Discrepancy Between Hemocytometer and Electronic Counts of Blood Cells

Author: Whrttington, R. J.

Source: Journal of Wildlife Diseases, 20(3) : 258-260

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-20.3.258

Journal of Wildlife Diseases, 20(3), 1984, pp. 258–260

LETTER TO THE EDITOR . . .

Discrepancy Between Hemocytometer and Electronic Counts of Blood Cells

R. J. Whittington,[1] Crown Street Veterinary Hospital, 424 Crown Street, Wollongong, New South Wales 2500, Australia; **and D. A. M. Comer,** Department of Haematology, Wollongong Hospital, Wollongong, New South Wales 2500, Australia

Hematological investigations of non-domestic animals are becoming increasingly common; however, standard hematological methods may not always be appropriate. During a recent study of the hematology of platypus (*Ornithorhynchus anatinus* (Shaw)) (Whittington and Grant, 1983, Aust. J. Zool. 31: 475–482) it was noticed that electronic counts of leukocytes were lower than those obtained by several manual methods. This was investigated further.

Blood collected from the bill sinus of 10 wild-caught platypuses was added to tubes containing EDTA. After thorough gentle mixing each sample was divided into three aliquots for analysis by a Model S Plus Phase II Coulter Counter (Coulter Electronics, Hialeah, Florida 33010, USA), a Royco Cellcrit Model 921 (Royco Instruments Inc., Menlo Park, California 94025, USA) and manually using the Unopette Method (Becton-Dickinson, Rutherford, New Jersey 07451, USA). Handling and storage of each sample prior to analysis was identical. Results obtained by the three methods were compared using the one-way analysis of variance and Scheffé test (Bailey, 1959, Statistical Methods in Biology, English Univ. Press, London, 200 pp.; Roscoe, 1975, Fundamental Research Statistics for the Behavioral Sciences, 2nd Ed., Holt, Rinehart and Winston, Inc., New York, 483 pp.). The results are shown in Table 1.

Received for publication 23 August 1983.

[1] Present address: Regional Veterinary Laboratory, New South Wales Department of Agriculture, Wollongbar, New South Wales 2480, Australia.

Although the Royco Cellcrit gave results similar to the manual method a large discrepancy (mean 39%) was observed between results from the Coulter Counter and the manual method. This discrepancy was likely to have been a result of the calibration of the Coulter Counter, which is preset to an optimum level for human hematology. The Coulter Counter S Plus classifies a leukocyte as a particle (actually leukocyte nucleus) larger than 45 fl remaining in suspension after the erythrocytes have been lysed (Anon., 1981, Coulter Counter S Plus Operators Reference Manual, Coulter Electronics, Hialeah, Florida). A visual display unit graph of leukocyte size distribution showed that the leukocyte population contained a significant proportion of cell nuclei in suspension that were below this threshold size. The calibration of the Royco Cellcrit was adjusted to suit platypus blood, resulting in leukocyte counts similar to those obtained by the manual method.

The platypus is an unusual mammal and has unusual hematological values, however discrepancies between manual and electronic blood cell counts occur in conventional species. Electronic particle counters give unreliable results for the leukocyte count in domestic cats due to the counting of clumped platelets as leukocytes (Schalm et al., 1975, Veterinary Hematology, 3rd Ed., Lea and Febiger, Philadelphia, 807 pp.; Weiser, 1981, Vet. Clin. North Am. Small Anim. Pract. 11: 189–208). Leukocyte counts should be determined by hemocytometer in this species. Similarly, discrepancies of between 8% and 48% were observed in leu-

TABLE 1. Discrepancy between methods of leukocyte counting in 10 platypuses. Values are mean ± standard deviation.

Method	Leucocyte count ($\times 10^9$/liter)	Analysis of variance and Scheffé test
Manual Unopette	36.30 ± 13.42	$P < 0.01$
Coulter Counter S Plus	22.23 ± 7.60	$P < 0.01$
Royco Cellcrit 921	36.81 ± 10.17	

kocyte counts determined by Coulter Counter and hemocytometer in neutrophilic dogs. These large discrepancies were due to leukocytes lysing to different degrees in the two diluting solutions used (Faulkner et al., 1982, Vet. Rec. 110: 202). Preleukemic and leukemic bovine lymphocytes may be lysed by saponin resulting in erroneously low leukocyte counts determined electronically (Schalm et al., 1975, op. cit.). These authors recommend the checking by hemocytometer of all bovine leukocyte counts exceeding 12,000/μl. In contrast, in healthy cattle, Halliday et al. (1979, Med. Lab. Sci. 36: 353–358) found that the standard Coulter method calibrated for cattle gave markedly higher leukocyte counts than the hemocytometer method and recommended an alternative method of sample preparation for Coulter counting.

Manufacturers of electronic particle counters are aware of the limitations of their machines. Coulter Electronics states that increased leukocyte fragility in certain diseases, cell size lower than the preset lower threshold, erythrocytes resistant to the lysing agent, nucleated erythrocytes and carryover of samples may result in erroneous leukocyte counts. Similar limitations are discussed in relation to the other electronically measured blood parameters (Anon., 1981, op. cit.). Schalm et al. (1975, op. cit.) also discuss factors that result in erroneous leukocyte and erythrocyte parameters determined electronically.

It is clear that electronic particle counters have limitations and that the extent to which these limitations apply to non-human species is largely unknown. Electronic particle counters are designed primarily for use with human blood and need to be individually calibrated for use in other species. Failure to do so constitutes inappropriate use of technology. This report is not intended to criticize or promote any particular electronic particle counter, however it illustrates that instruments with adjustable thresholds are preferable in veterinary hematology.

Although veterinary hematologists may be aware of the material discussed here we believe that many researchers who use or produce hematological data are not familiar with the problem.

Hematological studies on non-human species should commence with blood evaluation by manual methods, in order to verify results coming from electronic methods that can then be used to advantage. Reports of hematological reference values that were determined by particle counter should state whether the electronic counter was calibrated to give agreement with manual cell counts in that species. Veterinarians wishing to interpret hematological results from individual animals in the light of published reference values should ensure that they have used identical methods of blood analysis, or methods known to yield similar results in that species.

We would like to thank Tom Grant, Bi-

ology Department, Wollongong University, for capture of platypuses; Christine Medhurst, Pathology Department, Sydney University, for processing blood samples; and Paul Canfield, Pathology Department, Sydney University, for his valuable comments. Daphne Tulk typed the manuscript.