Automated Analysis of Salmonid Blood Cells

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Standardization of laboratory procedures for handling and processing Atlantic salmon *Salmo salar* blood samples were evaluated for: *blood cell counting* (Coulter Models T660, S-PLUS IV, ZBI, ZM Channel analyzer) and *hemoglobin determination* (Coulter Models T660, S-PLUS IV, Spectrophotometer, Hemoglobinometer). Correlation coefficients indicated no significant difference between automated versus manual cell counts. The ZBI was chosen as the instrument of choice for cell enumeration. Automated hemoglobin measurements were higher than results of the manual method. The hemoglobinometer was chosen for determining hemoglobin concentration.

Introduction

There are various diagnostic methods (both manual and automated) available to determine hematological profiles for mammalian species. When available, electronic counting systems (Coulter models) provide faster and more reliable results in determining hematological profiles than visual methods. Hemocytometer counts are routinely used for enumerating fish cells.[2] The cyanmethaemoglobin assay[4] is regarded as the method of choice for determining hemoglobin concentration for finfish. The objectives of this experiment were: (1) to evaluate the reliability of 4 automated blood cell counters/sizers for enumerating fish cells and, (2) to compare two automated methods against a manual assay and a spectrophotometrically determined standard curve, in order to identify the most reliable and efficient means of calculating hemoglobin levels for salmonid blood.

Methods

Fifteen Atlantic salmon were hand netted and anesthetized using tricaine methanesulfonate (MS 222) at a concentration of 100 mg/L. Blood was collected via the dorsal aorta with samples being mixed thoroughly by hand and placed on ice. For enumeration, blood samples were diluted 1:1000 in cold Cortland saline without glucose.[3] Before analysis, blood samples were further diluted 1:50,000 in counting diluent. Triplicate counts of each of the 1:50,000 dilutions were made with the final result expressed as a mean of nine. For hemoglobin determination blood samples were diluted 1:500 in lytic agent (Lyse II), incubated for 10 minutes at 37°C and spun for 5 minutes (1890*g) to remove cellular debris. Hemoglobin concentrations were read from the resultant supernatant directly on the hemoglobinometer. Resultant values were expressed as a mean of three.

Table 1. Correlation coefficients comparing automated cell counters for the enumeration of salmonid blood cells.

Coulter Models	By Hand	Coulter models		
		ZBI	C256	T660
ZBI	0.832	–	–	–
C256	0.836	0.977	–	–
T660	0.824	0.980	0.983	–
S-Plus-IV	0.823	0.982	0.981	0.998

Table 2. Comparison of hemoglobin values determined by hemoglobinometer, spectrophotometer and Coulter models T660 and S-Plus IV.

Fish	Coulter Models T660	Coulter Models S-PLUS IV	Hemoglobinometer	Spectrophotometer
1	12.5	12.5	7.8	7.75
2	12.2	12.5	7.2	8.10
3	12.3	12.5	7.2	7.8
4	18.4	18.2	10.8	15.8
5	18.1	18.2	10.8	15.8
6	18.3	18.2	10.9	10.8
7	18.2	17.0	10.2	9.8
9	17.0	17.0	9.7	9.6
10	18.0	16.8	9.2	10.7
11	15.8	15.7	9.6	9.5
12	15.3	15.6	9.4	10.3
13	15.7	15.7	9.6	9.8
14	20.4	20.3	12.3	11.8
15	20.5	20.1	12.9	12.7

Results and Discussion

Table 1 shows the correlation coefficients for equipment examined. The GLM (general linear model) indicated no significant differences between manual and automated procedures in the enumeration of fish cells. The ZBI was chosen for enumerating fish cells because it yielded an undifferentiated cell count. It allowed for upper and lower thresholds to be manipulated to ensure that all cells were counted. The T660 and the S-PLUS IV consistently yielded higher hemoglobin concentrations (g/dL) than the hemoglobinometer or the spectrophotometer. Hemoglobin concentrations are presented in Table 2. The hemoglobinometer was chosen for determining hemoglobin concentration.

Fish erythrocytes are easier to count than mammalian cells because they are larger and fewer in number.[2] With the aid of a hemocytometer both erythrocytes and leucocytes can be enumerated simultaneously. Coulter models such as the T660 and the S-PLUS IV determine differential cell populations for mammalian species based on the fact that only leukocytes are nucleated and cell populations fall within specified size ranges. Automated procedures are not commonly used for finfish due to the fact that all cells are nucleated and cell populations overlap.

Nuclear debris within the hemoglobin sample accounted for the falsely elevated hemoglobin readings. Automated methods can only be used to determine hemoglobin concentration for finfish if nuclear debris is removed by centrifugation before analysis.

Automated methods can provide rapid and reliable means of assessing finfish blood if samples are processed in the correct manner. Blood being the most accessible element in the teleost system can provide a means of nonlethally monitoring fish health status.

Notes and References

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