A COMPARISON OF HAEMATOLOGY ANALYSERS.

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ABSTRACT

There has been a rapid development in haematology analysers over the last decade. As a result, veterinarians in clinical practice are faced with a number of options when it comes to laboratory services. Choices include using an in-clinic analyser, making use of government and private veterinary diagnostic laboratories, or private medical and hospital laboratories.

Fundamental problems exist with using animal blood on analysers designed for human blood. Erythrocytes from some animals are much smaller than those of humans and may be confused with platelets. Furthermore there are species differences with regards to both total white cell count and the proportions of the different leucocytes.

In this study a widely used veterinary haematology analyser (ABX Minos Vet) was compared with two medical analysers (Technicon H1 and the Coulter VCS) using blood from cats and dogs with normal and abnormal haemograms. Also included in the comparison were the Automated and Manual QBC-V analysers which are being marketed to Veterinarians for in-clinic use.

The values obtained by all analysers were in close agreement when estimating the packed cell volume of both cats and dogs. Total white cell counts in dogs were also relatively consistent across all analysers , but in cats there was considerable variation in estimates of total white cell count between analysers and when compared with manual estimation using a haemocytometer. This variation highlights the difficulty in obtaining accurate total white cell counts in cats, probably due to interference by clumping of platelets.

Platelet counts obtained by the ABX Minos Vet in dogs correlated well with those counts obtained by both medical analysers but not with the QBC-V analysers. In cats, there was poor correlation of platelet counts between all analysers thus emphasising the problems caused by platelet clumping in this species. The total platelet counts in cats, and to a lesser extent in dogs,

should not therefore be interpreted rigidly and should be checked by visual appraisal of blood smears.

Measurements obtained by the Automated QBC-V most closely correlated with those of the ABX Minos Vet rather than with the Manual QBC-V, suggesting that it is capable of providing more accurate results.

A further study was carried out to determine the effects of time on blood parameters as, in a normal clinical setting there can be considerable variation in the time elapsing between collection of the blood sample and its analysis.

Blood from five cats and five dogs was tested on the three specially adapted veterinary haematology analysers (both QBC-V models and the ABX Minos Vet), over a 24 hour period. The packed cell volumes in both dogs and cats remained consistent over this time period.

The platelet counts in four of the five cats dropped into the thrombocytopenic range at either two or four hours post collection, on all of the analysers. This coincided with a peak in the white cell count observed on the ABX Minos Vet. It is likely that aggregated platelets were being recognised as white cells by the ABX Minos Vet. These results suggest that measurement of the total platelets and white cell counts in cats at two and four hours after blood collection may be less reliable than measurements made either immediately after collection or later than four hours. In dogs, the total platelet counts and white cell counts were relatively consistent over the 24 hour period and any variation encountered would not have altered the interpretation of the results.

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TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER ONE

GENERAL INTRODUCTION.

Veterinarians in clinical practice are faced with a number of options when it comes to laboratory services. In addition to government and private veterinary diagnostic laboratories, private medical and hospital laboratories are often asked to analyse blood from animals, even though their haematology analysers are designed for use on human blood. Modern haematology analysers currently used in medical institutes automatically provide differential white blood cell counts on human patients, but there is little published data on the accuracy of such counts on the blood from animals, although falsely elevated white cell counts in cats on electronic analysers have been observed (Schalm *et al*, 1986).

There are many inherent problems with using a human analyser for the analysis of animal blood, the most obvious of which is the morphological variation between the species with regard to red and white blood cell parameters. It has been observed that non-human mammalian blood cells are often too small to behave properly with respect to the cell counting threshold on instruments designed for human blood (Weiser, 1987). The size and number of erythrocytes vary amongst the animal species; the smaller the erythrocyte in size, the greater the number per unit volume of blood. Very small erythrocytes may be misinterpreted as platelets by analysers which differentiate cells on their size. Heinz bodies, clumps of denatured haemoglobin on the internal surface of the red blood cell membrane which reflect oxidative damage in other species can occur naturally in the cat. Likewise, Howell-Jolly bodies, small densely staining spherical bodies within erythrocytes, considered to be nuclear remnants are occasionally seen in the erythrocytes of cats (Thompson, 1993) These are not normally seen in the dog, except as a pathological condition.

Species differences occur not only with regard to the total white cell count but also in the

proportion of the different leucocytes. Neutrophils predominate in the human, dog and cat, but in ruminants and pigs, lymphocytes are the most abundant leucocyte in peripheral circulation (Schalm *et al*, 1986). Leucocyte morphology, except for some minor differences, is generally similar in various species. However nuclear segmentation in animal neutrophils is not as prominent as in human neutrophils. This may result in overestimation of band neutrophils in manually performed differentials performed by medically trained technicians. The eosinophil of the cat is quite unique in that it contains pink,rod-shaped granules, compared to the more circular granules of the dog and human. Canine basophils have very few granules while the granules of feline basophils lack metachromasia and stain pale grey. An immature feline basophil may exhibit both darkly and lightly stained granules.

Standard haematology analysers contain in-built thresholds which are set within certain limits to recognise cells by different methods such as size, volume, conductivity and light scatter. The incorporation of animal software allows these parameters to be altered to accommodate differences between species. Animal software is available for some haematology analysers presently in use but is unlikely to be installed in medical laboratories.

Another option for veterinary practitioners is to perform their own haemograms using an in- clinic analyser , such as the Q.B.C-V. With the increasing cost of diagnostic services this has become an attractive and viable option for some practices. The Q.B.C-V analyser is based on a different principle to other haematology analysers. It involves the use of a specially adapted micro-haematocrit tube internally coated with acridine orange and into which is placed a tight fitting cylindrical float. During centrifugation this plastic float spins down into the buffy coat layer expanding it several fold between the outer circumference of the float and the inner circumference of the haematocrit tube. The different cell layers can then be measured in a similar manner to the routine measurement of PCV. More traditional methods of white cell analysis such as those

employed by the Coulter VCS and Technicon H1 analysers involves the principles of volume, conductivity and scatter. Results from the Q.B.C-V correlate well in comparison on normal cats, dogs and horse (Levine *et al*, 1986). There is little information however on animals with altered haemograms.

The purpose of this study is to compare five different haematology analysers in the analysis of blood from dogs and cats with both normal and abnormal haemograms. Three of these analysers have been specifically adapted for veterinary use, while two are medical haematology analysers. Additionally, a time course study was conducted using the veterinary haematology analysers on five dogs and five cats to determine whether or not there are significant changes in any of the blood parameters over 24 hours.

CHAPTER TWO

REVIEW OF HAEMATOLOGY ANALYSERS.

2.0 Introduction

The standard haematology analysers use the properties of cell volume, conductivity and light scattering properties to recognise different cell types. More recently new generation analysers have developed specific cytochemical staining channels which allow further classification of cells based on their staining and morphological properties. A range of relatively inexpensive in-clinic analysers based on a different principle has also been developed. The Quantitative Buffy Coat (Q.B.C) analysers use a combination of fluorescent staining and direct measurement of the thickness of different cell layers in an expanded buffy coat, following centrifugation.

2.1 Q.B.C-V Analyser

The Quantitative Buffy Coat- Veterinary (Q.B.C-V) analyser measures PCV, total white blood cell count and a platelet count. The white blood cells are further quantified into: granulocytes (including: neutrophils, eosinophils and basophils), and a combination of lymphocytes and monocytes.

This system consists of a glass capillary tube with an inner diameter of 1.683mm (sd=0.0035mm) and a cylindrical float which has an outer diameter of 1.596 mm (sd=0.0035mm). A standardised pipette then aspirates 111 µl of venous blood into the capillary tube and the bottom of the tube is then sealed with a plastic stopper. The cylindrical float is then inserted into the top of the tube and the tube is centrifuged at 12 000 revs per minute for five minutes.

In studies of cell density gradients, it has been known for many years that the thin white buffy coat in the haematocrit tube consists of packed leucocytes and platelets, and that the platelets, being

less dense, settle in a separate layer above the leucocytes (Rayson, 1989). Wintrobe and Olef in the 1930's described methods for estimating the white cell and platelet populations based on the thickness of the buffy coat. Quantitative methods proved difficult because of the very small size and non-homogenicity of the cell layers. In later studies of cell density gradients, further subdivisions or layering was found to occur between two subpopulation of leucocytes by virtue of their different specific gravities. The upper layer was reported to contain predominantly lymphocytes and monocytes, the lower predominantly granulocytes, ie neutrophils, eosinophils and basophils.

The Q.B.C-V tube is lined with a number of solutions: potassium oxalate, acridine orange, an agglutinating agent and heparin. The potassium oxalate osmotically removes water from the erythrocytes thus increasing their density. This enhances the separation of erythrocytes from granulocytes which would otherwise have a similar density and finish up in the same layer.

The agglutinating agent is a mixture of species-specific antibodies against an erythrocyte antigen "glycophorin". This results in a clumping of erythrocytes and prevents damaged or lysed red cell fragments from collecting at the top of the red blood cell column and blurring the granulocyte -erythrocyte interface.

Acridine orange is a supravital fluorochrome. Such diamidines have long been employed in cytological labelling and clinical diagnostics because of their uptake by cellular nucleoproteins and by glycosamines in the granulocytic series (Levine *et al*, 1986). Under excitation by blue violet light the nucleated cells differentially fluoresce. Since erythrocytes do not have nuclei they are unaffected by the acridine orange and do not fluoresce.

Following centrifugation, one end of the float extends down into the packed red blood cell layer (Figure 1) and, the buffy coat layer is spread out between the outer wall of the cylindrical float and the inner wall of the capillary tube, a vertical expansion of ten times magnitude.

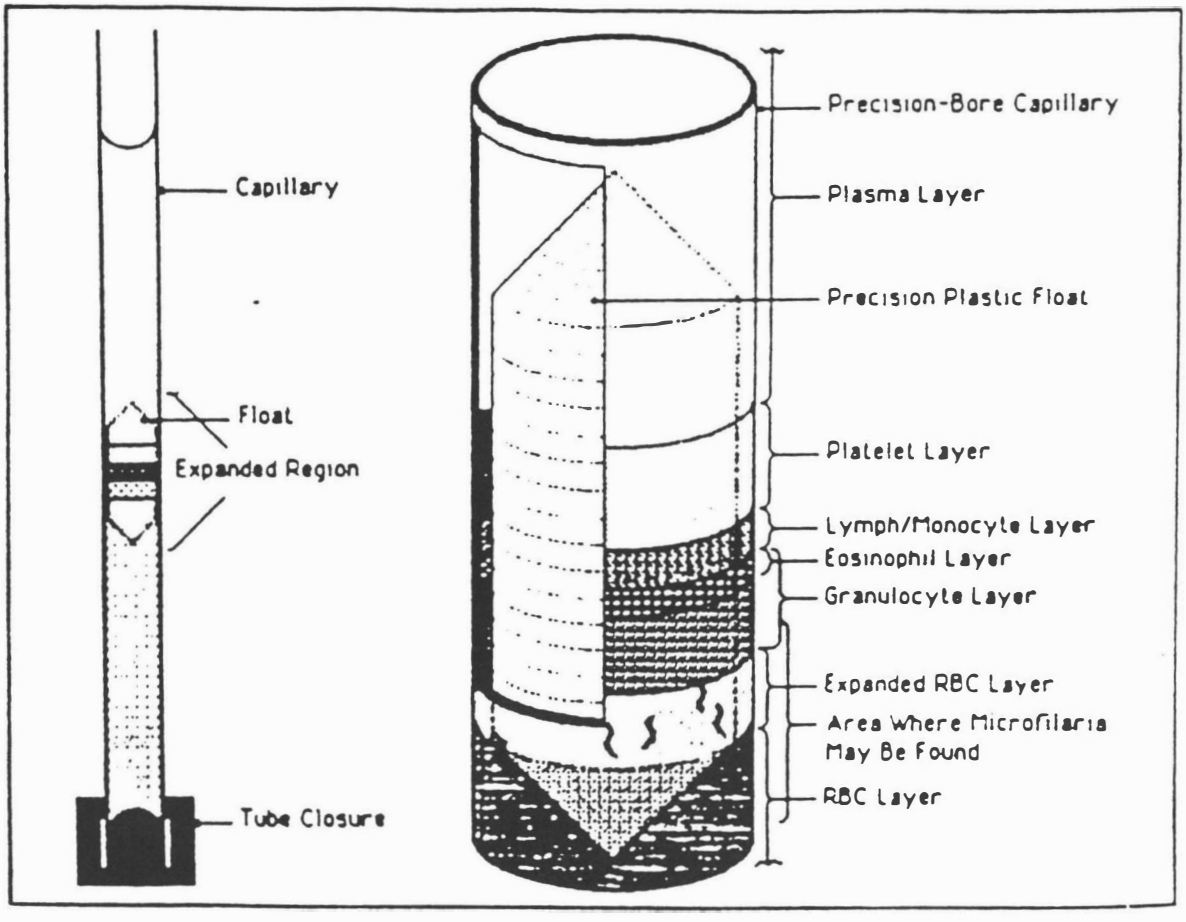

Figure 1. Left:Quantitative buffy coat analysis tube of canine blood, after centrifugation with the float in the buffy coat area. Right: An expanded view of the float.

The Manual Q.B.C-V analyser has a reading instrument into which the capillary tube is placed. The tube is illuminated at an excitation wavelength of 460 nm, and magnified by ten fold. Within the instrument is an electric micrometer that allows measurement of the thickness of each layer band lengths by moving the Q.B.C-V tube longitudinally and recording the position of each interface with a cursor. The erythrocytes appear a deep red colour, while the granulocytes fluoresce green to yellow-green. If there are sufficient eosinophils (ie $>1.0 \times 10^9$/l) then they may be visualised directly beneath the lymphocyte- monocyte layer as a separate orange-green band distinct from the neutrophils, but the eosinophil layer is not measured separately. The lymphocyte-monocyte layer fluoresces a very bright green colour, on top of which lie the orange coloured platelets.

A converting co-efficient allows direct conversion of the individual cell layers into standard percentages and actual numbers.

The Automated Q.B.C-V works in exactly the same manner , except that the thickness of each layer is measured automatically. The capillary tube is inserted into a scanner which electronically scans the tube and provides a sophisticated printout (Figure 2) which includes histograms of red cell, platelet and white cell parameters. Additionally, the Automated Q.B.C-V provides a haemoglobin concentration, which the Manual does not. It does this by calculation derived from a measurement of the depth that the float descends into the packed red cells (a function of red cell density) and the haematocrit. A reticulocyte percentage is available, expressed as a percentage of the haematocrit within the range 0.2-4.0%. This allows calculation of erythrocyte indices and gives the user more insight into the nature of an animal's erythrocyte status.

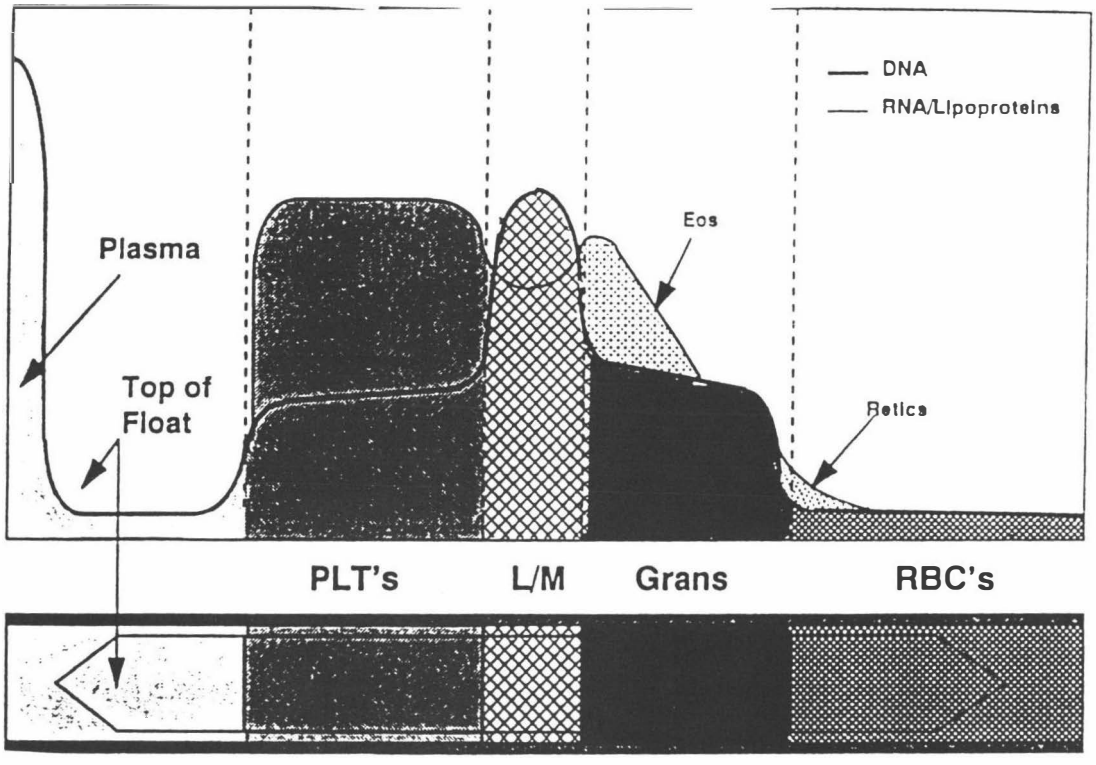

Figure 2. The Automated QBC-V readout creates a graph of the buffy coat profile by analysing the different fluorescencefrom both the DNA and RNA/ lipoprotein sources. The width of each band reflects the number of cells present in each population.

2.2 Coulter VCS Analyser

In this analyser electrical impedance is used for the routine measurement of the erythrocyte parameters. Following blood aspiration the stream of red blood cells is split. One portion is used for hemoglobinometry, one for enumeration of the red blood cells and one for counting of the leucocytes.

The haemoglobinometry is accomplished after red cell lysis with a reagent "Erythrolyse."(Coulter VCS Casebook, 1989). Absorbence spectrophotometry at 540 nm in a prepared cyanomethaemoglobin solution measures the haemoglobin concentration of the individual red blood cells.

The red blood cell counting is performed by passing the red blood cells singly through a small direct current . Resistance is generated as the red cells are drawn through a small aperture and is measured as a small pulse due to the temporary increase in impedance, (Figure 3). The height of the impulse is proportional to the cell volume, while the time it takes for the cell to travel through the aperture is indicated by the width of the pulse generated. A problem arises when red cells do not take a central path through the aperture and generate an aberrant pulse. Hydrodynamic focusing (Figure 4) has been designed to minimise this problem. It involves the use of a surrounding fluid sheath stream to force the cells through the centre of the aperture. When cells thus pass through the centre of the aperture a narrower and therefore more concise cell volume distribution can be produced than that with conventional flow conditions (Corash *et al*, 1995).

Mean corpuscular haemoglobin (M.C.H) and mean corpuscular haemoglobin concentration (M.C.H.C) and haematocrit are not measured directly by electrical impedance systems, but by calculation from the directly measured parameters of haemoglobin, red blood cell count and

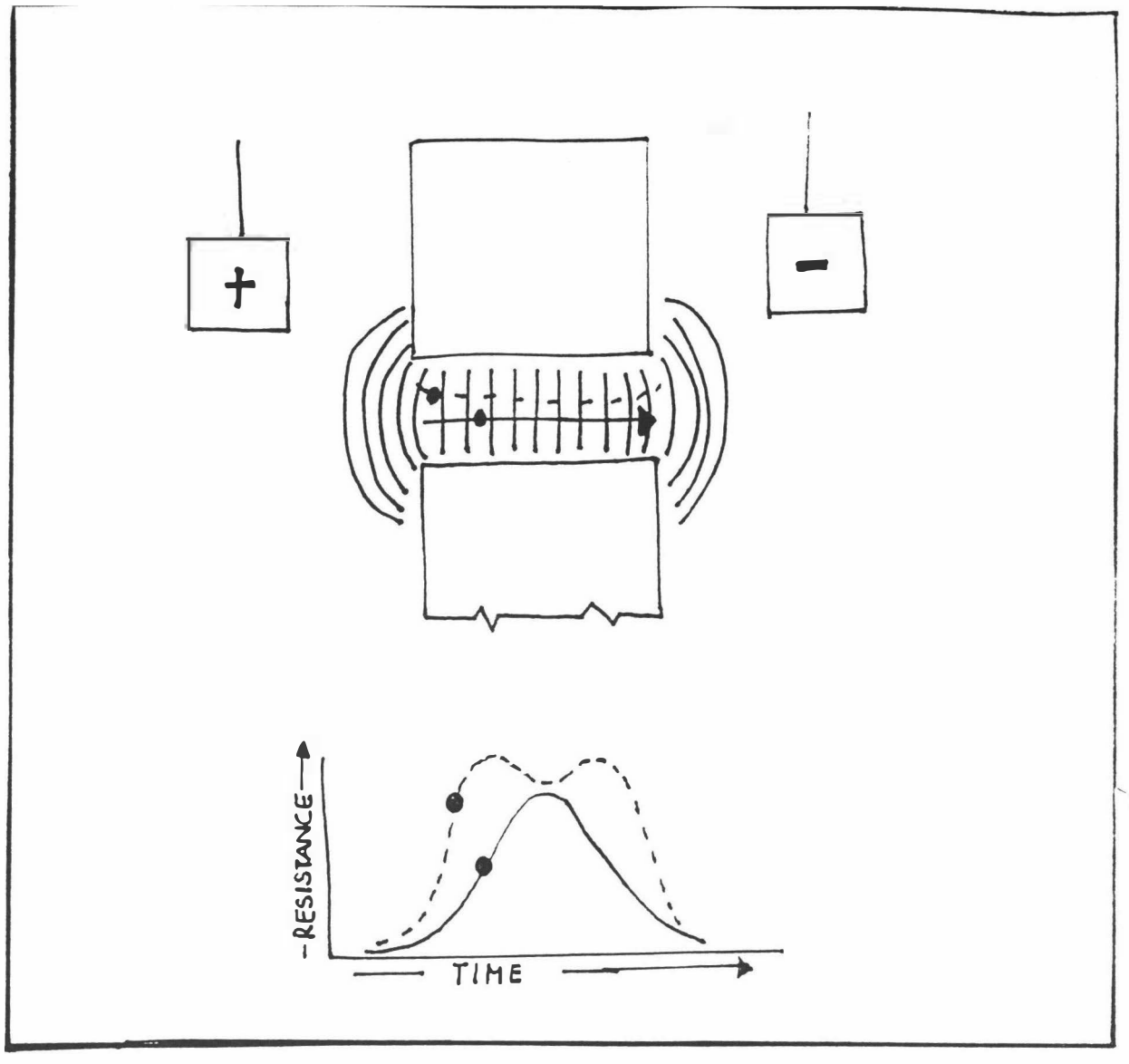

Figure 3. Electrical impedence cell counting. Cells are diluted in an isosmolar conducting solution and drawn through an oriface to which a small electric current has been applied. As the cell passes through the oriface it generates resistance, measured as pulse height, which is proportional to cell volume.

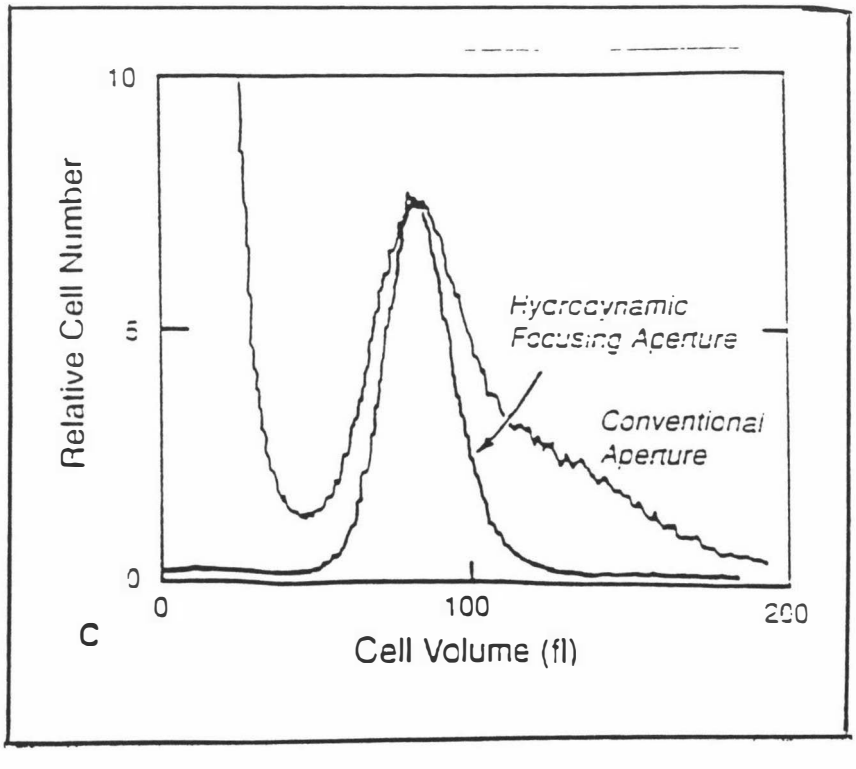

Figure 4. Hydrodynamic focusing.

red blood cell volume, according to the following formulae:

MCH=haemoglobin (g/l)/RBC (10^6m / l)

MCHC= haemoglobin(g/dl)/PCV%

PCV= Mean Corpuscular Volume(fL) x RBC (10^6m / l) (Corash *et al*, 1995)

The most recent Coulter VCS analysers produce histograms of erythrocyte volume distribution which provides information about the subpopulation of abnormal cells and a measure of dispersion for red blood cell volume distribution; this is termed the erythrocyte distribution width. The erythrocyte distribution width can then provide information about anisocytosis (ie variation in red blood cell size), and identify abnormal subsets of red blood cells. For example, hypochromic microcytic red blood cell populations can be identified and aid in the diagnosis of an iron-deficiency anaemia.

Platelet count is assessed on the Coulter VCS via electrical impedance, similar to the red blood cells. Using this technology there has been some evidence that over time platelet volume increases upon exposure to EDTA anti-coagulant (Levine *et al* , 1983).

Leucocyte analysis by this instrument involves simultaneous analysis of volume, conductivity, and light scatter, which are analysed simultaneously. During each instrument cycle, eight thousand individual particles are analysed (Coulter Casebook, 1989). Individual cell volume is measured by electrical impedance as described above for the erythrocytes.

Conductivity is a measurement of cellular internal content using a high frequency electromagnetic probe. Cell walls act as conductors when exposed to high frequency current. As the current passes through the cell, measurable changes are detected as the result of the chemical composition of the

nucleus and cytoplasm.

The light scatter characteristics of cell surfaces also provide distinguishing information regarding cell types. Each cell is scanned by a monochromatic light from a laser source. Flow through an optical sensing system then detects the scatter pattern.

From this, a three dimensional plot of cell populations and sub populations is then constructed, an example of which is presented in Figure 5. The scatter plot printed represents a different view of this three dimensional plot, as if the plot was rotated on its axis.

2.3 Technicon H1 Analyser

The Technicon H1 (Bayer, Tarrytown, NY) analyser is a new- generation model which measures red blood cell volume by low angle forward light scatter, and haemoglobin concentration by high angle light scatter.

Five to ten thousand red blood cells are sphered with a diluent reagent that contains surfactant and KCN, buffered to a pH of 11.3. The surfactant lyses the red blood cells, causing release of haemoglobin. The protein is denatured, the haemoglobin is solubilised and combined with cyanide and drawn through a flow meter, with simultaneous measurement of low angle (2-3 degree) and high angle (5-15 degree) scatter. Utilising the "Mie" scatter theory the red blood cell refractive index is directly proportional to the intracellular haemoglobin concentration, thus providing a direct measure of corpuscular haemoglobin concentration (Tycko *et al*, 1985). The spread of the

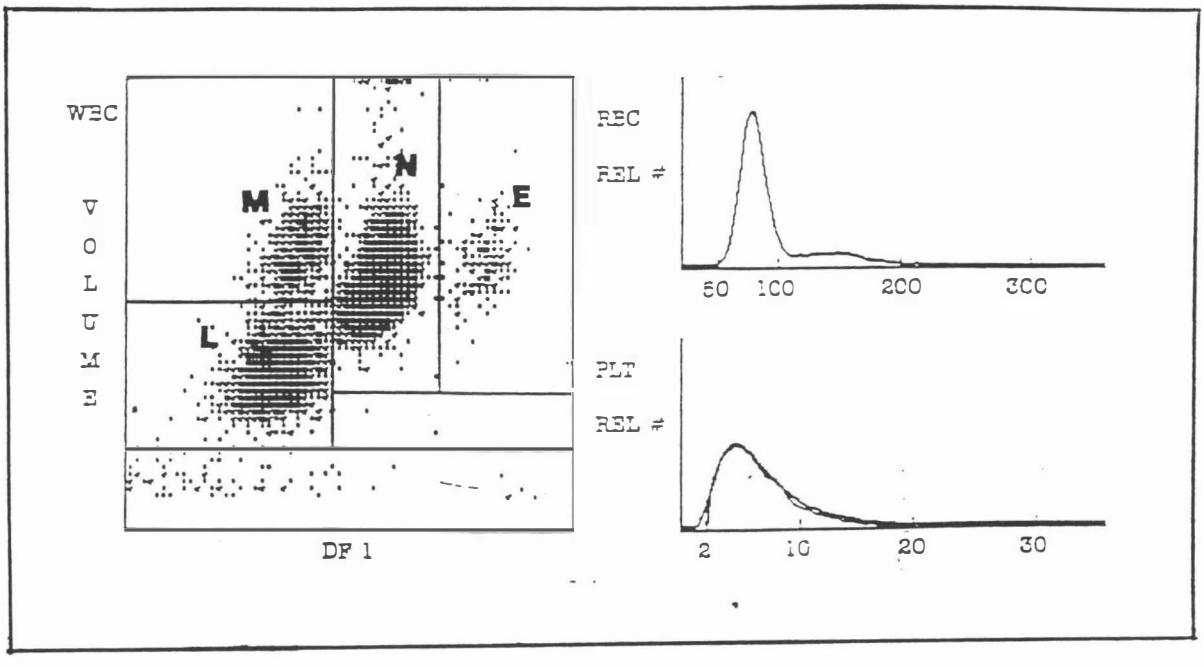

Figure 5. Coulter VCS instrument display. The left panel illustrates the discrimination of four leucocyte classes using volume and scatter measurements.

corpuscular haemoglobin concentration is called the haemoglobin distribution width.

This provides an excellent means of detecting subpopulations of cells with abnormal haemoglobin concentration . The direct measurement of mean corpuscular haemoglobin concentration (MCHC) is an improvement over electrical impedance in which automated MCHC did not agree well with manual determinations, due to the poor deformability of red blood cells with MCHC levels above 360 g/l.

Thus the H1 analyser provides the ability to directly measure four red blood cell parameters: haemoglobin, haemoglobin concentration, volume and number of red blood cells.

Platelet counts are determined via an electro-optical detection system. The platelets are sized using the high gain setting from the high angle light scatter used to determine the haemoglobin concentration (Cresce, 1986). Platelet histograms can then be produced (Figure 6), however the biologic significance of variation in platelet volume remains controversial. Platelet volume is still used as a diagnostic aid to determine thrombocytopenia or thrombocytosis.

The Technicon H1 also provides a sophisticated six part differential . The white blood cells are classified as neutrophils, eosinophils, large unstained cells, monocytes, lymphocytes and basophils. Both forward-angle light scatter and peroxidase cytochemical staining are used to detect the granulocytes and monocytes, while a separate channel is used to enumerate the basophils.

Technicon were the first to develop an improved version of peroxidase cytochemistry in the early 1980's. The peroxidase channel is where the white blood cells are fixed and stained (Simson *et al* 1988). When a chromogen and hydrogen peroxide as the substrate is added a precipitate forms in the granules of the leucocytes which already contain endogenous hydrogen peroxidase. Eosinophils have the most significant peroxidase activity and hence stain the most intensely, whilst neutrophils have modest activity and monocytes have little peroxidase activity. Both lymphocytes and the large unstained cells contain no peroxidase and so do not stain.

The cell specifics are then determined based on their size (via scatter) and their peroxidase activity, via absorbence, as the cells move single file down a tungsten-light based optics channel. A dark field detector records the light scattered, while the detector in the bright field assesses the degree of staining. A graph is then plotted of scatter on the y-axis and absorbance on the x axis. The positions of the cells are represented by a dot. This data is used in partial determination of both the total white blood cell count and the differential count, excluding the basophils (Figure 7).

The basophil/lobularity channel differentiates leucocytes based on nuclear shape and also counts the basophils. The enumeration of basophils is based on the principle that the cytoplasmic membranes of neutrophils, eosinophils, lymphocytes and monocytes are destroyed if these cells are exposed to a surfactant at a low pH, but the cytoplasmic membranes of the basophil in the same conditions remains intact.

Light scattering properties are used to determine the nuclear shapes of the leucocytes. Two different light angles are measured. The low angle forward light scatter consists of light angled between 0 and 5 degrees and provides a measure of cell size. The intact basophils have the greatest size and hence the greatest low angle forward scatter.

The high angle forward scatter is where light is deflected through 5 to 15 degrees of angle, which allows a lobularity index to be calculated. The greater the lobularity of the cell the greater the high angle scatter. The lobularity index is of special relevance to the neutrophil population, since it can quantify the degree of neutrophil segmentation. Immature neutrophils ie band forms are less lobulated and hence have a lower lobularity index, allowing identification of a left shift.

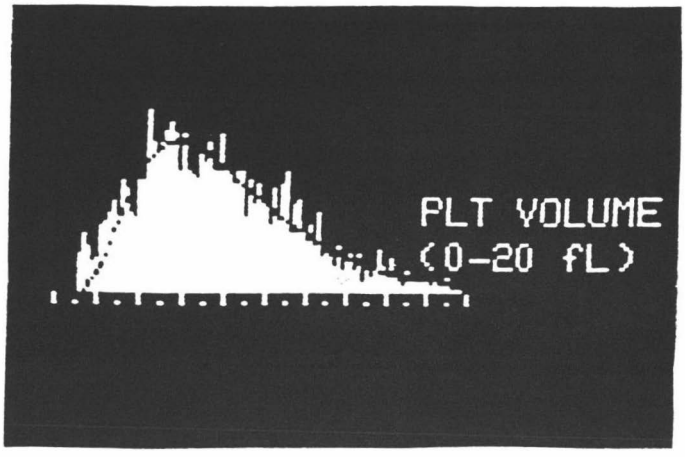

Figure 6. The platelet histogram is derived from measurements made with the high angle detector.

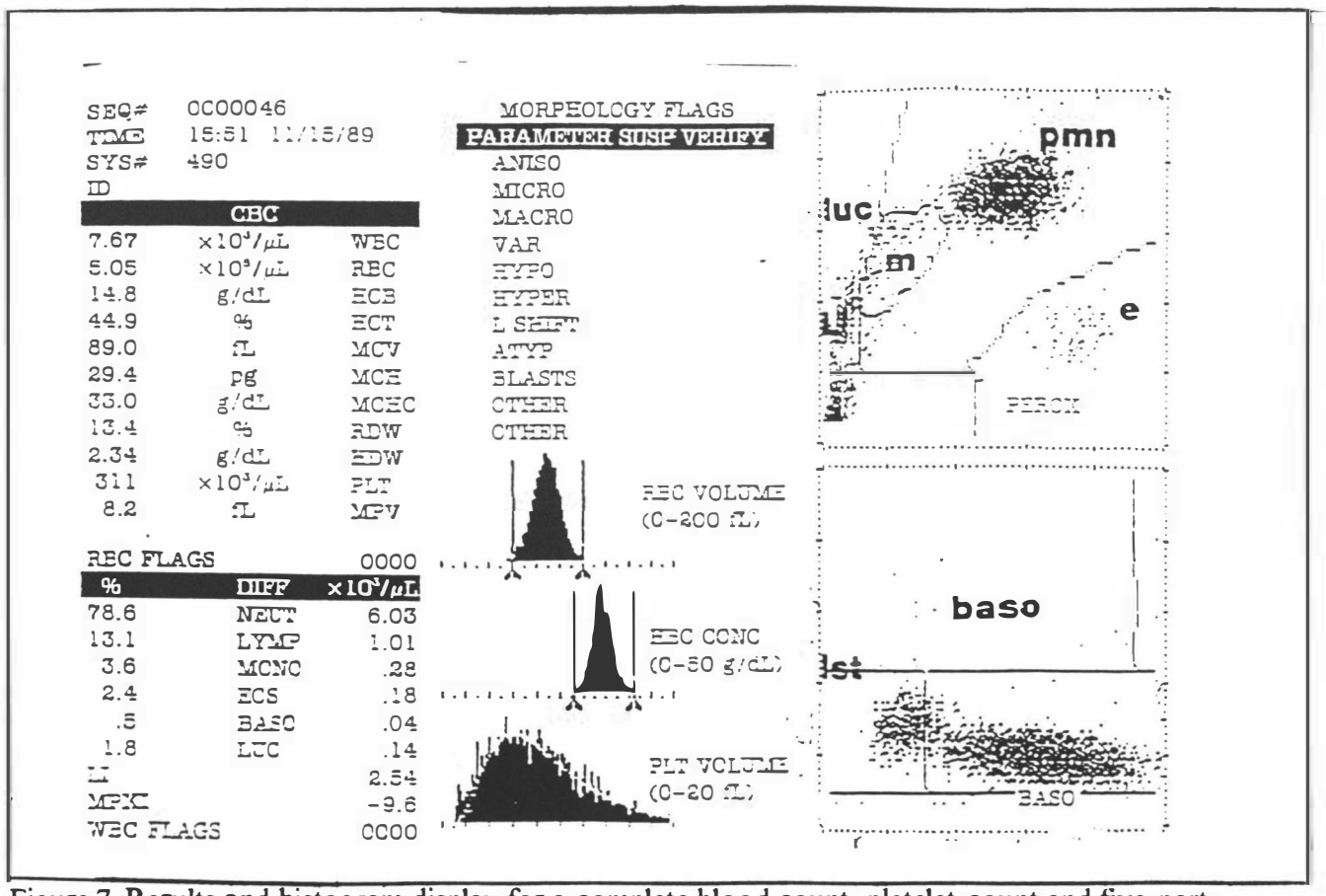

Figure 7. Results and histogram display for a complete blood count, platelet count and five-part differential obtained with the Technicon H1 system.

An immunoperoxidase method is used for lymphocyte subtyping, in order that specific subclasses of lymphocytes can be identified in the case of malignancy or in immunodeficiency diseases. Firstly, a specific monoclonal antibody is mixed with whole blood. This antibody will bind only to specific receptors of a given type. A second biotinylated antibody is added, followed by a peroxidase reagent. The biotinylated antibody binds only to the monoclonal antibody and can thus be identified. Lymphocytes labelled by the immunoperoxidase reaction appear between the unlabelled lymphocyte population and the monocytes. This is compared with endogenous peroxidase-containing cells which stain intensely and appear to the far right of the graph (Figure 7).

2.4 A.B.X. Minos Veterinary Analyser

This compact haematology analyser has been specifically designed by A.B.X. Haematologie, Parc Euromedecine, France and adapted for veterinary use.

The basic principle is similar to the Coulter VCS and the Technicon H 1 analysers. The erythrocyte parameters are calculated based on an impedance variation generated by the passage of the cells through the calibrated microaperture. Two electrodes are placed either side of the aperture and a continuous electric current passes between these. When the cell passes through the aperture there is an impedance generated according to the equation V=I.R, where V=voltage, I= current and R = resistance. The resistance increases proportionately with the volume of the cell. The impulses generated are very low, so the amplifier increases them such that they can be

analysed and the background noise eliminated.

The haematocrit is measured by a special electronic circuit which adds up all the impulse heights and a mathematical process is applied to the sum obtained to compensate for simultaneous passages in the aperture.

The haemoglobin is freed by the lysis of the cells following the addition of potassium ferracyanide and potassium cyanide. This results in the formation of a chromogenous cyanmethemoglobin compound. This compound is then measured using spectrophotometry with a wavelength of 540 nanometers.

The white blood cells and platelets are also measured by electrical impedance.

The Veterinary adaption of this analyser involves a threshold adjustor which is a separate part, that is plugged into the analyser (Technical manual Minos RAA, Roche Diagnostic Systems Haematology). A control button on the front of the adjustor allows the species whose blood is to be tested, to be selected. The threshold for the size of erythrocytes for that species is set. This prevents the erythrocytes being misinterpreted as platelets which could happen in sheep and goats for example, that have relatively small erythrocytes.

CHAPTER THREE

COMPARATIVE STUDY OF HAEMATOLOGY ANALYSERS

3.1 Introduction

The purpose of this study was to compare medical and veterinary haematology analysers that are commonly used in private and government-funded laboratories. There are more options today than ever before for veterinarians in practice, with the recent development of a number of competitively priced in-clinic haematology analysers on the market (Knoll *et al*, 1996). Clinicians however should be made aware of the limitations of these different analysers and the possible pitfalls of accepting automated haematology results at face value.

3.2 Materials and Methods

3.2.1 Animals Used

The study included 58 dogs and 55 cats. Of these, 42 cats and 23 dogs were research animals and were considered clinically normal. The remaining 13 cats and 35 dogs presented to the Massey University Veterinary Clinic with a variety of clinical syndromes.

3.2.2 Specimen Collection

In the dogs the blood was collected from the cephalic vein, while in the cats venipuncture from the jugular vein was used. All blood was collected into tubes containing the E.D.T.A.

anticoagulant (purple top) and blood smears were made within 15 minutes of collection.

3.2.3 Haematology Analysers

The three analysers located in the Clinical Pathology Laboratory at Massey University were used first, followed by the Coulter VCS and the Technicon H1 at the Palmerston North Hospital and the Private Medical Laboratory respectively. In all cases, blood smears were made within ten minutes of collection. Samples were then tested on the three analysers at the Massey University laboratory within ten minutes of collection and on the remaining two analysers had tested the blood samples within 90 minutes of collection.

3.2.4 Manual Methods

A 500 cell manual differential count was chosen in order to maximise accuracy. Manual white cell counts were also performed on cat blood because of potential problems associated with platelets clumping in this species. The Unopette method (Becton- Dickson and Company) was used to perform the manual white cell counts . A plastic capillary tube is filled with blood by capillary action then emptied into a reservoir containing a red cell lytic agent. This mixture is left to sit for ten minutes during which time the erythrocytes lyse, but leucocytes and platelets remain intact. A counting chamber (haemocytometer) is then loaded with the solution and leucocytes counted in the nine large squares of the counting chambers. The resultant number of cells counted is then divided by nine, giving the total number of white blood cells x 10^{9}/l.

3.2.5 Statistical Analysis

Analysis of variation (A.N.O.V.A) was used for this study.

A comparative study was done between the analysers, using a gold standard for comparison where there was one, otherwise comparing the analysers with each other. From this, a line of best fit was drawn and a regression coefficient determined using the SAS Statistics programme. The coefficient of regression, otherwise known as the coefficient of determination (r^2) is a value which explains the overall variation in y values by the fitted regression model. That is, the greater the r^2 value the better is the straight line fit of the graph. The y- intercept and the gradient of the slope was calculated using the equation $y= mx + c$.

The regression coefficient (r^2) was classified as excellent if it was between 1 and 0.901, very good if between 0.900 and 0.801, good if between 0.800 and 0.701 , fair if between 0.700 and 0.601 and possible if between 0.600 and 0.501. Any regression coefficient less than 0.400 was classified as poor and less than 0.200 was classified as non existent.

Certain outlier (anomalous data points which give a response inconsistent with the remaining data points) were occasionally removed. Using Cooks D analysis influential points could also be identified and excluded from the data. In all cases where this was done it was noted.

A correlation matrix was also used for easy comparison between all of the analysers for a certain haematology parameter such as white blood cell count or packed cell volume.

3.3 Results

3.3.1 Packed Cell Volume in Dogs and Cats.

For this comparison between the haematology analysers the ABX Minos Vet analyser in the Clinical Pathology Department at Massey University was used as the reference with which the other analysers were compared . This analyser has been used satisfactorily at Massey and in several Ministry of Agricultural Veterinary Diagnostic Laboratories.

As Figure 8 demonstrates, the Coulter VCS machine gave an excellent correlation when PCV's of canine blood samples were compared (r^2=0.931).

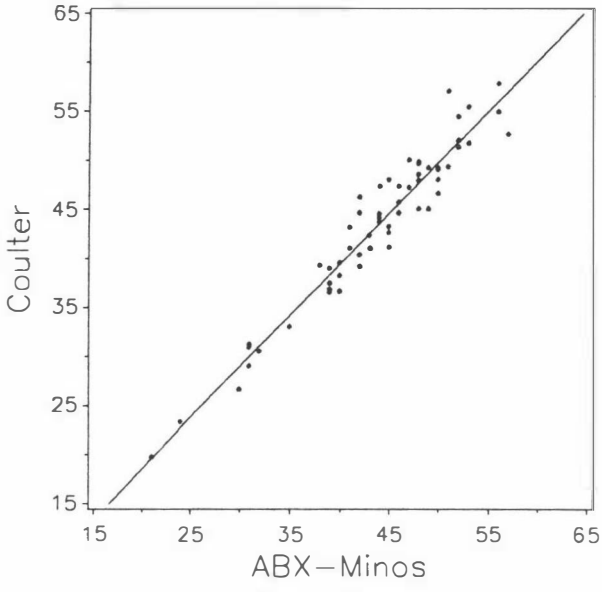

Figure 8. Scattergram and line of best fit for the ABX Minos Vet versus the Coulter VCS for packed cell volume in dogs. [r^2=0.931; y=1.02x -1.59]

The Technicon H1 also had a very good correlation with the ABX Minos Vet (r^2=0.895) The Manual QBC-V and the Automated QBC-V also gave very good and excellent correlation coefficients respectively (r^2=0.858 and r^2=0.909), (Figure 9).

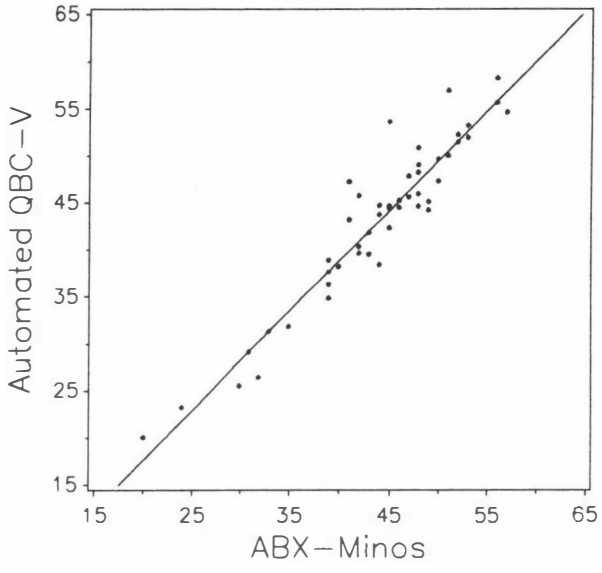

Figure 9. Scattergram and line of best fit for the ABX Minos Vet versus the Automated QBC-V analyser for packed cell volume in dogs. [r^2=0.909; y=1.064x - 3.687].

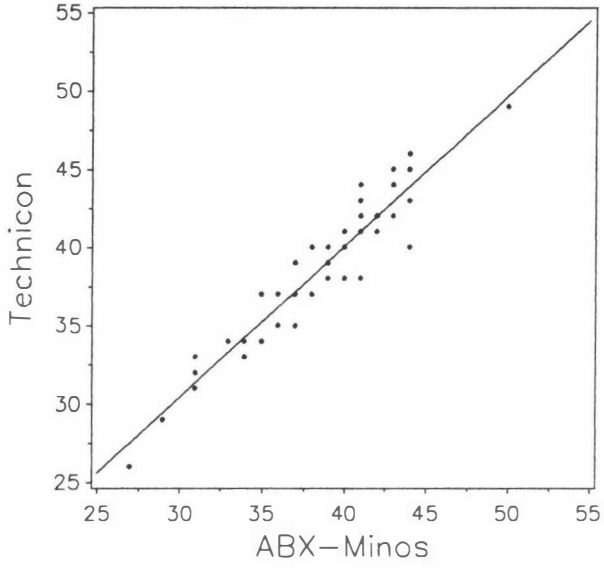

Figure 10. Scattergram and line of best fit for the ABX Minos Vet versus the Technicon H1 for packed cell volume in cats. Outliers removed. [r^2=0.905; y=0.964x + 1.53]

When the cat blood samples were analysed there were poor correlations between the medical analysers and the ABX Minos Vet (r^2= 0.135 for the Coulter VCS and r^2=0.142 for the Technicon H1). However once three outliers were removed the correlations improved to very good for the Coulter VCS (r^2=0.857) and excellent for the Technicon H1 (r^2= 0.905), (Figure 10).

The three outliers on the Coulter VCS were all cats that had much higher PCV's on the ABX Minos Vet than on the Coulter VCS. The reason for this inconsistency was not apparent although would support other studies done on Coulter models in cats.

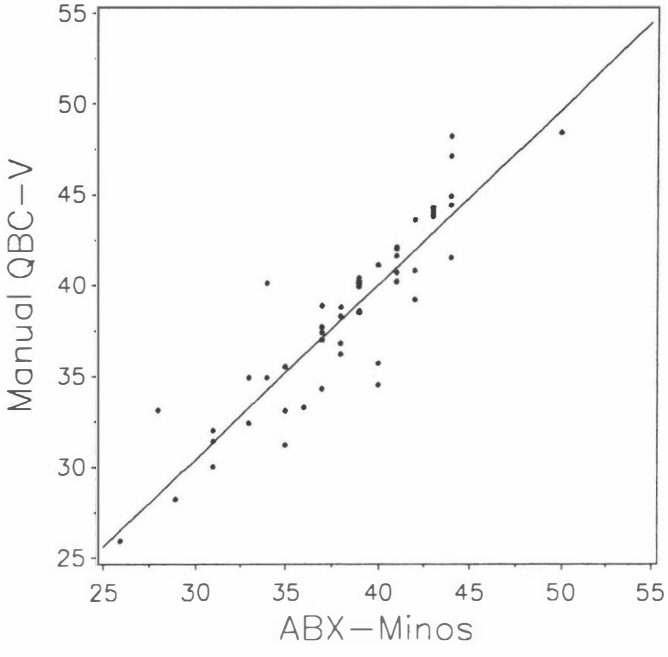

Figure 11. Scattergram and line of best fit for the ABX Minos Vet versus the Manual QBC-V for packed cell volume in cats.[r^2=0.841; y=0.989x +0.457].

Both the Manual QBC-V analyser and the Automated QBC-V gave poor correlation when compared with ABX Minos Vet. However when cats 93, 95 and 96 were removed as outliers, and the statistics repeated, the correlation coefficients improved significantly (r^2=0.841 for the Manual, see Figure 11 and r^2=0.914 for the Automated). These were different cats from those outliers removed for the Coulter VCS, and in this case the outliers had very high PCV readings on the QBC-V analysers when compared with the ABX Minos Vet.

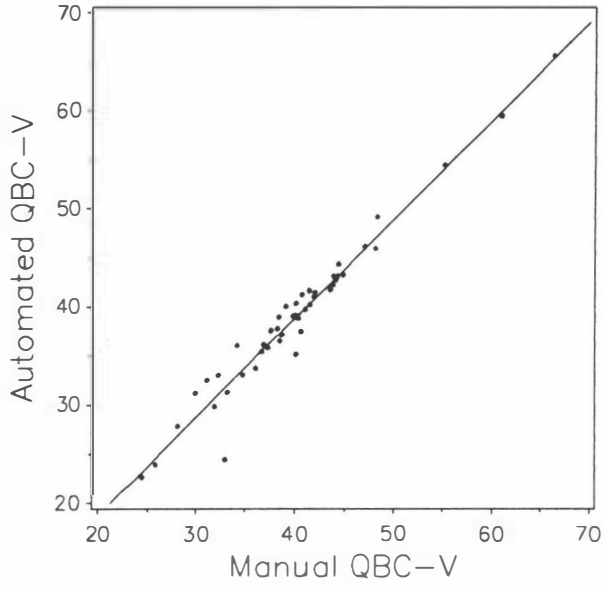

Figure 12. Scattergram and line of best fit for the Automated QBC-V versus the Manual QBC-V for packed cell volumes in cats.[r^2=0.813; y=0.949x + 3.16].

Using the correlation matrix there is a very good correlation (r^2=0.813) between the Automated and the Manual Q.B.C-V analysers in cats, see Figure 12. This was also the case in dogs.

It is interesting to note however that this correlation is not as good as that obtained in the previous comparisons even though the Automated QBC-V and the Manual QBC-V are measuring the same tube.

3.3.2 Total White Cell Counts in Dogs and Cats.

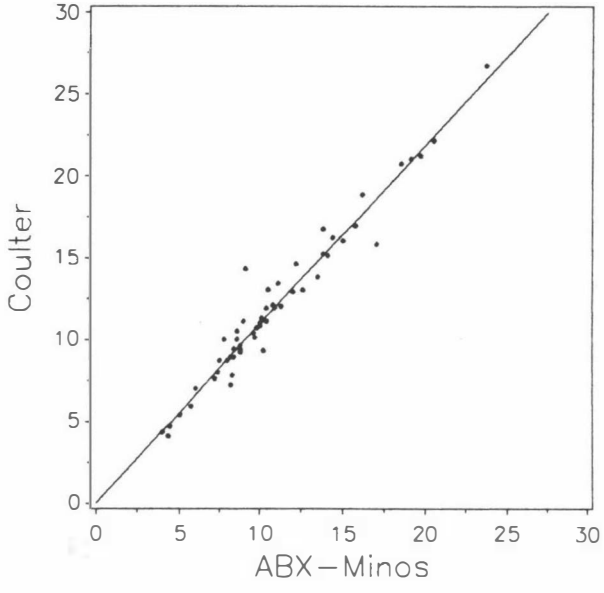

Figure 13. Scattergram and line of best fit for the ABX Minos Vet versus the Coulter VCS for white cell counts in dogs. [r^2= 0.954; y=1.088x + 0.030].

In dogs, the total white cell counts made by the Technicon H1 and the Coulter VCS analysers were strongly correlated with those obtained by the ABX Minos Vet analyser (r^2=0.952 and r^2=0.954 respectively). One influential point was removed and three outliers were removed from the raw data for the Coulter VCS , (Figure 13). The influential point was a 12 year old Labrador that had carcinoma of both the liver and the lung and a profound leucocytosis of 41.1 x 10^9/l on the ABX Minos Vet.The Coulter VCS analyser also registered a high white cell count (54.8 x 10^9/l) for this dog.

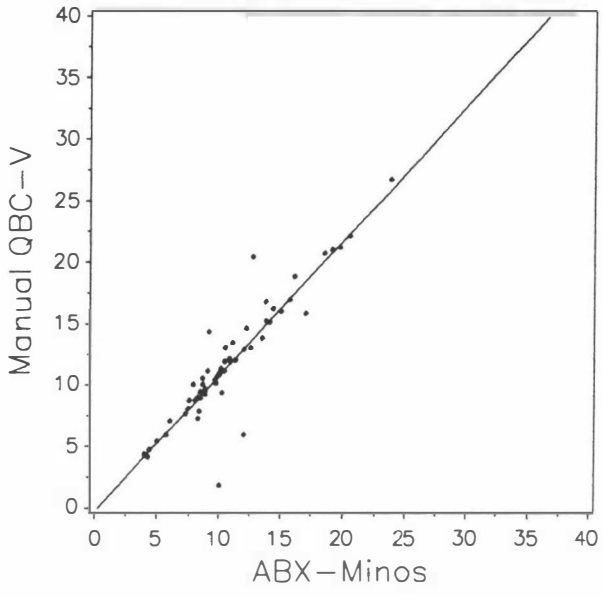

Figure 14. Scattergram and line of best fit for the ABX Minos Vet versus the Manual QBC-V for white cell counts in dogs. [r^2=0.709; y=1.067x - 0.949].

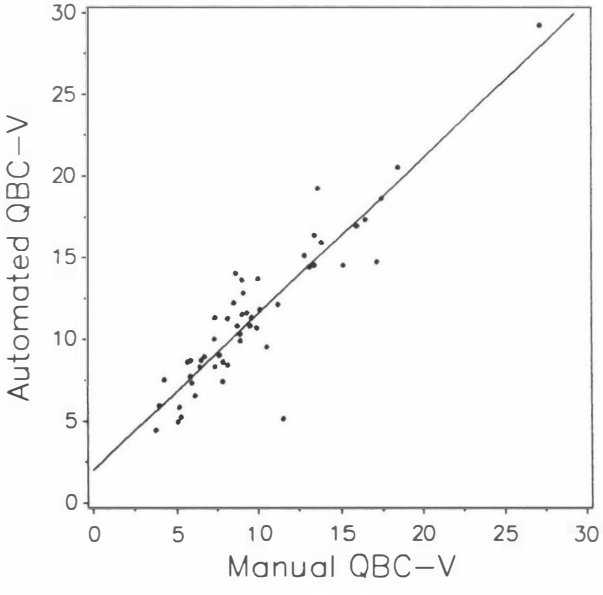

Figure 15. Scattergram and line of best fit for the Manual versus the Automated QBC-V for white cell counts in dogs.[r^2=0.834; y=0.873x -0.085].

The Manual QBC-V appeared to have a good correlation when compared with the ABX Minos Vet in dogs (r^2=0.709), (Figure 14) while the Automated Q.B.C-V had a very good correlation when compared with the ABX Minos Vet (r^2=0.866). This once again suggests that the Automated QBC-V gave a better result than the Manual QBC-V analyser for total white cell counts for dogs.Using the correlation matrix there was however an excellent correlation between the two QBC-V models (r^2=0.913).

A very good correlation was demonstrated when total white cell counts in the dog were compared on the Manual versus the Automated QBC-V analysers (r^2=0.834), (Figure 15). Following the removal of three outliers in the cats the WCC comparison was modest only (r^2=0.551), and certainly not as convincing as the dogs.

Initially, the correlation between the A.B.X. Minos Vet and the Coulter VCS analyser in cats appeared good. However Cook's D statistic identified two outliers and one influential point. Once these were removed and the correlation rerun the regression analysis was much poorer (r^2= 0.683) indicating only modest correlation. The influential point was a cat with a profound leucocytosis (63.2 x 10^9/l on the ABX Minos Vet) which had a marked basophilia and eosinophilia in addition to a neutrophilia.

It was noted that one of the outliers (cat number 25) had only given a small amount of blood, so the inconsistency may have been due to too much anticoagulant for the amount of blood in the E.D.T.A. tube, ie inadequate sample size.

The Technicon H1 also demonstrated poor compatability with the ABX Minos when assessing cats white cell counts, especially after removal of an influential point and an outlier (r^2=0.550). The influential point was again the cat with the marked leucocytosis.

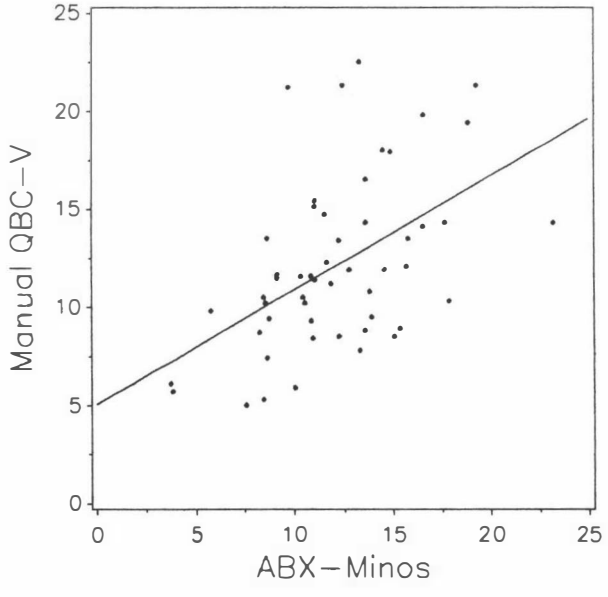

Figure 16. Scattergram and line of best fit for the ABX Minos Vet versus the Manual QBC-V for white cell counts in cats.[r^2=0.195; y=0.630x +5.164].

The ABX Minos versus the Manual QBC-V initially appeared to have a good correlation (r^2=0.727) however once an influential point was removed (again the cat with the leucocytosis, cat number 15)the correlation was poor (r^2=0.195), (Figure 16). The Automated QBC-V also demonstrated no correlation (r^2=0.253) when compared with the ABX Minos after two influential points were removed, including cat number 15. The

fact that both the QBC-V analysers detected this cat with the extremely high white cell count does however demostrate that these analysers are capable of detecting animals with abnormally high leucocyte values.

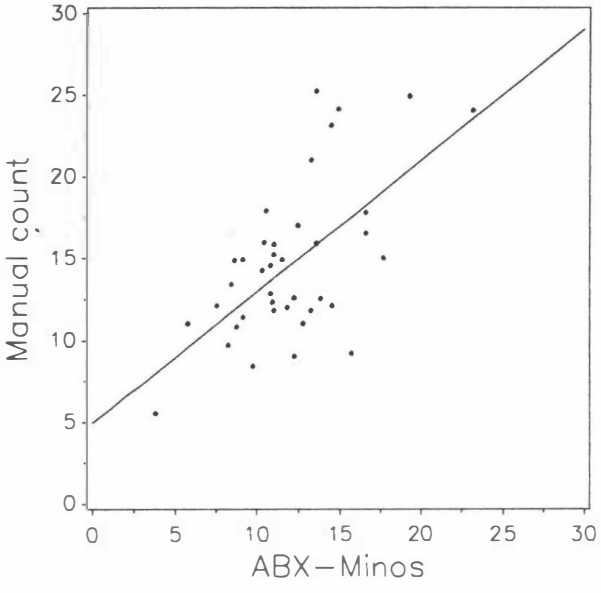

Figure 17. Scattergram and line of best fit for the ABX Minos Vet versus the manual white cell count in cats. [r^2=0.382; y=0.803x =4.958]

A comparison between a manual white cell count and the ABX Minos Vet analyser in cats demonstrated a poor relationship, with less than 40% of the manual white cell count being able to be explained by the fitted regression (Figure 17), (r^2= 0.382). This is of concern, assuming that the manual white cell count is the gold standard in this species.

3.3.3 Platelet Counts in Dogs and Cats.

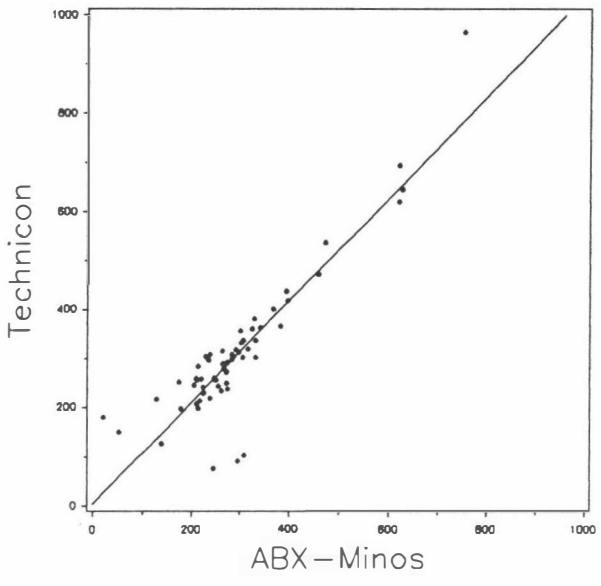

Figure 18. Scattergram and line of best fit for the ABX Minos versus the Technicon H1 for platelet counts in dogs.[r^2= 0.805; y=1.038x + 4.121].

In the dog, the total platelet counts obtained from each analyser were compared with the results from the ABX Minos Vet as the reference analyser. The Coulter VCS machine gave a good correlation when compared to the ABX Minos Vet ($r^2 = 0.773$). The correlation between the ABX Minos Vet and the Technicon H1 was very good, (r^2 =0.805), (Figure 18).

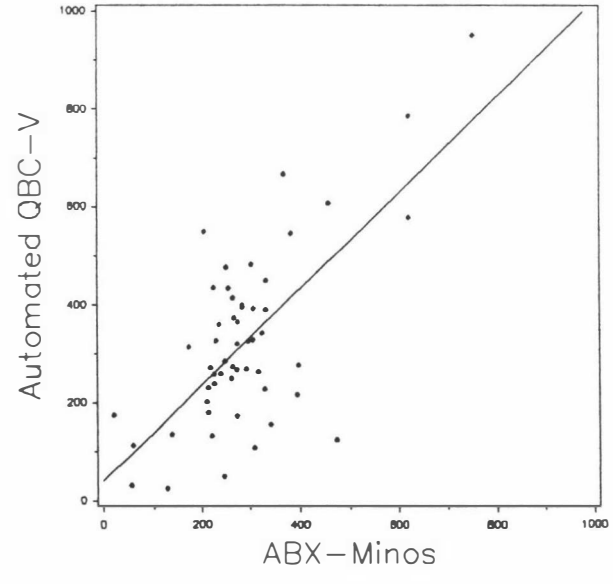

Figure 19. Scattergram and line of best fit for the ABX Minos Vet versus the Automated QBC-V for platelet counts in dogs. [r^2=0.474; y=0.978x +41.760].

Platelet counts obtained from the Automated QBC-V were poorly correlated with those from the ABX Minos Vet in dogs, (r^2=0.474), (Figure 19). A very poor correlation in canine platelet counts between the Manual QBC-V and the ABX Minos Vet was also demonstrated (r^2=0.237).

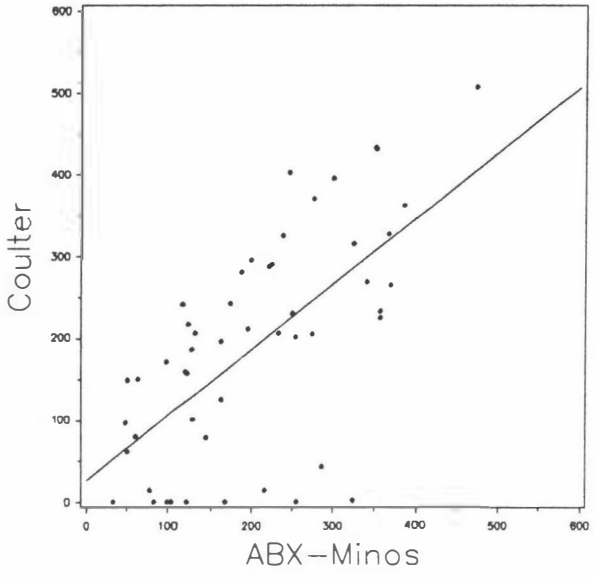

Figure 20. Scattergram and line of best fit for the ABX Minos Vet versus the Coulter VCS for platelet countsin cats. [r^2=0.418, y=0.800x =26.942].

In contrast to samples from dogs, the Technicon H1 and the Coulter VCS were poorly correlated with the ABX Minos Vet (r^2= 0.227 and 0.418 respectively) in cats. Figure 20 illustrates the correlation between the Coulter VCS and the ABX Minos Vet in cats. The correlation matrix comparison between the Technicon H1 and the Coulter VCS indicated modest correlation (r^2=0.607).When platelet counts in dogs were compared on the two QBC-V analysers there was little correlation (r^2=0.428), despite them reading the same tubes.

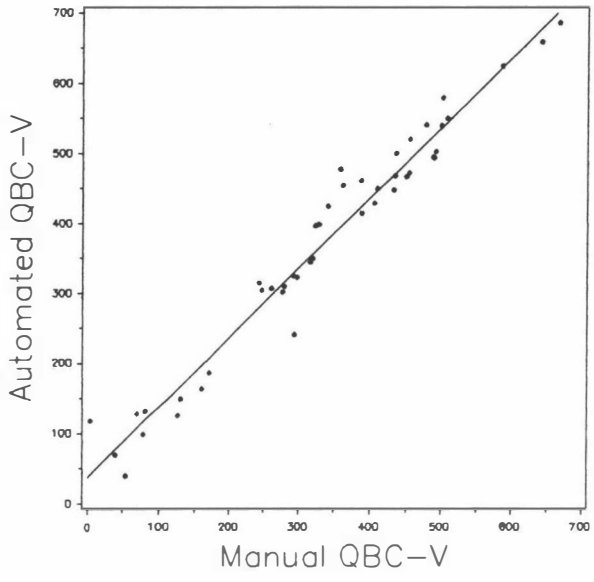

Figure 21. Scattergram and line of best fit for the Manual versus the Automated QBC-V for platelet counts in cats. [r^2=0.961, y=0.964x - 22.924].

In cats, both QBC-V models were poorly correlated with the ABX Minos Vet (r^2=0.146 for the Automated QBC-V and 0.068 for the Manual QBC-V). Although there was an excellent correlation between the QBC-V Manual and Automated analysers once three outliers were removed (r^2 = 0.961), (Figure 21). This only indicates consistency in platelet counts between the two analysers, not accuracy.

3.3.4 Comparison of Automated and Manual Differential White Cell Counts.

All the manual differential counts (done in percentages) differed significantly from the automated differential counts generated by all of the analysers. There was no apparent correlation between results obtained from any of the analysers and the manual counts. For example,the granulocyte percentage on manual differentials versus the Manual QBC-V had no correlation (r^2=0.273 in dogs and r^2=0.202 in cats), and manual granulocyte percentage versus the Automated QBC-V showed only modest correlation in either the cat or the dog (r^2=0.336 and 0.446 respectively).

3.3.5 Comparison of the QBC-V Analysers in Dogs and Cats with Abnormal White Cell Counts.

Of the animals involved in this study 12 had abnormal total white cell counts. Seven of these had an elevated white cell count, (six dogs and one cat) and five had low white cell counts (three dogs and two cats). Although these numbers were not high enough for statistical analysis they were adequate for comparisons.

The two QBC-V analysers were compared with the reference ABX Minos Vet.

In the animals with high white cell counts, similiar results were obtained on each of the three analysers in five of the six dogs, although two of the dogs registered lower white cell counts on the ABX Minos Vet than on the QBC-V analysers, they were still considered to be comparable.

In one dog there was a marked disparity; 11.6 x 10^9/l on the Manual QBC-V , 5.1 x 10^9/l on the Automated QBC-V and 17.1 x 10^9/l on the ABX Minos Vet.

The cat with the leucocytosis registered an elevated total white cell count on two of the analysers, although there was some variation in its magnitude (79.2 x 10^9/l on the Manual QBC-V versus 63.2 x 10^9/l on the ABX Minos Vet). The Automated QBC-V would not give a reading for this cat.

In the leucopenic animals the total white cell counts in the three dogs were very similiar. There was a marked disparity on the analysers when interpreting one cat's total white cell count, 23.1 x 10^9/l being the white cell count on the Manual QBC-V, 9.8 x 10^9/l on the Automated QBC-V and 5.4 x 10^9/l on the ABX Minos Vet (the basis of it being determined to be leucopenic). The manual white cell count in this cat was most consistent with the ABX Minos Vet at 6.8 x 10^9/l. It was observed on the Manual QBC-V that there was no obvious bright green band visible, representing the lymphocyte-monocyte layer,so it is possible that the white cell count may have included the platelets as well. The other cat which was leucopenic on the ABX Minos Vet (3.7 x 10^9/l) was within the normal reference ranges on the two QBC-V analysers (6.1 x 10^9/l on the Manual and 7.4 x10^9 /l on the Automated) The manual white cell count was 4.0 x10^9/l which supported the leucopenia detected on the ABX Minos Vet. This difference is important because leucopenia is a significant clinical finding and it was not detected by the two QBC-V analysers.

3.4 Discussion

The results of the packed cell volume (PCV) estimates in dogs and cats indicate that medical haematology analysers produce accurate measurements in the cat and the dog and can be relied upon to identify changes in PCV. It is possible that the initial poor correlations between the medical analysers and the reference ABX Minos Vet in the cat blood was due to the probe of the ABX Minos Vet analyser selecting sedimented red blood cells at the bottom of the capillary tube, due to insufficient mixing. It was noted that the three outliers that were removed in the Coulter VCS comparison with the ABX Minos Vet, all had a very low PCV as measured on the Coulter VCS, with a normal PCV measured on the ABX Minos vet. These findings are consistent with previous studies which have shown that unless the current amplitude is increased for the erythrocyte channel, then the smaller erythrocytes of the feline species are not detected (Weiser, 1987), resulting in false results consistent with a diagnosis of anaemia.

The QBC-V analysers are also well correlated with the ABX Minos Vet for packed cell volume in dogs and cats, although there was much better correlation between the ABX Minos Vet and the Automated QBC-V than between the ABX Minos Vet and the Manual QBC-V. This suggests greater error with the manual method. It probably also accounts for the surprisingly low correlation between the Manual and Automated QBC-V (r^2=0.813 in cats) when assessing PCV's on the same capillary tubes. These analysers determine the PCV directly by centrifugation and so they would be expected to be more accurate than the automated analysers which measure the PCV by calculation. The direct measurement of haemoglobin by the medical haematology analysers would perhaps be a more specific determination of red blood cell status than the PCV in cats and dogs.

The Technicon H1 and the Coulter VCS medical analysers provide reliable estimates of total white cell counts in dogs. The Manual and Automated QBC-V analysers were also reliable with respect to total white cell counts in dogs, both in normal dogs and dogs with altered leucograms. Again the Automated QBC-V analyser was more strongly correlated with the ABX Minos Vet than was the Manual QBC-V analyser (r^2=0.866 and r^2=0.709 respectively). Clinicians may rely on these in-clinic analysers as being accurate analysers in dogs more especially the Automated QBC-V, but not in cats. However the poor correlations demonstrated by both the medical haematology analysers and the QBC-V analysers with respect to each other and the manual white cell count demonstrate that it is difficult to get an accurate total white cell count in the cat. These findings would support the practice that some haematology laboratories follow, whereby all cats' total white cell counts are determined manually on a Unopette or similiar counting grid. There are limitations also associated with this method of enumerating total white cell counts, such as incorrect filling of the counting chamber and the limitations of testing such a small aliquot of blood to determine the total white cell count (Schalm *et al*, 1986). Cell counts may also be inaccurate if not performed in a systematic way (Knoll *et al*, 1996). The reasons for these discrepancies on all of the automated analysers may lie in the clumped platelets being misinterpreted as white blood cells, or as studies have shown in the relative resistance of feline erythrocyte membranes to be lysed, resulting in residual erythrocyte stroma which can then be included in the white cell count, giving a falsely high white cell count (Weiser, 1987). Additionally, both cat's and dog's leucocytes may be affected by sample age, with nuclear pyknosis and fragmentation occuring most obviously. These changes may prevent accurate identification by both manual and automated methods (Knoll *et al*, 1996).

The automated differential counts from the Coulter VCS and the Technicon H1 for both cats and dogs showed no correlation with the ABX Minos Vet or the QBC-V analysers. It would be

essential for clinicians who send their blood to either hospital or private medical laboratories to be aware whether the differential white cell counts have been performed by the analyser, or whether the analyser was 'disarmed' and the differential count was performed manually. Explanations for the poor correlation between the medical analysers and the manual differential counts may be due to the medical analysers not having had their white cell channels recalibrated to recognise dog and cat leucocytes. The medical analysers are set to recognise the lobularity and cellular content of human neutrophils and other human leucocytes. The falsely high basophil count commonly seen on both the Coulter VCS and the Technicon H1 may be due to the relative resistance of the feline and canine leucocyte membrane to lyse with the diluent used. Weiser (1987) found that it was necessary to increase the voltage amplitude of the white cell counting chamber, because of the resistance of the feline erythrocytes to lysing. It was also noted that the behaviour of animals leucocytes in lysing reagent systems needed to be characterised due to the variability in shrinkage of the leucocyte membrane.

There was poor correlation between both QBC-V analysers partial differentials and the manual differential. There are obvious limitations in having a 500 cell manual differential as the gold standard method, the number of cells counted is a fraction of what is counted on the Automated analysers.There can also be an uneven distribution of leucocytes on the blood smear, resulting in over-representation of a particular cell type. The quality of the blood smear is another important determinant in the accuracy of the manual differential. Leucocyte morphology deteriorates with time (Knoll *et al* , 1996) which may be misleading both for manual and automated differentials. The Manual QBC-V analyser has the limitation of requiring manual input to determine the length of the white cell bands. The positioning of the cursor accurately is not easy especially when there is only a very thin band as commonly occurs in the lymphocyte-monocyte layer of stressed animals. While the Automated QBC-V does not require the manual placement of a cursor the

principle is similiar in that thin bands are being read.

With respect to platelet counts in dogs, the Technicon H1 and the Coulter VCS gave consistently strong correlations with the ABX Minos Vet but the Automated and the Manual QBC-V analysers were unreliable for platelet counts in this species. Possible explanations for the QBC-V analysers not giving counts consistent with the ABX Minos Vet are that the orange band of platelets which lies on top of the white cell band is not always clear and therefore may not be entered correctly on the Manual QBC-V, or that the clumping of the platelets is blurring the cell to cell interfaces as reported by Rayson (1989). Platelet clumps may be visible on top of the inserted floating rod and although this allows confirmation of their presence, it does not allow quantification. Another possibility is that the platelet clumps in these dogs are being misinterpreted as white blood cells, although if this were the case then it would be expected that the medical haematology analysers would also have produced inaccurate results. The Automated QBC-V analyser was again more closely correlated with the ABX Minos Vet than the Manual QBC-V was.

All of the analysers provided unreliable platelet counts in cats. The two QBC-V analysers demonstrated a very good correlation with each other, but both were poorly correlated with the ABX Minos Vet, indicating consistency but not accuracy. We know that cats platelets are prone to clumping during venous collection. In addition platelets have a tendency to clump over time in spite of the presence of an anticoagulant (Knoll *et al*, 1996). Thrombocytopenias should always be interpreted with caution in this species and confirmed by examination of a blood smear.

The QBC-V analysers are capable of detecting accurately elevated total white cell counts in both dogs and cats, although there may be some variation in its magnitude when compared with the reference ABX Minos Vet. The QBC-V analysers also detected leucopenias in dogs but there was an inability to detect both of the leucopenic cats in the study. The manual white cell count

supported the findings of the ABX Minos Vet in both cases, although there are obvious limitations to the Unopette method, as previously mentioned. The most logical reason for the apparent false increase in leucocyte numbers on the two QBC-V analysers would be the clumping of platelets being misinterpreted as white cells. Although this study involved only very limited numbers, it would be important for clinicians to be aware that the QBC-V analysers may not be so accurate with respect to total leucocyte counts in cats.

3.5 Conclusions

The medical analysers can be relied on to produce accurate PCV's in both dogs and cats. The Automated QBC-V would appear a more accurate analyser with respect to PCV's in both cats and dogs, than the Manual QBC-V when compared with the reference analyser.

Total white cell counts can be deemed reliable in dogs on both the Technicon H1 and the Coulter VCS analysers. Both in-clinic analysers were also reliable for total white cell counts in both normal and altered leucocyte status. The Automated QBC-V analyser performed better though. In cats total white cell counts were difficult to assess on any of the analysers. The one leucocytic cat was detected on the QBC-V analysers, but neither of the leucopenic cats were. Clinicians should be aware of the hazards of total leucocyte counts determined by either automated, semi-automated and even manual methods. The assessment of a well made blood smear would be benficial. In light of the poor performance of all of the analysers with respect to the manual differential, it would be recommended to have a well trained technician perform these manually, or use adapted veterinary software with the medical analysers.

Platelet counts in dogs were reliable on the medical analysers but not in cats. This emphasised the difficulty in assessing platelet counts in cats and highlighted the importance of examination of a smear. The QBC-V analysers did not produce platelet counts similiar to the reference analyser in either dogs or cats, something for clinicians to be aware of who are routinely using these analysers in their clinics.

The Automated QBC-V was more closely correlated with the ABX Minos Vet in many of the comparisons than was the Manual QBC-V. This included PCV's in both cats and dogs, platelet counts in dogs and cats and white cell counts in dogs. It would appear a superior model to its predecessor the Manual QBC-V.

CHAPTER FOUR

TIME COURSE STUDY.

4.1 Introduction

In addition to the comparison between the haematology analysers, a time course study was conducted on the three veterinary haematology analysers located at Massey University (ABX Minos Vet, and the Automated and Manual QBC-V analysers).

The reason for this study is that in a normal clinical setting it is not always possible to test the sample within an hour of collection. In most situations, the blood is transported via courier to the closest laboratory and may not be tested for several hours or even on the following day. It is known that over time the fragile nature of the cellular contents of blood change, and the white blood cells undergo morphological change, making both manual and automated differentials difficult. Platelets, especially in cats, are prone to clumping, despite the presence of EDTA anticoagulant (Knoll *et al;* Veterinary Clinics of North America, 1996).

The aim of the study was to determine whether or not any changes occurred in the packed cell volume, total white cell count, and platelet count over a 24 hour period in blood samples analysed on each of the three analysers.

4.2 Materials and Methods

Venous blood was collected from five dogs and five cats into evacuated tubes containing EDTA anticoagulant. The blood was then analysed within ten minutes of collection on the ABX Minos Vet, and both the Automated and the Manual QBC-V analysers.

The samples were then re-analysed at two hourly intervals, until eight hours post-collection, and then again at 24 hours post-collection.

4.3 Results

4.3.1 Cats

(i) Packed Cell Volume

The Manual QBC-V analyser gave consistent packed cell volume (PCV) readings over time (Figure22). Cat 3 showed a decline in PCV between eight and 24 hours after collection but the values remained within the normal range. PCV's of the other four cats also remained within the reference range for the entire 24 hour period and showed no consistent trends. The Automated QBC-V analyser also gave consistent haematocrit readings over the 24 hour period. Three of the five cats showed a slight increase in PCV over time, although all remained within the normal reference range for cats (Figure 23). One of these was cat 3 that had shown a decline in PCV on the Manual QBC-V. Results obtained from the ABX Minos Vet analyser were equally consistent up to eight hours. By 24 hours three of the cats had a PCV which had risen to lie just outside the reference range (Figure 24). Overall, the PCV's measured on the ABX Minos Vet seemed higher than on the other two QBC-V analysers.

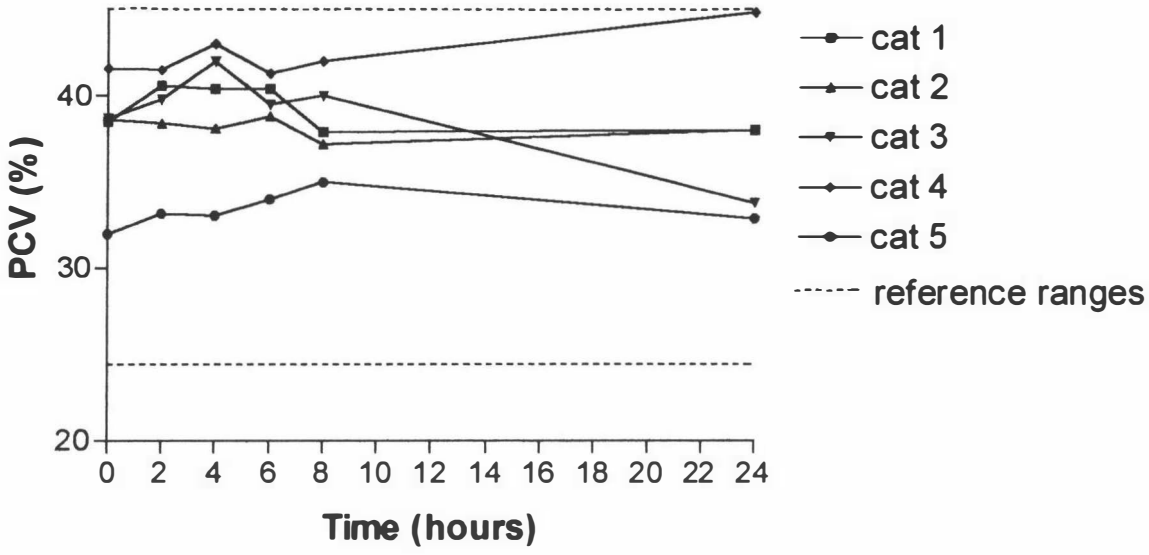

Figure 22: Line graph for the Manual QBC-V versus time for packed cell volumes in cats.

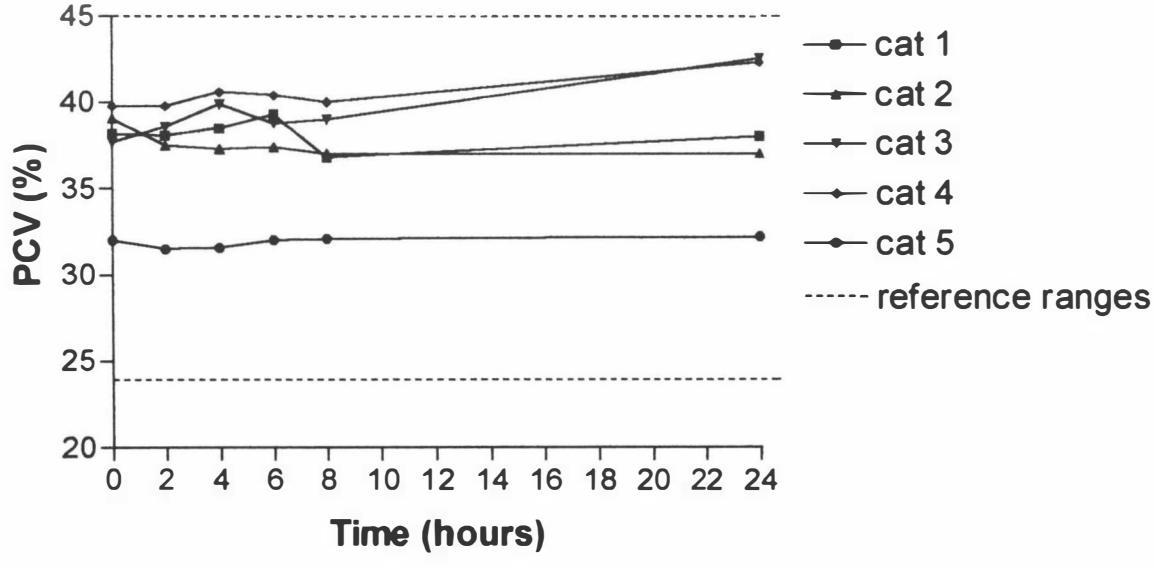

Figure 23: Line graph for the Automated QBC-V versus time for packed cell volumes in cats.

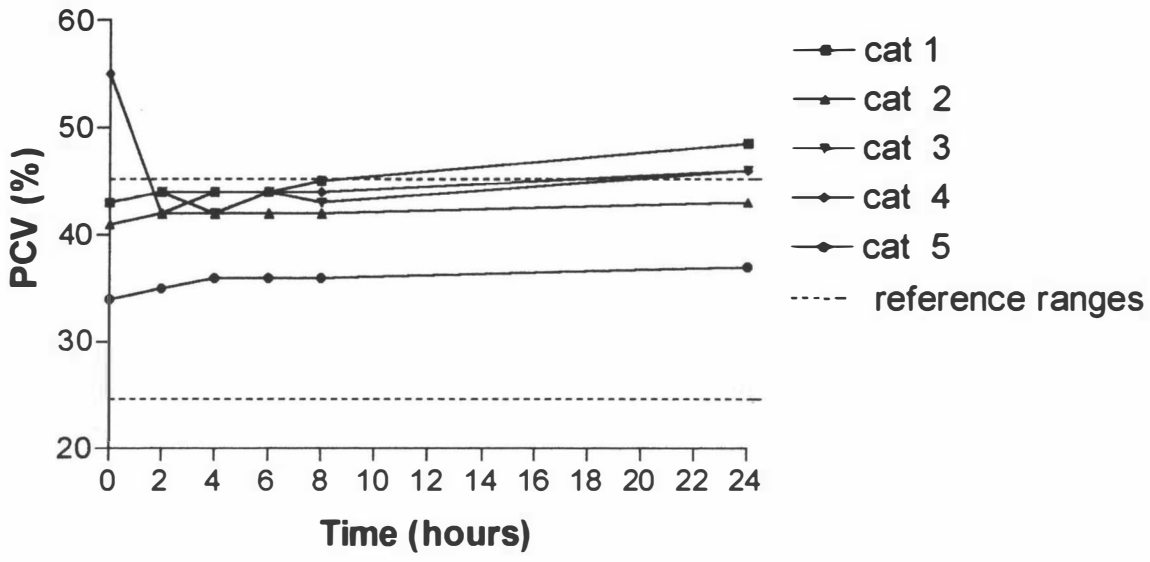

Figure 24: Line graph for the ABX Minos Vet versus time for packed cell volumes in cats

(ii) Platelet counts.

In four of the five cats, platelet numbers dropped below the reference range within two hours of sampling on the Manual QBC-V (Figure 25). In two of these cats the platelet counts returned to approximate their initial value by four hours and then remained in the reference range until 24 hours. In one of the two remaining cats the platelet counts remained in the thrombocytopenic range (<300 x 10^9/l) at 24 hours. In contrast, the platelet numbers in cat 5 showed a gradual decline throughout the 24 hour period and had fallen below the reference range by four hours after collection.

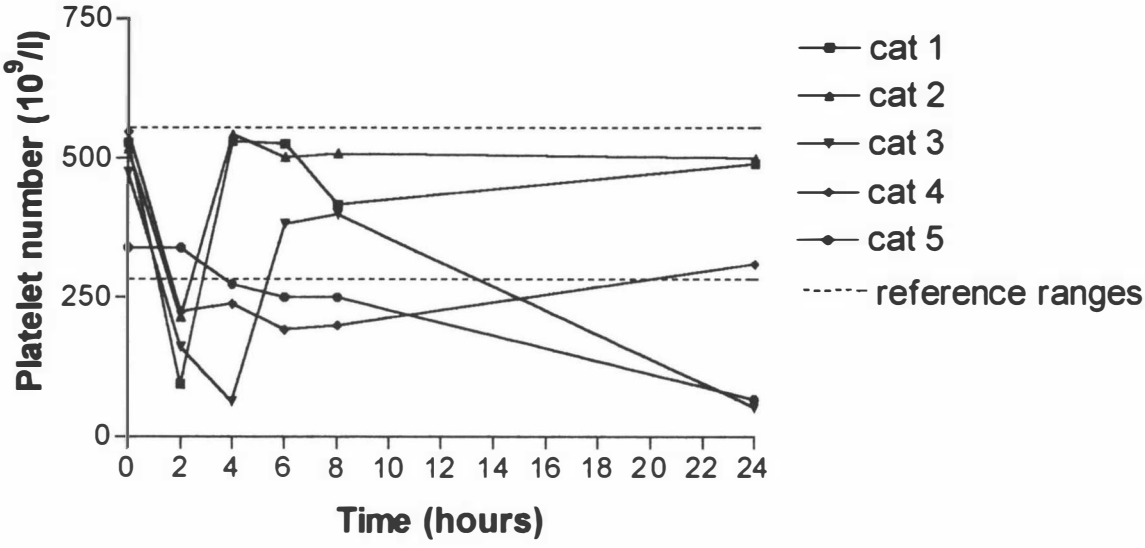

Figure 25: Line graph for the Manual QBC-V versus time for platelet counts in cats.

The blood samples tested on the Automated QBC-V analyser also demonstrated this trend (Figure 26). Not surprisingly the same four cats had a marked reduction in platelet numbers by two hours post sample collection. Again two of the cats showed a return of platelet numbers to the normal reference range by four hours (300-600 x 10^9/l) while in two cats the platelet counts remained low, after a rise at six hours. Using this analyser, cat 5 had a consistent platelet count in contrast to that obtained using the Manual QBC-V.

The platelet numbers determined by the ABX Minos Vet analyser showed a similar pattern (Figure 27).

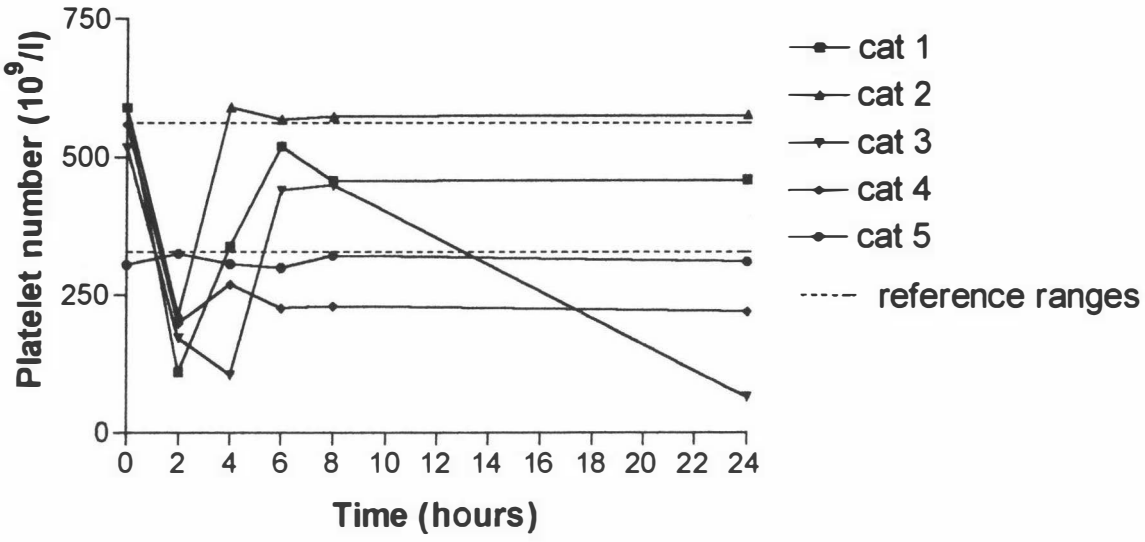

Figure 26: Line graph for the Automated QBC-V versus time for platelet counts in cats.

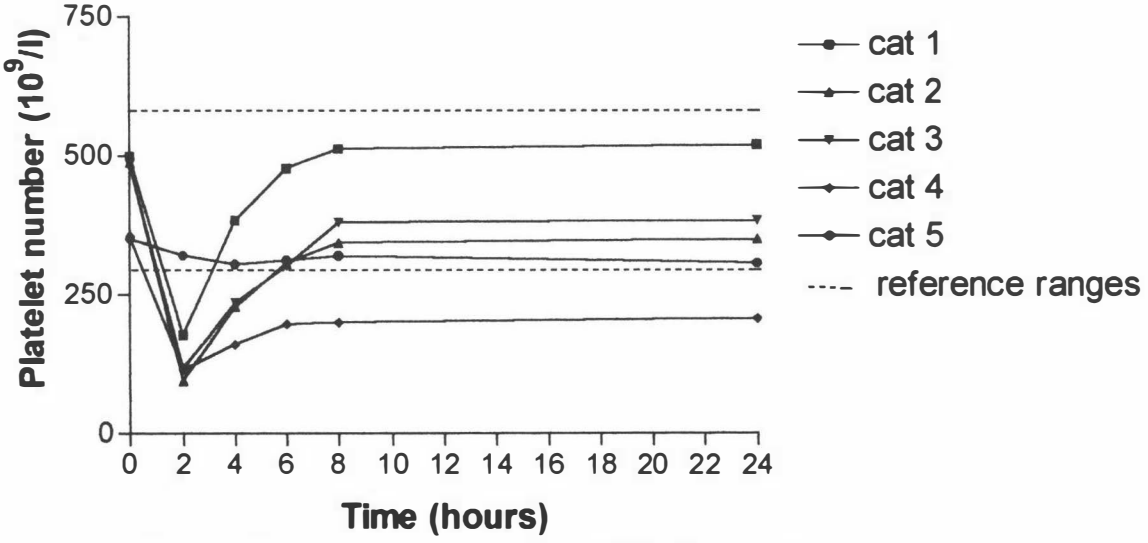

Figure 27: Line graph for the ABX Minos Vet versus time for platelet counts in cats.

(iii) Total White Cell Counts

There was very little variation in the total white cell counts (WCC) over the 24 hour period in any of the cats, when assessed on the Manual QBC-V (Figure 28). Similarly, the Automated QBC-V demonstrated little change in WCC over the 24 hours in three of the five cats, with a small amount of variation in cat 1 and 4 (Figure29).

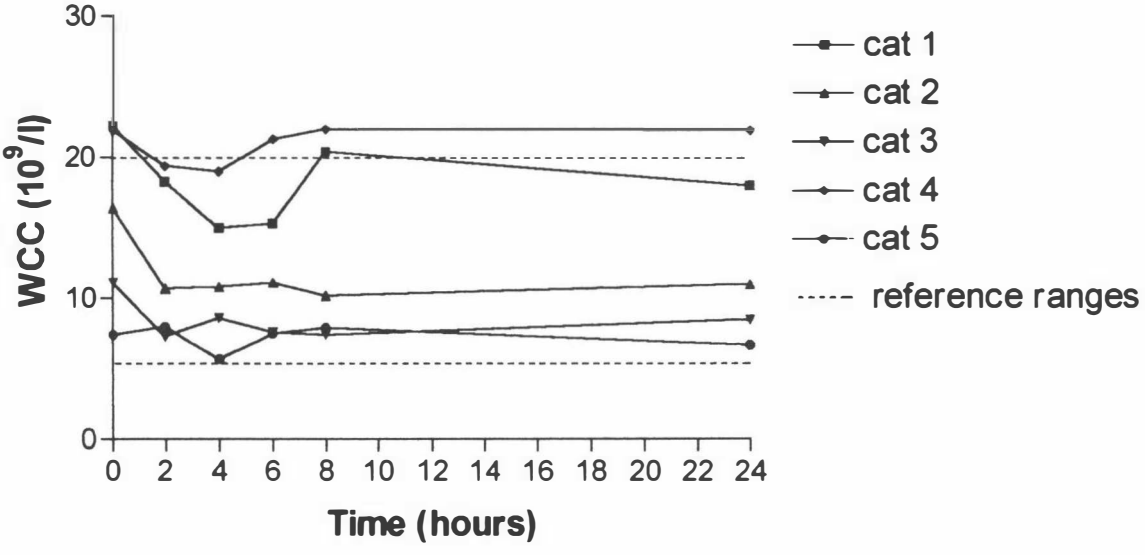

Figure 28: Line graph for the Manual QBC-V versus time for white cell counts in cats.

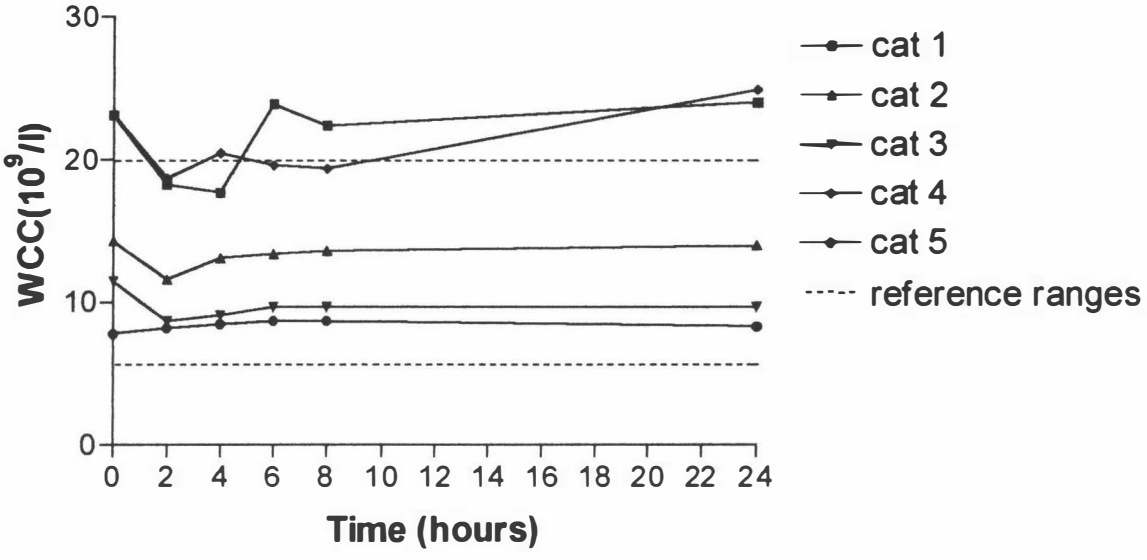

Figure 29: Line graph for the Automated QBC-V versus time for white cell counts in cats.

In contrast, the ABX Minos Vet showed more variation in WCC (Figure 30). Using this analyser a peak in WCC was observed at two hours in four of the five cats, followed by a return to normal in three of these cats by four or six hours. This corresponded to the trough in platelet counts observed in all three analysers. Cat 5 however had very little variation in WCC, remaining low throughout the 24 hour period.

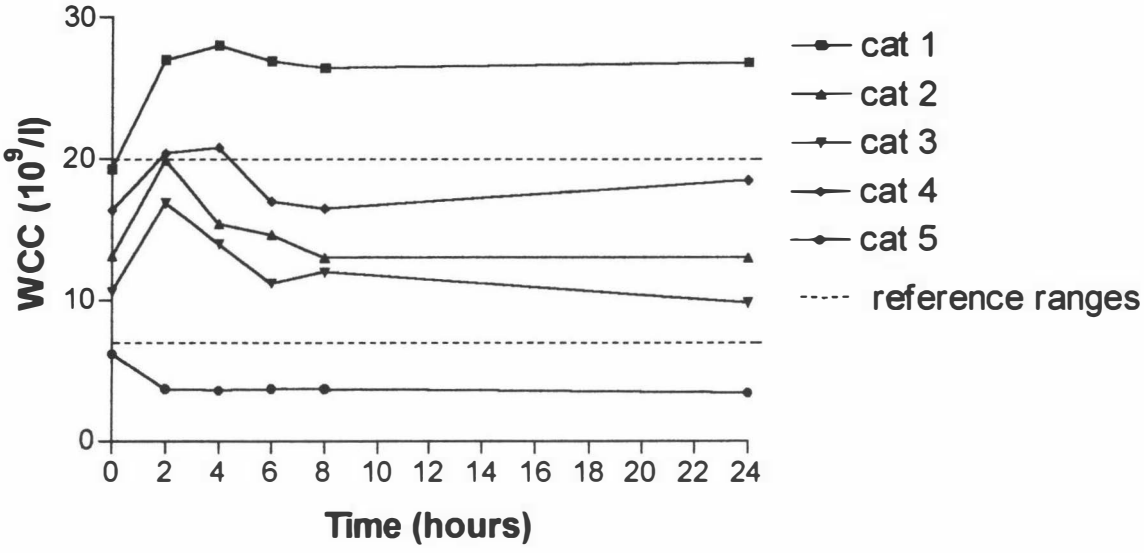

Figure 30: Line graph for the ABX Minos Vet versus time for the white cell counts in cats.

4.3.2 Dogs

(i) Packed Cell Volume

All five dogs had consistent PCV's over the 24 hour period as measured by all three analysers (Figures 31,32 and 33).

The PCV's of the dogs showed less variation than those of the cats.

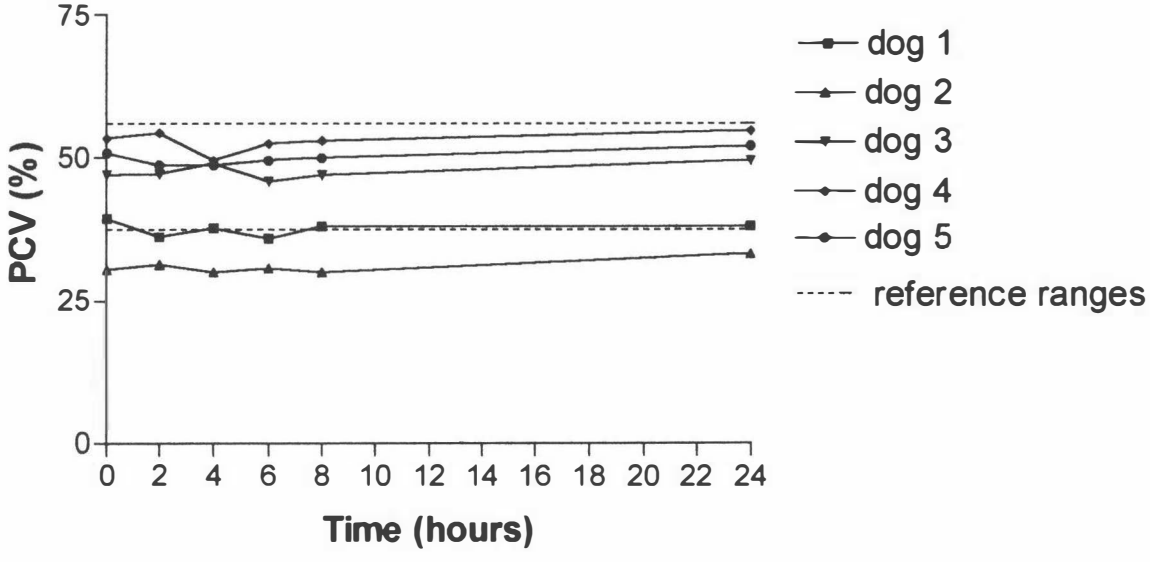

Figure 31: Line graph for the Manual QBC-V versus time for packed cell volume in dogs.

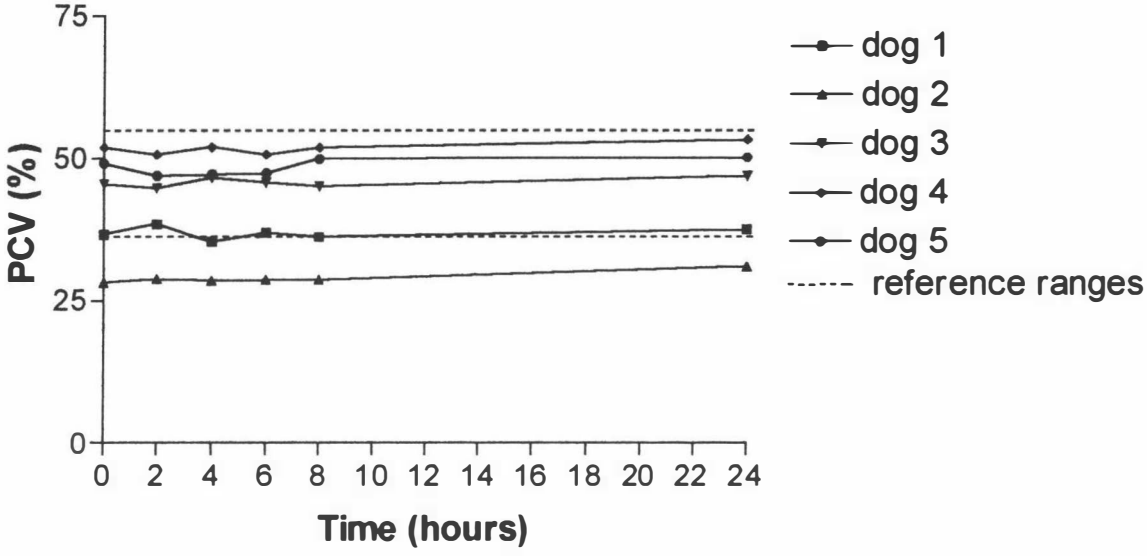

Figure 32:Line graph for the Automated QBC-V versus time for packed cell volume in dogs.

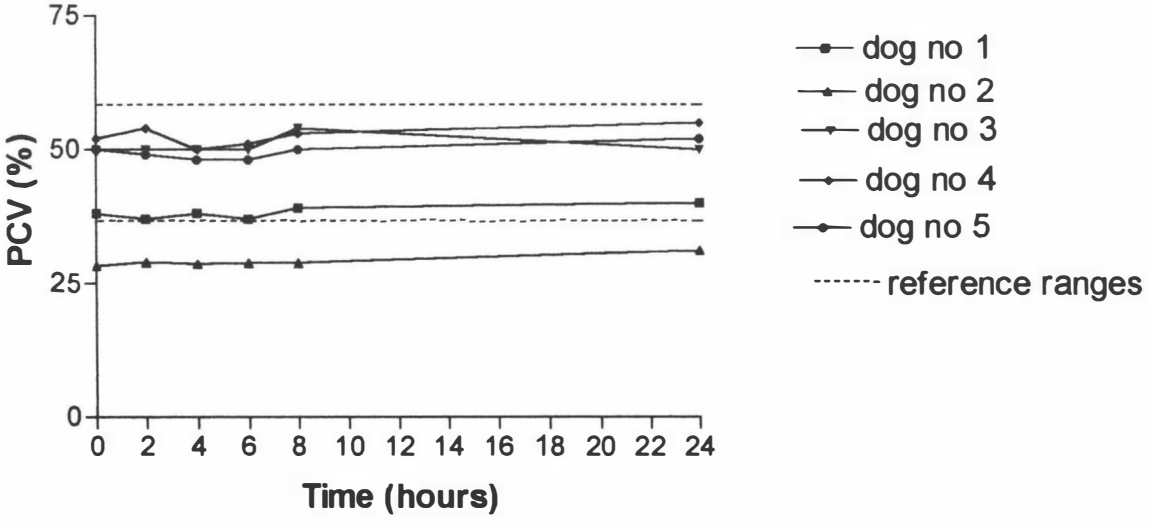

Figure 33: Line graph for the ABX Minos Vet versus time for packed cell volumes in dogs.

(ii) Platelet Numbers

There was some variation in platelet number over the 24 hour period when assessed on the Manual QBC-V (Figure 34). Three dogs had platelet counts which decreased at various times over the 24 hours. In one of the dogs the platelet count decreased gradually to fall below the reference range by 8 hours and was well below the original value by 24 hours. The remaining two dogs showed consistent platelet counts over the time.

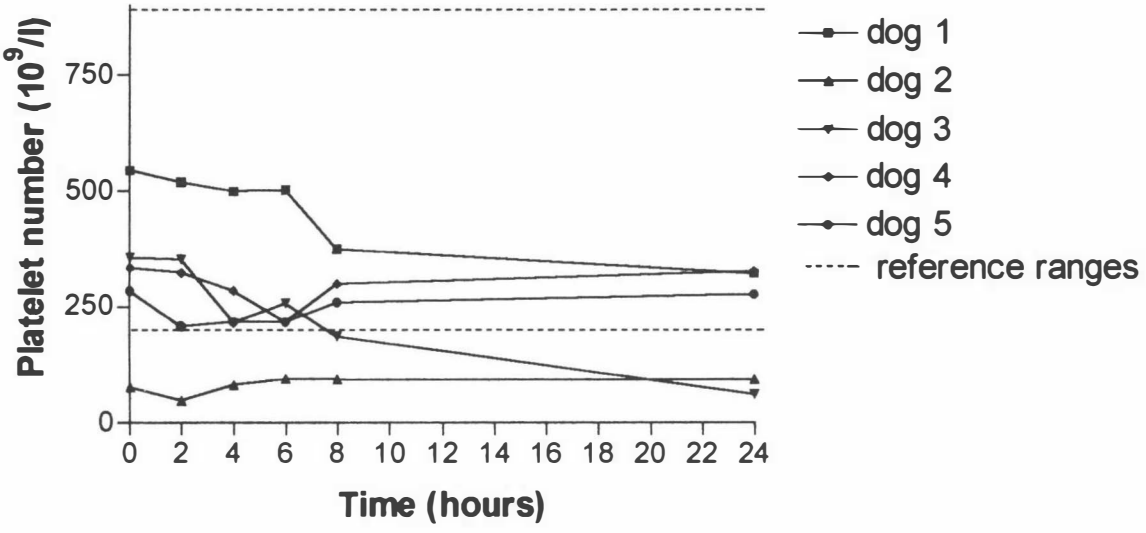

Figure 34: Line graph for the Manual QBC-V versus time for platelet counts in dogs.

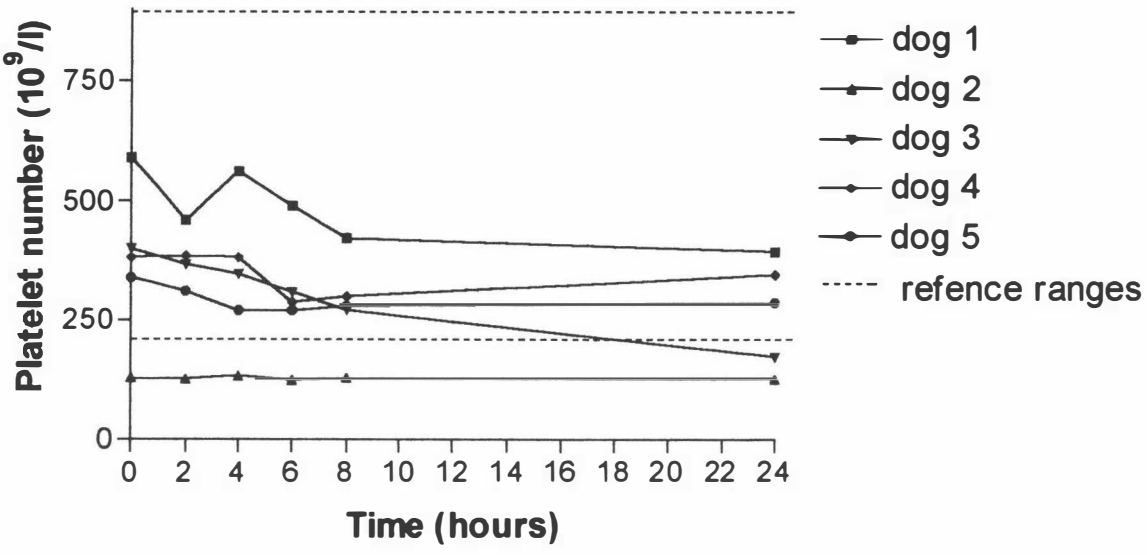

Figure 35: Line graph for the Automated QBC-V versus time for platelet counts in dogs.

The Automated QBC-V analyser produced results very similar to the Manual QBC-V (Figure 35). Dogs 2 and 5 again demonstrated consistent platelet counts over the 24 hours. In dog 3 the platelet count fell to below the reference range by 24 hours. The remaining two dogs also showed some variation in platelet counts over the time span but their values remained within the reference range.

In contrast to the QBC-V analysers the platelet counts of all the dogs, when assessed on the ABX Minos Vet, showed little variation over the 24 hour period (Figure 36). This included dog 3 which had demonstrated a marked decrease in platelet number on the other two analysers.

Overall the variation in platelet numbers of the dogs on all three analysers was not as great as that observed in the cats.

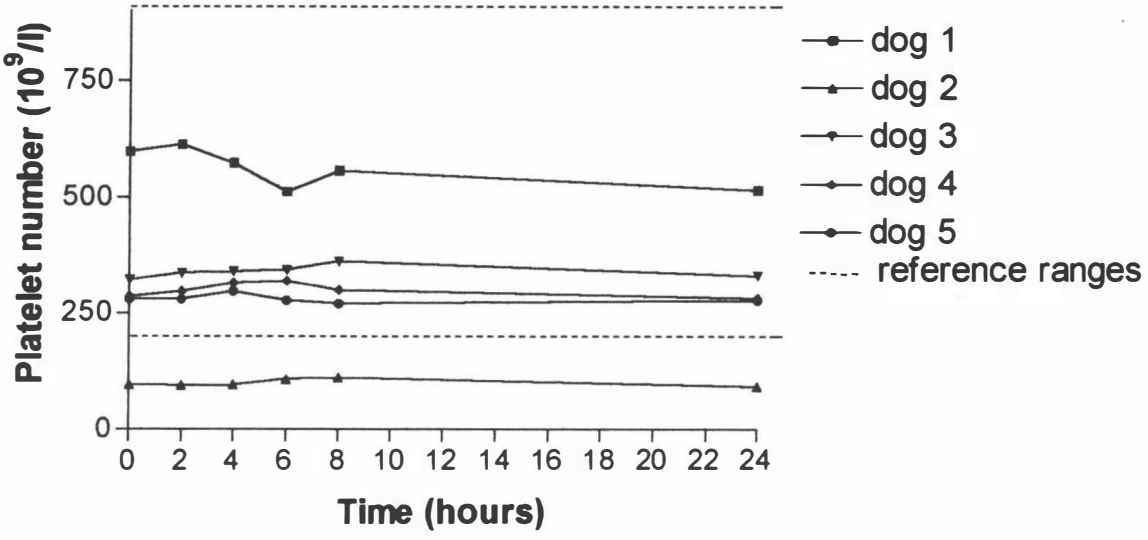

Figure 36: Line graph for the ABX Minos Vet versus time for platelet counts in dogs.

(iii) Total White Cell Counts

On the Manual QBC-V analyser all dogs had consistent WCC's until eight hours (Figure 37). The WCC then increased in dog 3 and decreased in dog 2 by 24 hours.

Using the QBC-V analyser four of the five dogs showed consistent WCC's over the 24 hour period (Figure 38). The WCC in the remaining dog showed erratic increases and declines which could not be explained.

In contrast, WCC's performed on the ABX Minos Vet showed little variation (Figure 39). This included the dog which had demonstrated marked variation in WCC when analysed on the Automated QBC-V.

Overall, the WCC of the dogs varied less than in cats over the 24 hour period.

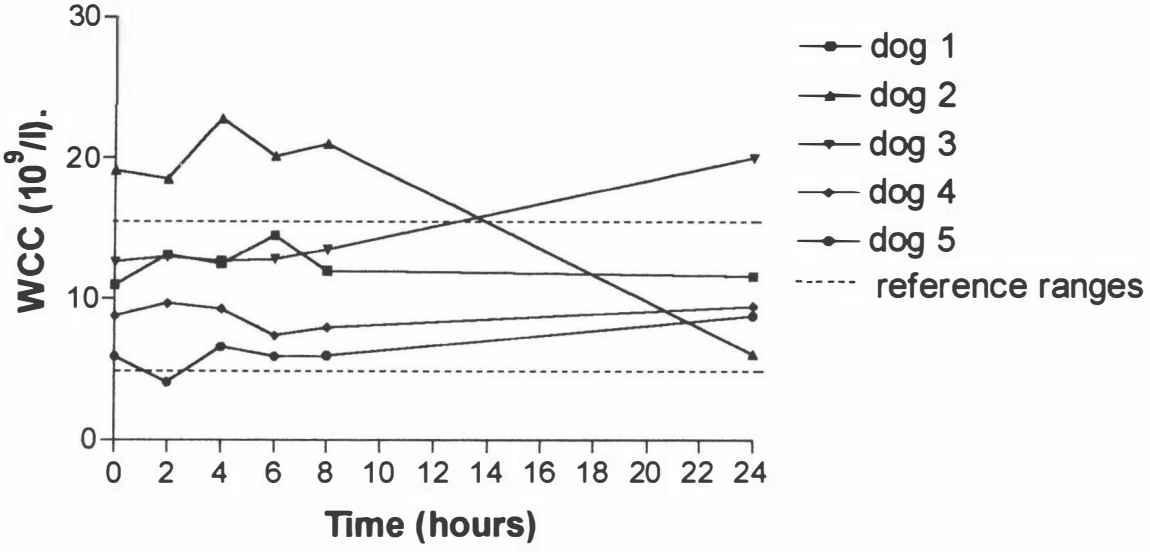

Figure 37: Line graph for the Manual QBC-V versus time for white cell count in dogs.

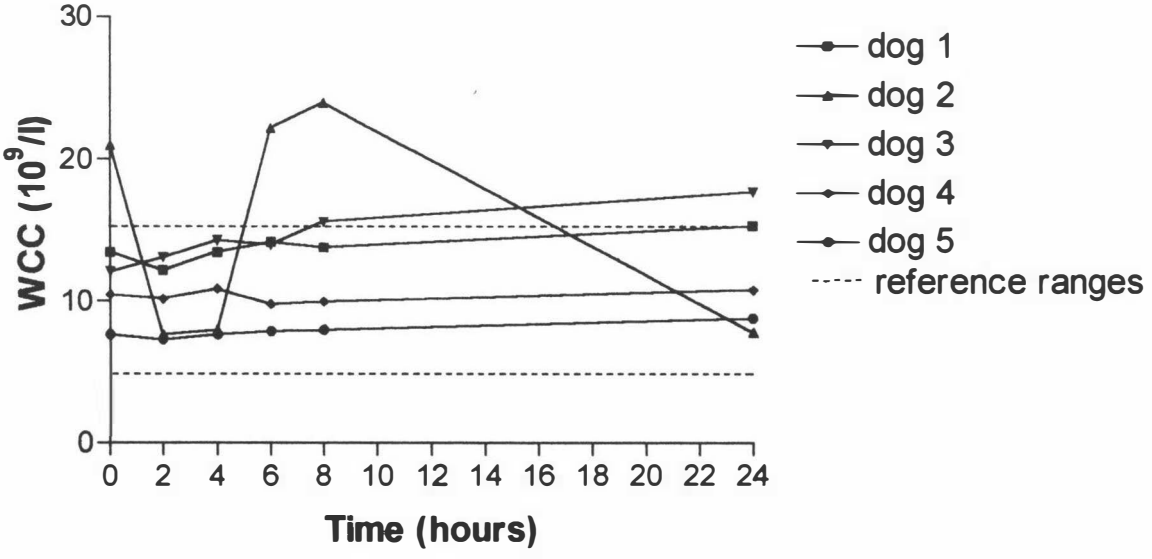

Figure 38: Line graph for the Automated QBC-V versus time for white cell counts in dogs.

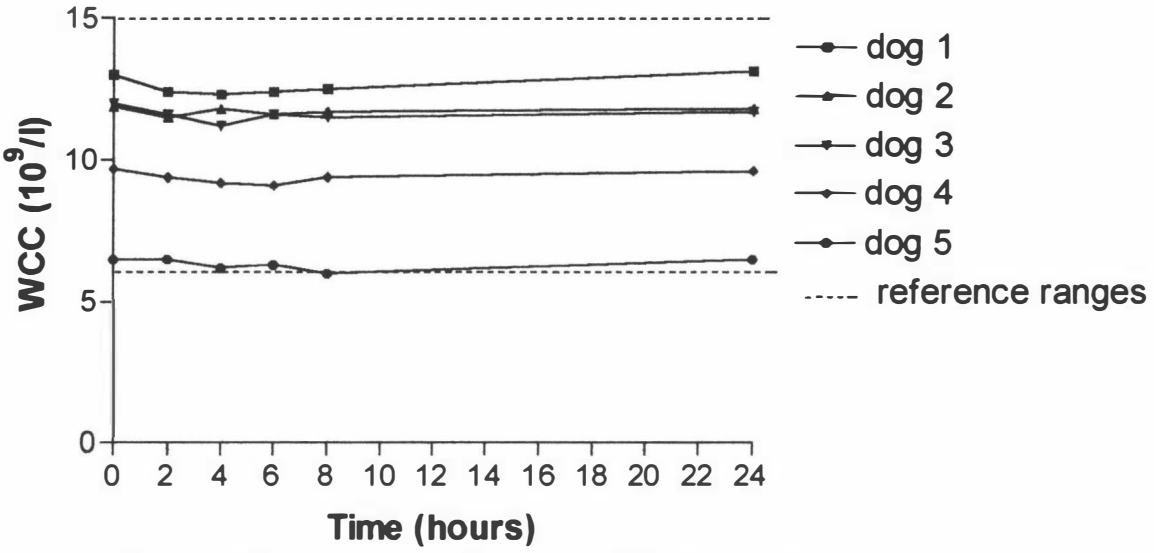

Figure 39: Line graph for the ABX Minos Vet versus time for the white cell counts in dogs.

4.4 Discussion

The time course study exposed several trends in haemograms over time, some of which could be significant from a clinical perspective.

Essentially there was little variation in the PCV's of cats over the 24 hours. The Automated QBC-V analyser demonstrated an increase in PCV in three of the five cats. Perhaps a gradual decline in ATP generation by the red blood cells during storage results in an influx of sodium and water into the cytoplasm, leading to a mild increase in cell volume and therefore PCV. Another possibility for the mild variation in cats PCV's may be due to clumped platelets being misinterpreted as erythrocytes. This would explain why there was more variation in the PCV results of the cats than the dogs, where platelet clumping would be less likely.

However, it is apparent that the PCV's of cats can be interpreted with confidence up to at least 24 hours on all three haematology analysers.

Platelet numbers in cats varied significantly over the 24 hour period when measured on all three analysers. This may suggest that platelets are aggregating and then disaggregating and therefore either being misinterpreted as white cells by the ABX Minos Vet or being deposited in different layers of the QBC-V. This is supported by the variation in WCC of cats as measured by the ABX Minos Vet. Four of the five cats tested on this analyser showed an increase in WCC at two hours after collection before returning to their initial values. This coincided with the decrease in platelet numbers that were observed on each of the analysers. It is likely that the aggregated platelets would have been recognised as white blood cells on the ABX Minos Vet, which classifies cells on the basis of their size. Low platelet counts in cats should therefore be interpreted with caution and checked by inspection of a well made blood smear. This study showed that measurements of platelets and white cell counts in cats at two and four hours after collection may be less reliable than measurements performed before or after this time. The Manual and Automated QBC-V analysers however demonstrated good consistency in white blood cell count.

The excellent consistency in the PCV's of all of the dogs on all of the haematology analysers suggests that PCV's in dogs can be interpreted with confidence up to at least 24 hours.

There was some disparity in the platelet counts of dogs when analysed on the Manual and Automated QBC-V, although this was not as marked as in cats. Again this supports some degree of aggregation and disaggregation of platelets in this species. The ABX Minos Vet showed minimal variation in platelet numbers, so it can be concluded that platelet counts performed by this analyser on dogs can be interpreted with confidence up to at least 24 hours after collection.

There was also good consistency in the total WCC of the dogs on each of the analysers although more so on the ABX Minos Vet. It can be concluded that total WCC can be interpreted with

confidence up to at least 24 hours in dogs in each of the analysers.

4.5 Conclusions

For each of the analysers used in this study, PCV's in cats and dogs can be interpreted with confidence up to at least 24 hours post collection. Platelet counts and white cell counts in cats are more variable and even early testing, i.e. within two hours of collection, can result in erroneous values. It would be recommended to assess a peripheral blood smear for both platelet and white blood cell morphology and numbers in cats.

Platelet counts in dogs measured on the ABX Minos Vet can be interpreted with confidence up to at least 24 hours. Some variation in platelet counts should be expected in this species when QBC-V analysers are used, but this is unlikely to lead to misdiagnosis.

White cell counts can be interpreted with confidence in dogs up to at least 24 hours post-collection on all three analysers.

CHAPTER FIVE

SUMMARY AND CONCLUSIONS.

Veterinarians in clinical practice today have many options when it comes to laboratory services. The purpose of this study was to assess the performance of five haematology analysers on blood from both sick and healthy dogs and cats and, in doing so highlight the potential pitfalls of the various analysers.

The medical analysers (the Technicon H1 model and the Coulter VCS) can both be relied on to produce accurate PCV's in dogs and cats, as can the Automated and Manual QBC-V in-clinic analysers.

The Technicon H1 and the Coulter VCS also appeared reliable with respect to total white cell counts in dogs. The Automated and Manual QBC-V were able to accurately determine white cell counts in both normal dogs and those with altered leucocyte status. The Automated QBC-V analyser was consistently more closely correlated with the ABX Minos Vet, than was the Manual QBC-V with respect to PCV's and platelet counts in both dogs and cats, and white cell counts in dogs. It can be concluded that the Automated QBC-V would be a sounder purchase, than the Manual QBC-V as an in-clinic analyser.

Total white cell counts in cats are difficult to determine accurately on all analysers, whether or not they are in the reference range. Possible reasons for this include the tendency for cats' platelets to clump, resulting in misinterpretation as white cells. Weiser (1987) reported that feline erythrocytes are also quite resistant to lysis, resulting in residual erythrocyte stroma further confusing the white cell count. Both QBC-V analysers detected the cat with the leucocytosis but not the leucopenic ones. The manual white cell count has its own inherent problems in that far fewer cells are counted than with the haematology analysers and a smaller sample size is therefore

used to represent the whole blood sample.

The two medical analysers cannot be relied on to produce accurate white cell differentials in dogs or cats. Similarly, partial differential counts performed on the Automated and Manual QBC-V analysers did not correlate well with those performed manually. It would therefore be recommended that a technician perform manual differentials from a well made blood smear to support the results from these analysers.

The medical analysers produced reliable platelet counts in dogs but not in cats. The QBC-V analysers produced different results from the ABX Minos Vet analyser in both cats and dogs. This highlighted the difficulty of obtaining accurate platelet counts especially in cats. Thrombocytopenias should always be interpreted with caution in this species and confirmed by examination of a blood smear.

A time course study (Chapter four) was done to demonstrate the changes which may occur in haematology values with time, so that clinicians may be more aware of any changes in blood values that may occur if testing is delayed. The time course study involved the use of the three veterinary specialised haematology analysers on blood from five cats and five dogs over a 24 hour period.

The dogs PCV's showed no significant variation with time, while in the cats there was mild variation in PCV's over the 24 hours. However in summary both cats and dogs PCV's may be interpreted with confidence up to at least 24 hours after collection.

Platelet counts and total white cell counts in cats were found to vary significantly over time. Interestingly, measurements of these cells at two and four hours after collection may be less reliable than measurements performed before or after this time. The marked decline in platelet numbers in four of the five cats at approximately time two hours coincided with an increase in total white cell count as measured on the ABX Minos Vet. This suggests that aggregated platelets

may be misinterpreted as white cells by this analyser which classifies cells on the basis of their size. There was some disparity in the platelet counts of dogs when assessed on the Manual and Automated QBC-V analysers, suggesting that there may be a degree of platelet aggregation and disaggregation in this species also. The ABX Minos Vet however showed minimal variation in platelet numbers, so platelet counts over 24 hours can be interpreted with confidence on this analyser.

Total white cell counts can be interpreted with confidence up to at least 24 hours in dogs on all three analysers.

REFERENCES.

Corash,L. (1995)
Laboratory hematology; methods for the analysis of blood, from Blood Principles and Practice of Hematology.JB Lippincott Company, Philidelphia.

Coulter VCS Casebook (1989)

De Cresce,R. (1986)
The Technicon H1; a discrete, fully automated complete blood count and differential analyzer. Laboratory Medicine **17**,1, 17-21.

Greendyke,R.M., Kanter,D.R., DeBoover,L., Savage,L.,VanGelder,S. (1985)
A comparison of differential white blood cell counts using manual technic and the Coulter S-Plus IV. American Journal of Clinical Pathology, September 1985, 348-350.

Groner,W., Boyett,J., Johnson,A., Scantlebury,M. (1986)
Variability of erythrocyte size and hemoglobin content observed in man and four selected mammals.Bloodcells **12**, 65-80.

Jain,N.C. (1986)
Schalm's Veterinary Hematology, 4th Edition.

Knoll,J., Rowell,S.L. (1996)
In-clinic analysis, quality control, reference values and system selection. Veterinary Clinics of North America: Small Animal Practice **26**,5, 981-1003.

Levine,R.A., Hart,A.H.,Wardlaw,S.C. (1986)
Quantitative buffy coat analysis of blood collected from dogs, cats, and horses. Journal of the American Veterinary and Medical Association **189**, 6, 670-673.

Rayson,C. (1989)
Quantitative blood counter.Clinical Pathology, JD Stewart Refresher Course.

Simson,E. (1988)
The Technicon H1 system. Technicon H*1 Haematology Symposium.

Simson,E., Ross,D.W., Kocher,W.D. (1988)
Atlas of Automated Cytochemical Hematology.1st Edition, Technicon Corporation.

Technical Manual Minos RAA
Roche Diagnostic Systems Haematology.

Thompson,K.G. (1993)
Veterinary clinical pathology, Student study guide, Massey University Publication.

Tvedten,H., Korcal,D. (1996)
Automated differential leucocyte count in horses, cattle, and cats using the TechniconH1E hematology system. Veterinary Clinical Pathology **25**, 1, 14-22.

Tycko,D.H., Metz,M.H., Epstein,E.A. (1985)
Flow-cytometric light scattering measurement of red blood cell volume and haemoglobin concentration. Applied Optics **24**, 1355-1365.

Weiser,M.G. (1983)
Comparison of two automated multi-channel blood cell counting systems for analysis of blood of common domestic animals. Veterinary Clinical Pathology **XII**, II, 25-32.

Weiser,M.G. (1987)
Modification and evaluation of a multichannel blood cell counting system for blood analysis in veterinary hematology. Journal of the American Veterinary and Medical Association, **190**, 4, 411-415.