American Journal of Animal and Veterinary Sciences 8 (4): 203-209, 2013
ISSN: 1557-4555

doi:10.3844/ajavssp.2013.203.209 Published Online 8 (4) 2013 (http://www.thescipub.com/ajavs.toc)

A Comparative Study of the Ovine Haemogram: Cell-Dyn 3500 Versus Manual Methods

[1]Labrini V. Athanasiou, [2]Constantinos G. Giannakopoulos, [3]Zoe S. Polizopoulou, [1]Eleni Katsogiannou and [4]Vassilis I. Kontos

[1]Department of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece
[2]Private Practitioner, Volos, Greece
[3]Diagnostic Laboratory, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
[4]Department of Veterinary Public Health, National School of Public Health, Athens, Greece

Received 2013-10-02, Revised 2013-10-10; Accepted 2013-10-23

ABSTRACT

Manual hematology techniques traditionally applied in farm animal medicine are time consuming and labor-intensive, especially when large numbers of samples have to be processed. As a result, several automated hematology instruments have been developed for use in these species. An automated hematology analyzer (Abbott Cell-Dyne 3500 system) was used in performing a complete blood cell count and differential counts of white blood cells in sheep blood samples. The system was compared with basic manual hematologic techniques. A linear regression was used to assess correlation between the two methods. Correlation coefficients (R2) were good for the hematocrit, the total white blood cell count, the neutrophils, the lymphocytes and the platelet count, while a poor correlation existed in monocytes, eosinophils and basophils. The automated and the manual technique were also compared in terms of sensitivity using the Sensitivity Ratio (SR). The automated analyzer was slightly more sensitive than the manual technique for all parameters tested except for monocytes, eosinophils and basophils were the difference was greater and the automated analyzer was 1.5, 2.5 and 2 times more reliable, respectively. The method bias was also calculated. It seems that the overall performance of the automated analyzer justifies its utilization in sheep blood analysis, although as for any analyzer used in any species, a stained blood film evaluation remains an indispensable technique to confirm the results being reported by the automated analyzer and provide additional information for the ovine haemogram.

Keywords: Sheep, Hematology, Evaluation, Analyzer

1. INTRODUCTION

The increasing demand for hematological tests in veterinary medicine during the last decades has lead to the development of automated haematology instruments for veterinary use. The widely used manual techniques are time-consuming and labor-intensive. The problem is even worse in the case of farm animals where diagnosis is based on the examination of many animals and therefore a larger number of samples had to be analyzed. A wide variety of instruments, initially developed for analysis of human samples, is currently available. Some of the hematology systems use light-scatter measurements of cells passing through a light source, impedance technology or flow cytometry to count and identify blood cells in veterinary samples (Weiser *et al*., 2007; Stockham and Scott, 2008).

The Abbott Cell-Dyn 3500 system is an automated hematology system which utilizes a combination of impedance and laser technologies to perform a complete blood cell count and differential counts of white cells (web site of Abbott Diagnostics). It is available with multiple preprogrammed settings for the commonest domestic species. Although comparative studies have been conducted between automated hematology systems and manual techniques using samples of most of the domestic species

Corresponding Author: Labrini V. Athanasiou, Department of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece

(Hennessy *et al*., 1998; Bienzle *et al*., 2000; Dawson *et al*., 2000; Papasouliotis *et al*., 2006; Roleff *et al*., 2007), such a comparison has not been made for ovine blood except for a comparison of blood samples from lambs collected into two types of vacutainer tubes, one containing EDTA and the other heparin (Vatn *et al*., 2000).

In countries like Greece where sheep production traditionally represents a large part of animal production minagric, 2008, 2009 the use of automated systems for blood analysis is important in diagnostic evaluation, food and drug safety assessment and research studies. The purpose of this study was to compare the results of the Abbott Cell-Dyn 3500 system in ovine haemogram with those of common manual techniques.

2. MATERIALS AND METHODS

2.1. Blood Samples

Blood samples of 35 clinically healthy sheep of both sexes and covering all age groups, were collected from the jugular vein into EDTA containing vacutainer tubes (Venoject, Terumo Europe, Leuven, Belgium). They were immediately mixed by gentle inverting for several seconds to avoid coagulation. Blood smears were prepared at once and kept dry at room temperature. Hematological tests were performed within 3 h and blood samples were mixed again before performing any tests to avoid erroneous data. The hematological tests were performed by both the Abbott Cell-Dyn 3500 system and a manual technique and the hematological parameters determined were: Hematocrit (HCT), Total White-Blood- Cell Count (TWBC) count, Platelet (PLT) count and White-Blood-Cell Differential (WBCD) count.

2.2. The Abbott Cell-Dyn 3500 System

The Abbott Cell-Dyn 3500 system is an automated hematology system that analyses blood cells using a combination of impedance and laser technologies. Electronic impedance is used to determine Red- Blood-Cell (RBC), TWBC and PLT counts. As cells are passed through a laser beam, light scatter measurements are taken at three different angles. In addition, a portion of the light beam is depolarized and light scatter is measured as cells pass through this beam. This information is used to count WBCs and classify the subpopulations. Eosinophils can be distinguished from other leukocytes by the tendency of their unique granules to scatter polarized light. Because of the water soluble nature of basophil granules, these cells fall with the mononuclear cells. Dynamic thresholds are used to determine the best separations between impedance and optical WBC counts can be caused by Nucleated RBCs, lyse-resistant RBCs or fragile WBCs. The system analyzes the data to determine which count is more appropriate (Weiser *et al*., 2007; Stockham and Scott, 2008).

2.3. The Manual Technique

A Wintrobe macrohematocrit system was employed for the determination of the hematocrit while the WBC and PLT counts were determined by the Unopette (Becton Dickinson Vacutainer Systems, Becton Dickinson end Co., Franklin Lakes, NJ, USA) microcollection system (Thrall and Weiser, 1997; Lassen and Weiser, 2004). Blood smears stained by Diff-Quick (Harleco) were used for the determination of the WBCD count (Thrall and Weiser, 1997; Lassen and Weiser, 2004). Blood smears were subjected to a triplicate observation by three experienced hematologists each reading a 400 leukocytes and the average of all these observations was used for comparison with the results of the Abbott Cell-Dyn 3500 system.

2.4. Statistical Analysis

Data for hematocrit, TWBC count, PLT count and WBCD count were subjected to a linear regression analysis (Petrie and Watson, 2006). Any bias between the two methods was evaluated for each sample and the result reported as the percentage difference with respect to the manual method. The data were also used to calculate Sensitivity Ratio (SR) (Davies and Fisher, 1991). This ratio takes account of the slope of the linear regression and the analytical error or variability associated with each method. When the SR is close to 1 the both methods are equally sensitive. A value greater than 1 indicates the Manual method to be more sensitive whereas a value of less than 1 indicates the Abbott Cell-Dyn 3500 system is the more sensitive method (Petrie and Watson, 2006). Furthermore, results were compared using Bland-Altman difference plot (Bland and Altman, 1986).

3. RESULTS

3.1. Regression Analysis

Regarding haematocrit a high correlation coefficient (R2 = 0.941) exists between the Abbott Cell-Dyn 3500 system analysis and the manual method. The same holds true for the total WBC count where R2 is 0.962 (**Table 1 and Fig. 1**). With respect to the WBCD count, a good correlation exists between neutrophils and lymphocytes (0.947 and 0.901 respectively) for the observed values between a single Abbott Cell-Dyn 3500 system determination and the average of three 400 cell manual differential counts (**Table 1 and Fig. 2**).

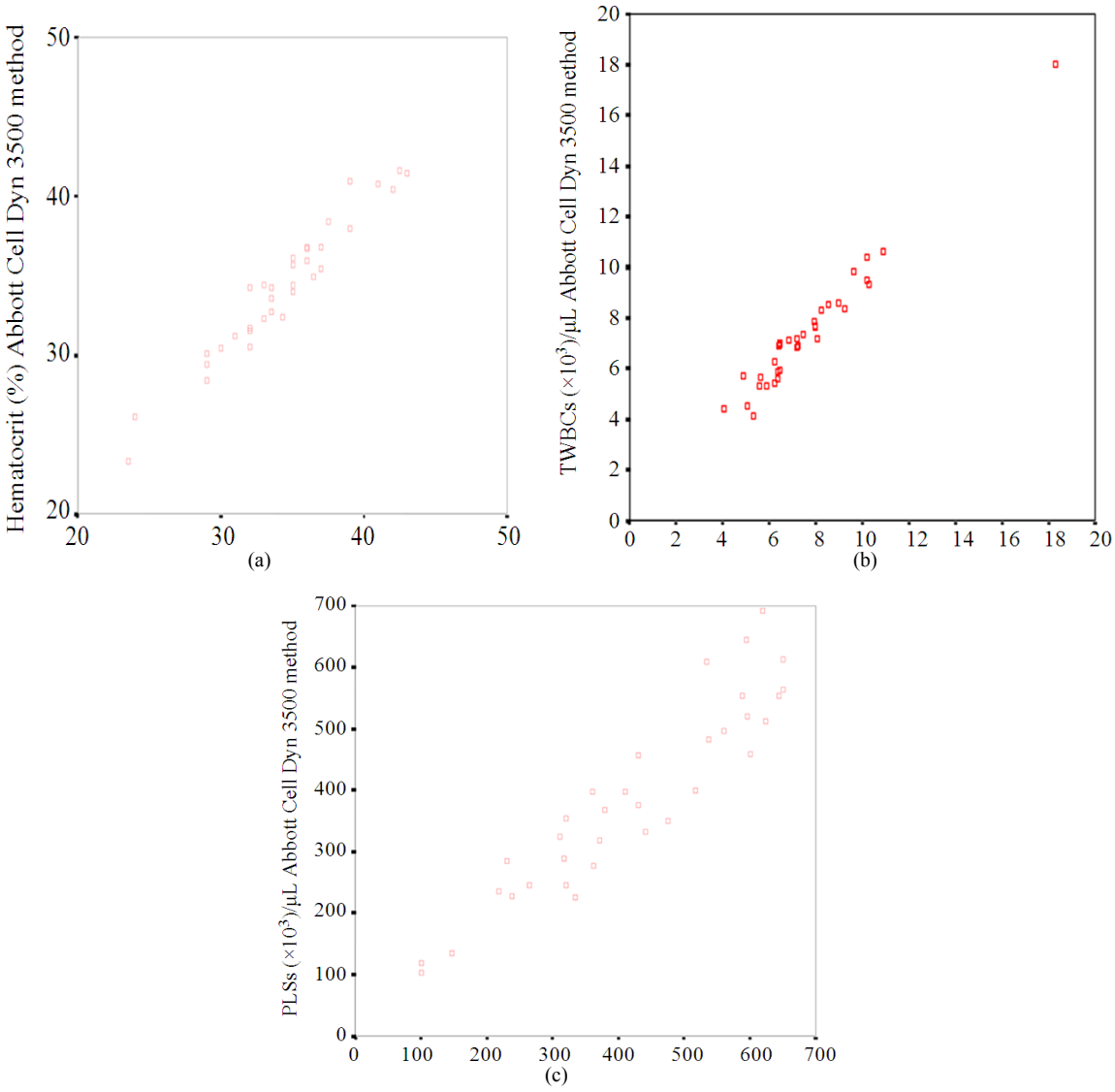

Fig. 1. Linear regression plot together with the correlation coefficient (R2) and equation of the line for the HCT, WBCs and PLTs (a) Hematoctir (%) manual method (b) TWBCs ($\times 10^3$)/μL manual method (c) PLTs ($\times 10^3$)/μL manual method

However, in case of monocytes, eosinophils and basophils there is a poor correlation (0.509, 0.166 and 0.408 respectively) between the two methods (**Table 1 and Fig. 2**). An acceptable correlation of R2 = 0.864 was observed for platelet count indicative of a good compatibility of the two methods (**Table 1 and Fig. 1**).

3.2. Sensitivity Ratio (SR) Analysis

The results of the SR analysis are presented in **Table 2**. Data indicate that Abbott Cell-Dyn 3500 system method is slightly more sensitive than the manual one for haematocrit and TWBC count (0.981 and 0.969 respectively). Regarding platelets count the Abbott Cell-Dyn 3500 system method was more sensitive than the manual one demonstrating a SR equal to 0.929.

As far as the WBCD count results are concerned, neutrophils and lymphocytes counts with Abbott Cell-Dyn 3500 system were slightly more sensitive than the manual differential count. The same was not demonstrated for the monocytes, eosinophils and basophils counts where Abbott Cell-Dyn 3500 system results were 1.5, 2.5 and 2 times more reliable, respectively, than the manual obtained ones.

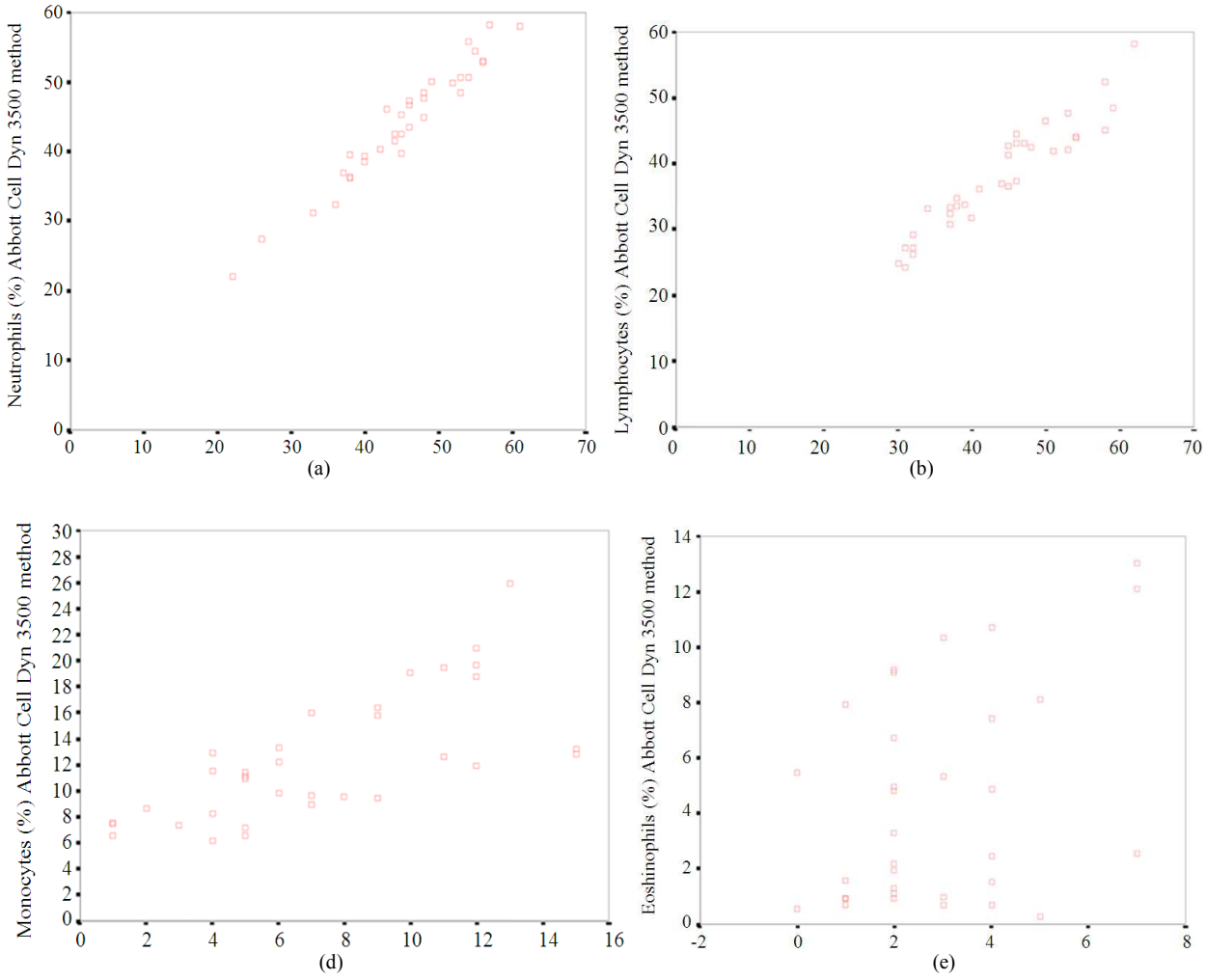

Fig. 2. Linear regression plot together with the correlation coefficient (R2) and equation of the line for the neutrophils, lymphocytes, eosinophils and monocytes (a) Nuetrophils (%) manual method (b) Lumphocytes (%) manual method (c) Monocytes (%) manual method (d) Eoshinophils (%) manual method

Table 1. Correlation coefficients for hematology parameters evaluated by the manual method and the Abbot-Cell Dyn 3500 analyzer

Hematology parameter	Correlation coefficient (R^2)
Hematocrit	0.941
Total white-blood-cell count	0.962
Platelet count	0.864
Neutrophils	0.947
Lymphocytes	0.901
Monocytes	0.509
Eosinophils	0.166
Basophils	0.408

Table 2. Sensitivity Ratio analysis for hematology parameters and white blood cell differential counts evaluated by the manual method and the Abbot-Cell Dyn 3500 analyzer

Hematology parameter	Sensitivity Ratio (SR)
Hematocrit	0.981
Total white-blood-cell count	0.969
Platelet count	0.929
Neutrophils	0.973
Lymphocytes	0.948
Monocytes	0.713
Eosinophils	0.400
Basophils	0.505

Table 3. Comparison between the manual method and the Abbot Cell-Dyn 3500 analyzer obtained results. The mean difference reflects the bias and is expressed as a percentage of the manual method

Hematology parameter	Abbot Cell-Dyn 3500 analyzer	Manual	Difference	Percentage BIAS(%)
Hematocrit	34.25	34.32	-0.07	-0.20
Total white-blood-cell count	7.41	7.63	-0.22	-2.88
Platelet count	387.12	420.18	-33.06	-7.87
Neutrophils	44.05	45.23	-1.18	-2.60
Lymphocytes	38.16	43.91	-5.75	-13.09
Monocytes	12.33	7.21	5.12	71.01
Eosinophils	4.28	2.79	1.49	53.41
Basophils	1.17	0.82	0.35	42.43

Table 4. Mean differences between the manual method and the Abbot Cell-Dyn 3500 system obtained results and limits of agreement

			Limits of agreement	
Hematology parameter	Mean difference (đ)	2sd	đ + 2sd	đ - 2sd
Hematocrit (Hct)	-0.0640	2.2480	2.1820	-2.3120
White blood cell counts	-0.2256	0.9712	0.7456	-1.1968
White Blood Cell Differential Count (WBCDC)				
Neutrophils	-1.1850	4.0272	2.8422	-5.2122
Lymphocytes	-5.7494	5.8458	0.0964	-11.5952
Monocytes	5.0962	6.9314	12.0276	-1.8352
Eosinophils	-2.9518	9.7784	6.8266	-12.7302
Basophils	0.3494	1.8282	2.1776	-1.4788
Platelet count (Plt)	-33.0590	121.3300	88.2710	-154.3890

3.3. Method Bias

The mean difference between the results of the Abbott Cell-Dyn 3500 system and the manual method reflects the bias of the Abbott Cell-Dyn 3500 system method and is expressed as a percentage of the manual method (**Table 3**).

3.4. Difference Plot Analysis

The results of the difference plot analysis are presented in **Table 4**. Regarding hematocrit, WBC count and neutrophils an acceptable aggrement of the two methods was observed. On the contrary in case of lymphocytes, monocytes, eosinophils, basophils and platelet count there can be considerable discrepancies between the two methods.

4. DISCUSSION

As instruments become easier to operate and results are available within few seconds, hematology analyzers are more and more used in every day veterinary practice. However, it is essential to compare their results to those obtained by commonly used manual methods.

The results of the present study indicate a good correlation between the hematocrit, the WBC and the platelets counts calculated by the Abbott Cell-Cyn 3500 system and those obtained by the manual method. This could be attributed to the large population of these cells as well as to the combination of both impedance and laser technology which permits a more accurate differentiation among cells. A common observation during the Abbott Cell-Dyn 3500 system evaluation was that the manual method for assessing the WBCD count gave a higher value for the most common cell type i.e. the lymphocytes and neutrophils which are the dominant populations in sheep blood (Byers and Kramer, 2011).

Regarding method comparison, use of appropriate statistical analysis has been the topic of many publications in scientific journals (Westgard, 1998; Jensen and Kjelgaard-Hansen, 2006) and a relevant guideline has been issued by the American Society of Veterinary Clinical Pathology (ASVCP Quality Control Guidelines). The difference plot analysis was used in the present study due to the absence of a reference method. The difference plot allows the identification of any possible relationship between the measurement error and the true value which is unknown and therefore the mean of

the two measurements is the best possible estimate. The mean of the difference (đ) is an estimate of the average bias of one method relative to the other. In orde to assess how well the measurements agree, limits of agreement are determined within which most of the differences lie. However, the decision whether the limits of agreement are acceptable or not is based on clinical judgement of the particular problem (Petrie and Watson, 2006).

As far as agreement between the two methods in the present study is concerned the statistical analysis revealed an acceptable agreement in hematocrit, WBC cound and neutrophils (**Table 4**). With respect to the platelet count, although not strong an acceptable correlation still exists while the agreement between the two methods is not acceptable. The SR shows that the Abbott Cell Dyn is more sensitive in counting platelets than the manual method. This could be attributable to the fact that the number of platelets observed on the 25 primary squares of the hematocytometer is usually large, cells are small and concequenlty some of them could have been left out. Moreover, the number of platelets observed is multiplied by 1000 which multiplies at the same time the error considerably.

Regarding the monocytes, eosinophils and basophils in normal blood, the percentage bias between the two methods are large. This may be due to the fact that the Abbott Cell-Dyn 3500 system evaluates thousands of leukocytes as opposed to the manual method. Even though in the present study the differential count was determined by assessing 400 cells by three different observers the number of cells is still limited in comparison with the assessed by the automated system. Therefore, the automated system derived results are considered to be more reliable. This explanation could stand for basophils and eosinophils which are rarely observed by the manual method.

A thorough examination of the results revealed that the difference in the percentage of monocytes by the two methods is nearly the same in absolute values with that of lymphocytes (5.75 Vs 5.12). It looks like some of the lymphocytes are mistaken by the Abbott Cell-Dyn 3500 system as monocytes. Moreover, the bias percentage is greater in monocytes than in lymphocytes (only 13.09% for lymphocytes and 71.01% for monocytes) because the lower incidence of the monocytes in normal blood tends to exaggerate any method difference when expressed as a percentage.

Potential limitations of this study include the imprecision that has been reported for manual WBC and platelet enumeration and the evaluation of healthy animals only. Similar comparative studies involving clinical cases with various hematologic abnormalities could be of value.

5. CONCLUSION

The Abbott Cell-Dyn 3500 system could be considered as a reliable alternative to the manual technique for most of the hematological parameters and could facilitate the diagnosis of hematological abnormalities in sheep. However, a stained blood film remains essential to confirm the numbers being reported by the hematology analyzer acting a quality control test, while it will also provide additional valuable information such as morphology of erythrocytes and leukocytes, detection of intracellular inclusions, hemoparasites and other organisms.

6. REFERENCES

Bienzle, D., J.B. Stanton, J.M. Embry, S.E. Bush and E.A. Mahaffey, 2000. Evaluation of an in-house centrifugal hematology analyzer for use in veterinary practice. JAVMA, 217: 1195-1200. DOI: 10.2460/javma.2000.217.1195

Bland, J.M. and D.G. Altman, 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet, 1: 307-310. DOI: 10.1016/S0140-6736(86)90837-8

Byers, S.R. and J.W. Kramer, 2011. Normal Hematology of Sheep and Goats. In: Schalm's Veterinary Hematology, Weiss, D.J. and K.J. Wardrop (Eds.), John Wiley and Sons, ISBN-10: 047096183X, pp: 836-842.

Davies, D.T. and G.V. Fisher, 1991. The validation and application of the technicon H1 for the complete automated evaluation of laboratory animal haematology. Comp. Hematol. Int., 1: 91-105. DOI: 10.1007/BF00422879

Dawson, H., B. Hoff, E. Grift, H. Tvedten and M. Shoukri, 2000. Validation of the coulter AcT diff hematology analyzer for analysis of blood of common domestic animals. Vet. Clin. Path., 29: 132-136. DOI: 10.1111/j.1939-165X.2000.tb00243.x

Hennessy, M., T.C. Buckley, D.P. Leadon and C.S. Scott, 1998. Automated analysis of blood samples from thoroughbred horses with the Abbott Cell DYN 3500 (CD3500) haematology analyser. Comp. Hematol. Int., 8: 150-158. DOI: 10.1007/BF02642506

Jensen, A.L. and M. Kjelgaard-Hensen, 2006. Method comparison in the clinical laboratory. Vet. Clin. Path., 35: 276-286. DOI: 10.1111/j.1939-165X.2006.tb00131.x

Lassen, E.D. and G. Weiser, 2004. Laboratory Technology for Veterinary Medicine. In: Veterinary Hematology and Clinical Chemistry, Troy D.B., (Ed.), Lippincott Williams and Wilkins, Baltimore, ISBN-10: 0683304151, pp: 3-38.

Papasouliotis, K., S. Cue, E. Crawford, M. Pinches and M. Dumont, 2006. Comparison of white blood cell differential percentages determined by the in-house LaserCyte hematology analyzer and a manual method. Vet. Clin. Path., 35: 295-302. DOI: 10.1111/j.1939-165X.2006.tb00134.x

Petrie, A. and P. Watson, 2006. Statistics for Veterinary and Animal Science. 2nd Edn., Wiley, Ames, ISBN-10: 1405127813, pp: 312.

Roleff, S., G. Amdt, B. Bottema, L. Junker and A. Grabner *et al*., 2007. Clinical evaluation of the CA530-VET hematology analyzer for use in veterinary practice. Vet. Clin. Path., 36: 155-166. DOI: 10.1111/j.1939-165X.2007.tb00202.x

Stockham, S.L. and M.A. Scott, 2008. Fundamentals of Veterinary Clinical Pathology. 2nd Edn., Blackwell, Ames, ISBN-10: 0813800765, pp: 936.

Thrall, M.A. and M.G. Weiser, 1997. Hematology. In: Laboratory Procedures for Veterinary Technicians, Pratt, P.W. (Ed.), Mosby, St. Louis, MO., ISBN-10: 0815173261, pp: 35-98.

Vatn, S., T. Framstad and W.O. Torsteinbo, 2000. Hematologic evaluation of normal and anemic lambs with the Technicon H*1 Using EDTA or Heparin as anticoagulants. Vet. Clin. Path., 29: 62-68. DOI: 10.1111/j.1939-165X.2000.tb00401.x

Weiser, M.G., L.M. Vap and M.A. Thrall, 2007. Clinical pathology and diagnostic techniques. Vet. Clin. North. Am., 37: 221-236. DOI: 10.1016/j.cvsm.2006.11.005

Westgard, J.O., 1998. Points of care in using statistics in method comparison studies. Clin. Chem., 44: 2240-2242. PMID: 9799748