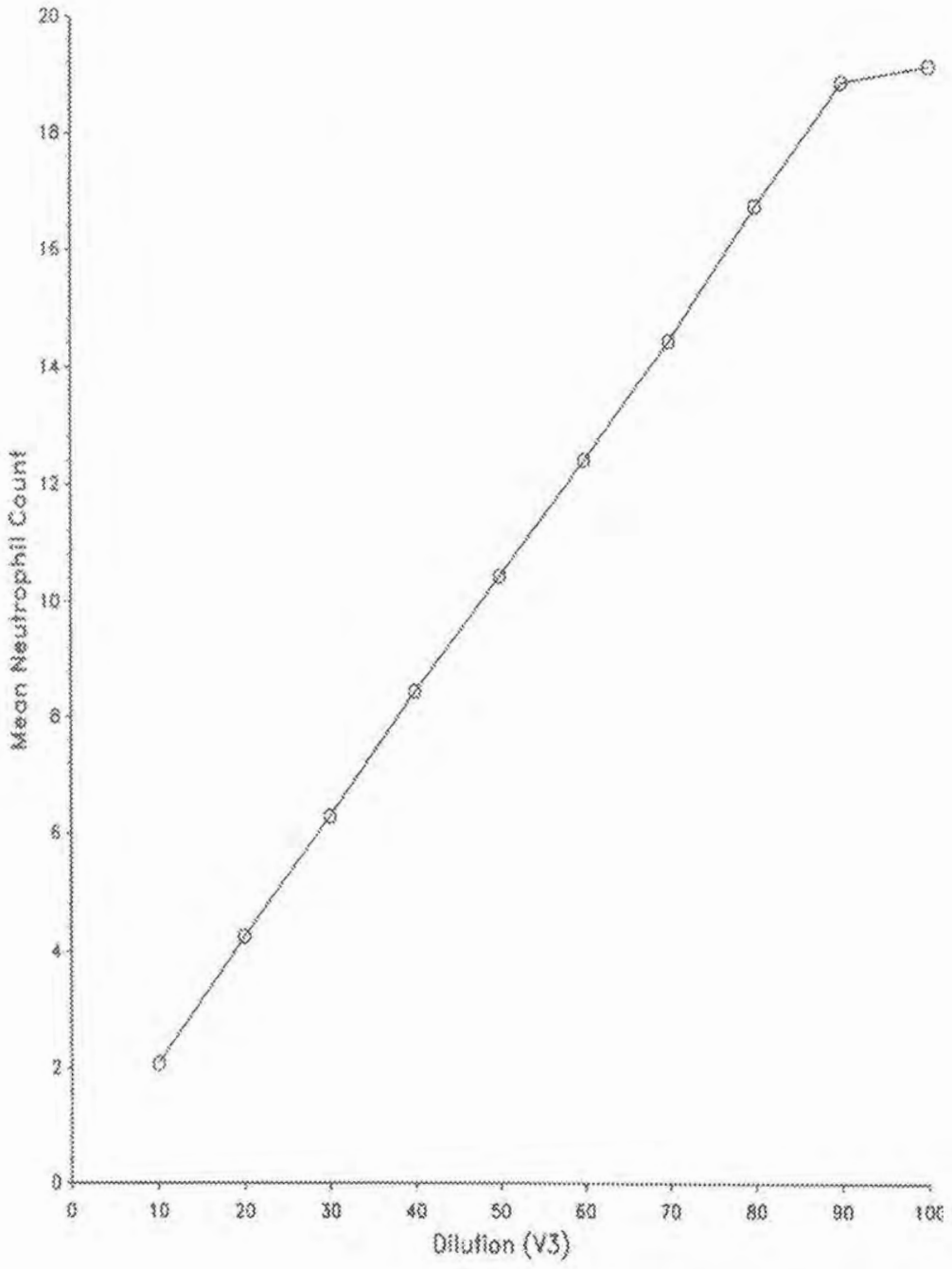


Figure 5.20: Mean Lymphocyte Count of the Cell-Dyn 3500 Vs Dilution Factor.

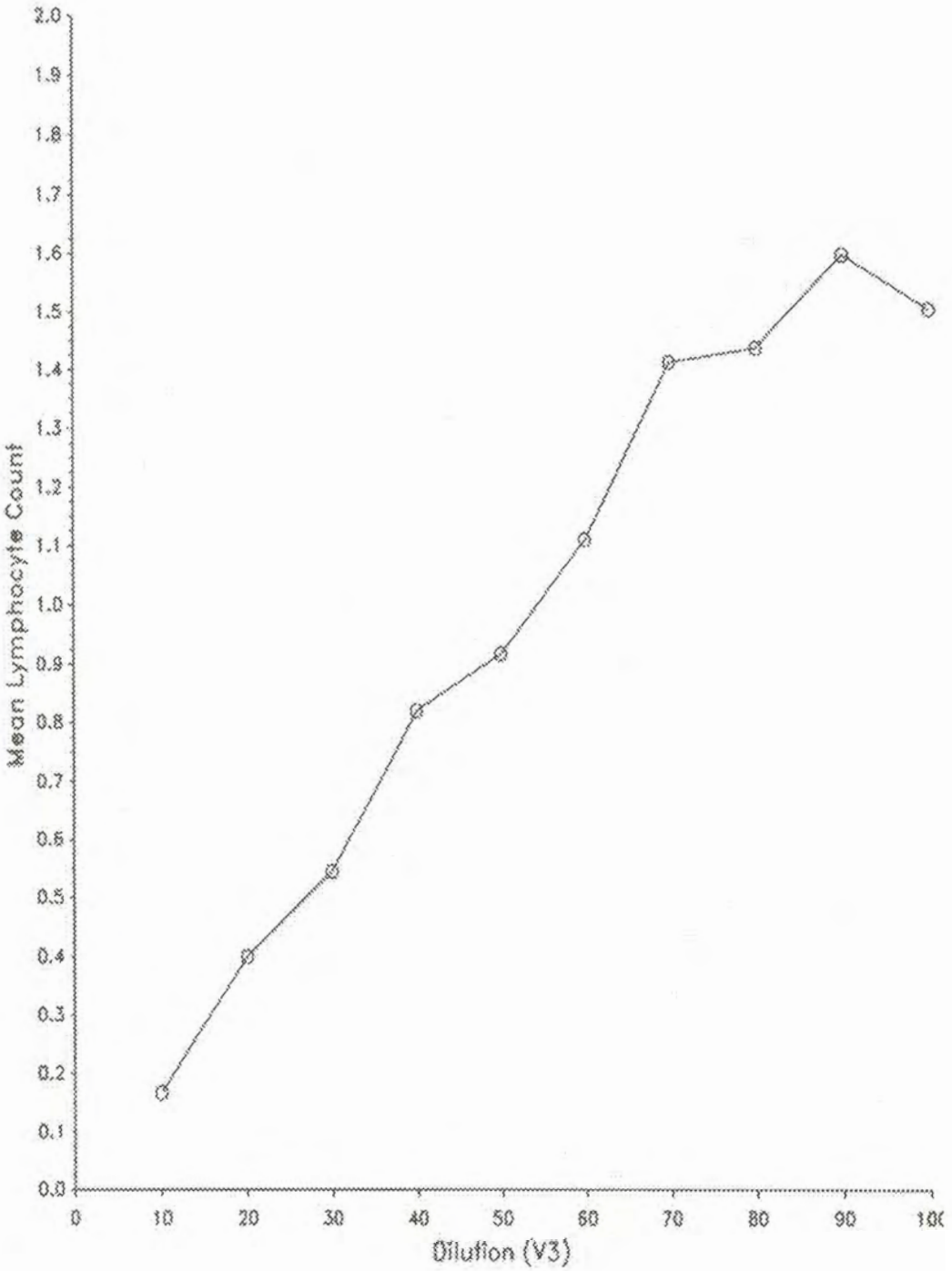


Figure 5.21: Mean Monocyte Count of the Cell-Dyn 3500 Vs Dilution Factor.

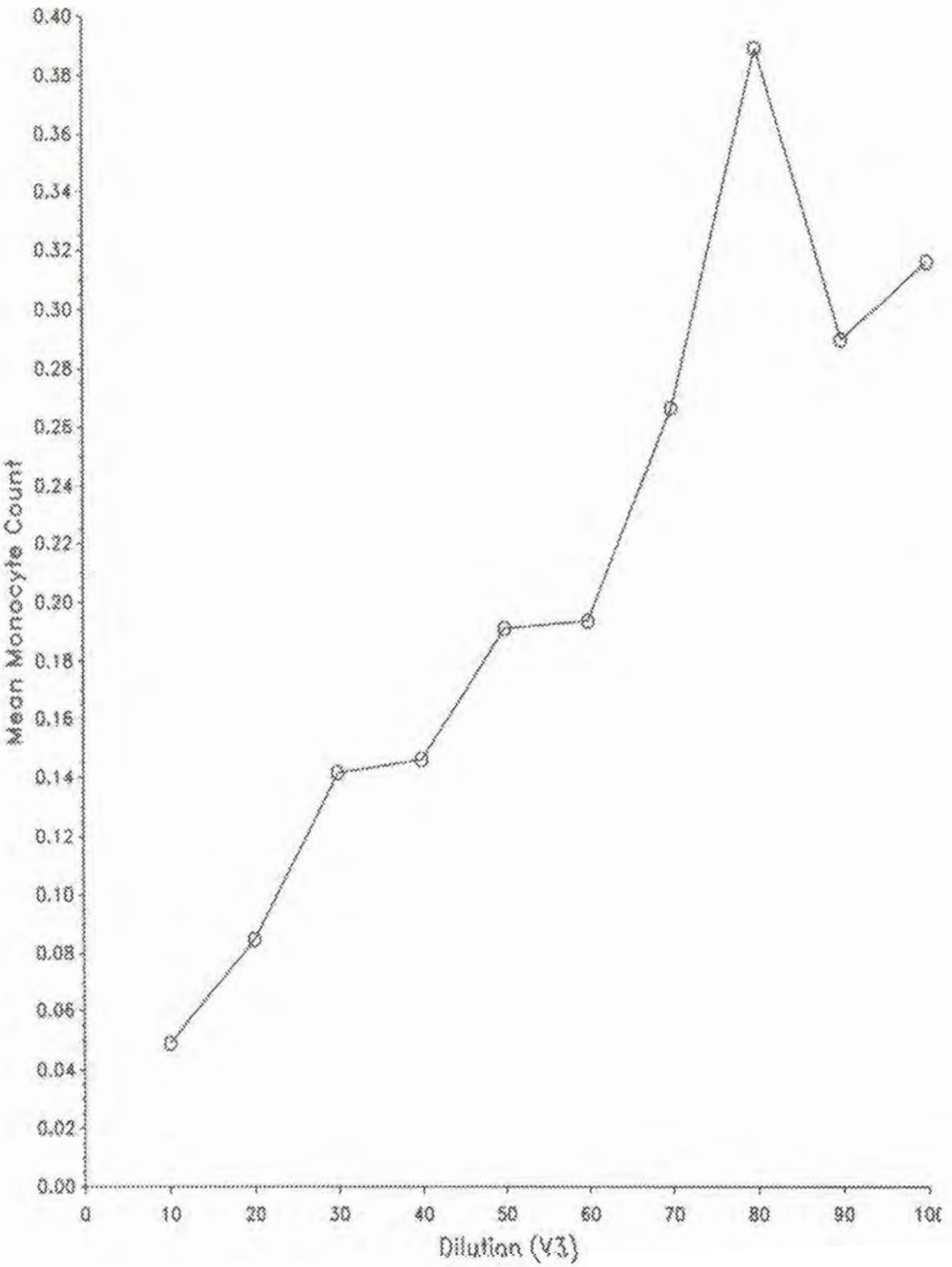


Figure 5.22: Mean Eosinophil Count of the Cell-Dyn 3500 Vs Dilution Factor.

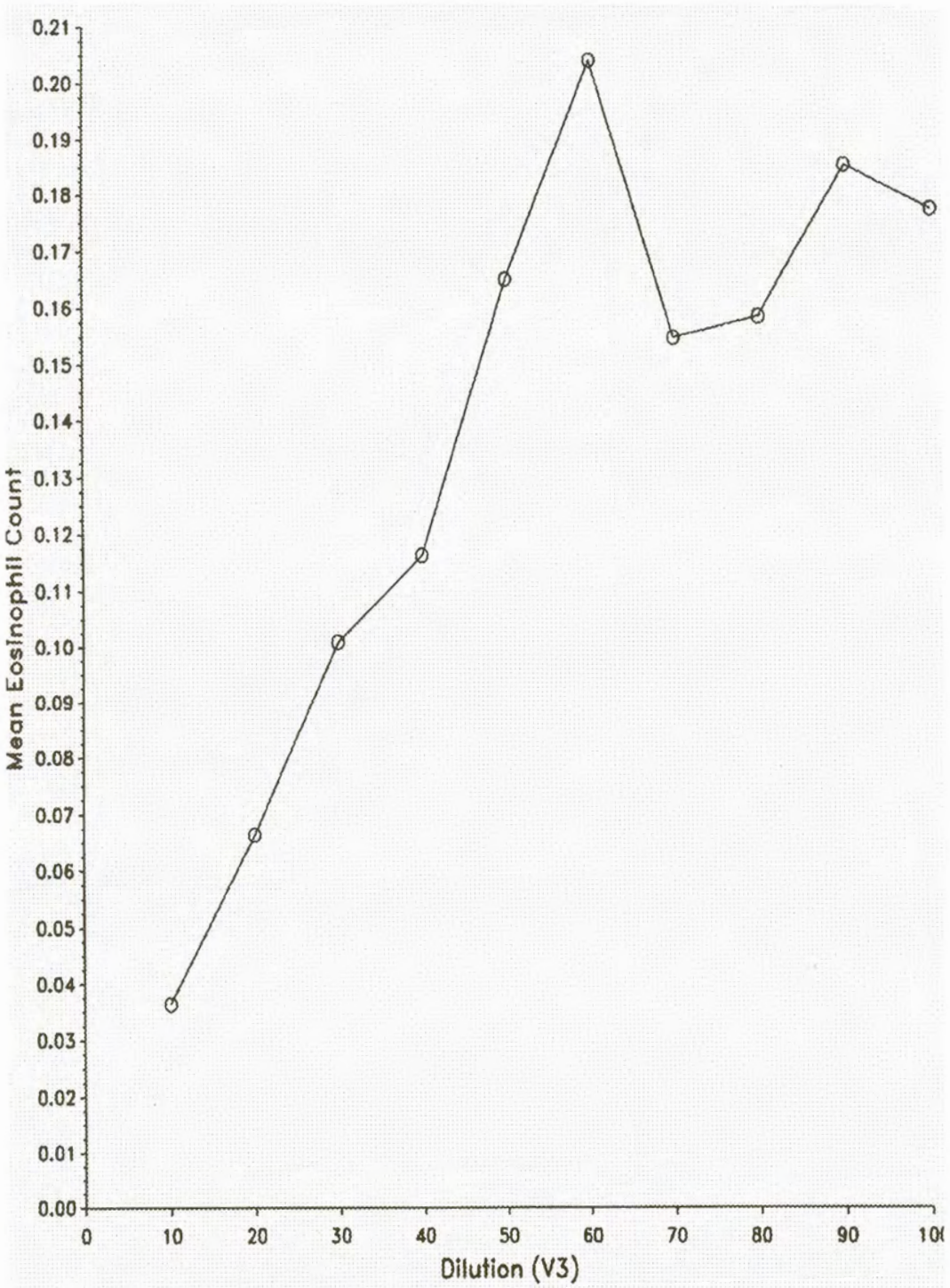
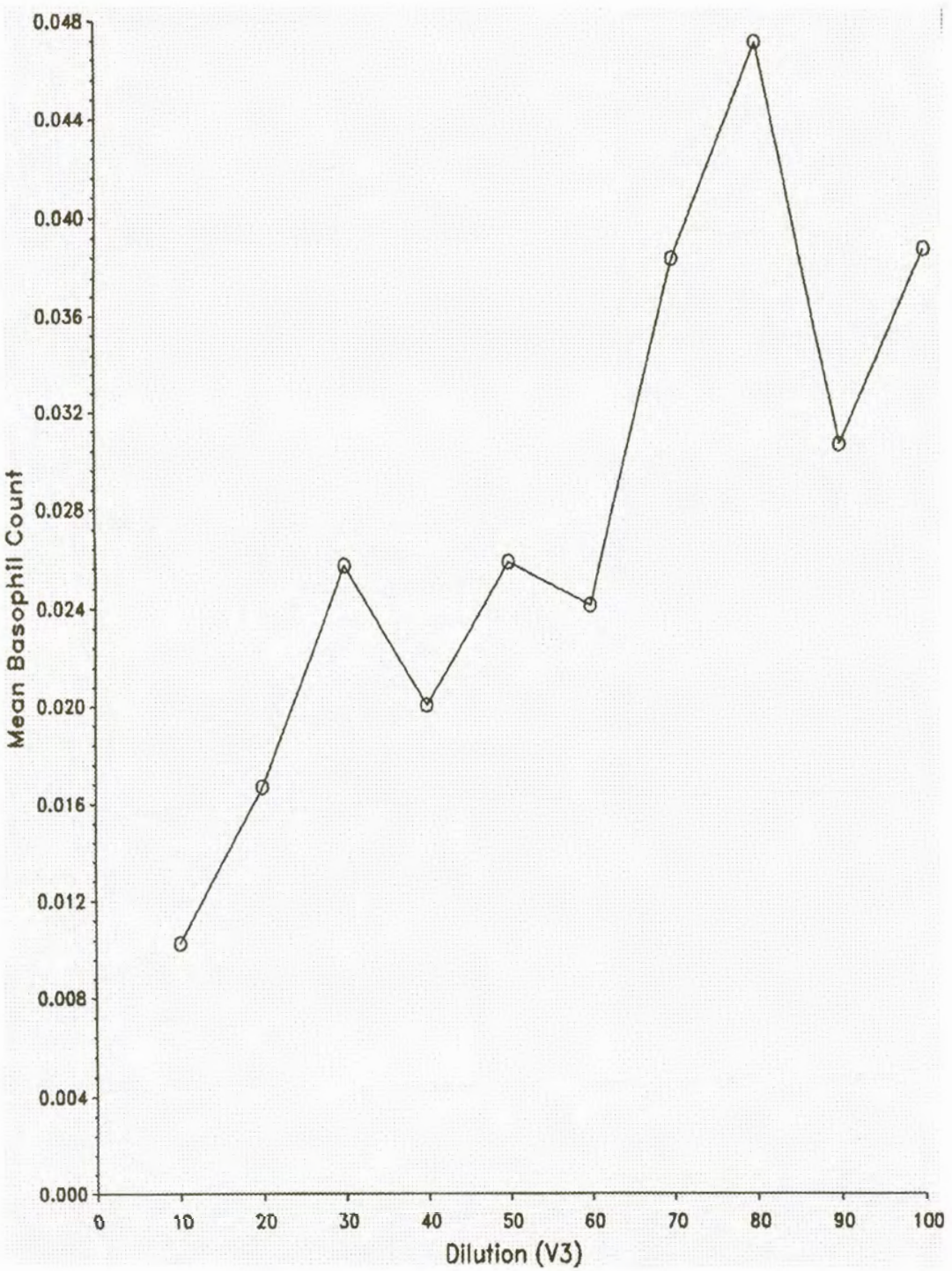


Figure 5.23: Mean Basophil Count of the Cell-Dyn 3500 Vs Dilution Factor.



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.

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