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Automated method and not automated: the evaluation of hematological parameters of rats*

Método automatizado e não automatizado: na avaliação dos parâmetros hematológicos de ratos

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Resumo

O objetivo deste trabalho foi verificar em sangue de rato a compatibilidade entre a metodologia automatizada e a não automatizada. Foram realizadas contagens das variáveis hematológicas de 10 ratos Wistar, *Rattus norvegicus*. Para o método automatizado foram utilizados os aparelhos ABX-Pentra-60c+ e Celldyn-3200. Para o método não automatizado foi utilizado o hemocitômetro nas contagens de eritrócitos, de leucócitos e de plaquetas, utilizando o líquido de Hayem (1:200), o líquido de Türk (1:20) e o líquido de Brecher e Cronkite (1:200), respectivamente. A determinação do hematócrito foi obtida utilizando-se uma centrífuga de micro-hematócrito, a concentração de hemoglobina pelo método de cianometa-hemoglobina (líquido de Drabkin) e os índices hematimétricos absolutos calculados através dos resultados da série vermelha. A contagem diferencial de leucócitos foi realizada em extensões de sangue (panótico rápido). Os dados foram analisados através da variância – ANOVA, seguida pelo teste Tukey, com nível de significância de 5%. Os valores obtidos pelo hemocitômetro comparadas aos contadores hematológicos mostraram diferenças significativas ($p < 0,05$) para neutrófilos, eosinófilos e basófilos. Os resultados permitiram concluir que as contagens realizadas com os aparelhos utilizados foram confiáveis e rápidas, mas não são indicadas para a contagem diferencial de leucócito.

Palavras-chave: automação, hematologia, hemograma, sangue.

Abstract

The aim of this study was to verify blood compatibility between automated and non-automated methodology in rats. Counts of haematological variables of 10 Wistar rat, *Rattus norvegicus* were made. For the automated method were used the ABX-Pentra-60c + equipment and Celldyn-3200. For non-automated method the hemocytometer was used to count the erythrocytes, leukocytes and platelets using the Hayem liquid (1: 200), the Türk liquid (1:20) and the Brecher and Cronkite liquid (1: 200), respectively. The determination of the hematocrit was obtained by using a microhematocrit centrifuge, hemoglobin concentration by cyanmethaemoglobin method (Drabkin liquid) and absolute RBC indices calculated by the results of red series. The differential leukocyte count was performed on blood extensions (fast Panotic). The data was analyzed through variance – ANOVA and then by Tukey test at 5% significance level. The values obtained by hemocytometer compared to hematological counters revealed significant differences ($p < 0.05$) for neutrophils, eosinophils and basophils. Through the results obtained, it was concluded that the counts made with the devices used were reliable and fast, but are not indicated for the differential count of white blood cell.

Keywords: automation, hematology, blood cell count, blood.

Introduction

The hemogram is made up by total erythrocyte count, total leukocyte count and platelet count and can be performed using non-automated methods (manual method) or automated method (hematology analyzers) with extensive variation associated with computers. Several scientific papers published confront the two methodologies and reveal that there are no statistically significant differences. Thus, the gradual replacement by the use of automated equipment is a tendency, as the cell counters have a high level of accuracy, depending on the equipment (Messias et al., 2009; Soares et al., 2012).

The most used counters in automatic hematology in animal routine are systems for impedance (electrical) and laser (flow cytometry). The impedance analyzers dissolve blood cells in an electrical conductor, which allows passage for cells through a small gap between two electrodes, the change in electrical impedance produced is proportional to cell size as it passes through the opening. While the count performed by the laser system measures the bending of light at two angles as the cells pass through the beam, being capable to obtain accurate counts using specific antibodies and color indicators, it is important to consider the variation between different methodologies (Silva et al., 2007).

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Nowadays it is noticeable that the use of automated hematology analyzers to determine the hematologic profile in experimental animals has grown, providing reduction of manual labor and increasing the accuracy of the count (Ebling et al., 2014). Many articles available in several bases of research (Gonçalves et al., 2007; Lima et al., 2008; Duarte et al. 2009; Messias et al., 2010; Melo et al., 2012; Taiwo Idowu et al., 2013; Achuba; Ogwumu, 2014; Lima et al., 2014; Mulata et al., 2015) show that it is still common the use counters to determine the blood cell count in animals, however, many of them correspond to standard equipment for human blood and not for laboratory animals. In this context, rodents are the most used animals for scientific studies in several areas as they have similar genetic and physiological characteristics to humans (Harkness; Wagner, 1993; Cubas et al., 2007, Melo et al., 2012; Lima et al., 2014). The aim of this work was to check if the automatic hematology equipment used for human beings could also analyze rat blood (*Rattus norvegicus*, Wistar).

Material and methods

This work was developed in the Laboratory of Histological Techniques and in the Biochemistry Laboratory of the Institute of Biological Sciences - University of Pernambuco (UPE / ICB), located in the Universitary Campus Santo Amaro.

Ten (10) wistar rats, *Rattus norvegicus* var. albinus, of both sexes were randomly selected, weighing 170 ± 20g, from the vivarium of the Department of Antibiotics, Federal University of Pernambuco (UFPE) and housed in the Experimental Surgery Center (NUCEX- UPE / ICB). The animals were kept in appropriate boxes and fed with water and diet ad libitum (Labina®) under controlled conditions of light (12h cycle light / dark) and temperature (23 ± 2 ° C). This study was approved by the Ethics Committee on Animal Use (CEUA), University of Pernambuco - UPE (nº003 Protocol / 09).

To perform the blood collection rats with 12 hours of fasting were anesthetized according to Messias et al. (2009; 2010), with ketamine 85mg/kg (Vetbrands®) and xylazine 3mg / ml (Vetbrands®), and immobilized. Afterwards, 1 ml of blood was collected by cardiac puncture. Subsequently, the smear was performed for subsequent reading of the specific leucometry. The blood was conditioned in tubes (13 x 100 mm) containing anticoagulant in the ratio of 1.8 mg EDTA to 1 ml of blood, aiming to finish hematological parameters, stored in thermal boxes for 60 minutes.

For the red blood cell count, a dilution 1/200 was made with Hayem liquid of blood (Newprov®). Subsequently, dilution was placed in a hemocytometer (Neubauer chamber), counting 5 of 25 quarters (4 ends and 1 central). The count was made in light microscope (Olympus CX31) with 40x objective and the result was multiplied by 10,000, representing the number of red blood cells per cubic millimeter (mm3) of blood.

The hematocrit was obtained using a microhematocrit tube filled with blood in about ¾ of its capacity and closed at one end. The capillary was placed in microfuge (Fanem IEC® 210) for five minutes at 12000 rpm, reading done at the specific card (Mello, 2005).

The hemoglobin determination was done spectrophotometrically (Coleman®) at 540nm cianetohemoglobina by employing the liquid Drabkin (Doles®) and the results were expressed in g/dL. The hematimetric indexes were calculated using the results determined in the red series (erythrocytes, hemoglobin and hematocrit) according to Mello (2005). The values were expressed in femtolitre (medium corpuscular volume - MCV), in picogram (mean corpuscular hemoglobin - MCH) and in g/dL (medium corpuscular hemoglobin concentration - MHCM).

The white blood cell count was performed using the hemocytometer, Türk liquid (Newprov®) was applied as a diluent for the count of white blood cells at a 1:20 dilution (400µL of diluent and 20µL of sample solution).

The differential counting of leukocytes was performed by means of a stained blood extension using fast panotic (NewProv®) and examined by optical microscope at 100x objective using immersion oil, counting 100 cells per slide, which were classified according to their morphology.

The Brecher and Cronkite method (direct method) was used at a 1: 200 light microscopy (4mL Brecher liquid diluent and 20µL of the sample). The final solution was homogenized by inverting tubes and filling the Neubauer chamber with the aid of a pipette. The platelet count was performed in 400x magnification optical microscope (Olympus CX31) by counting the cells in the twenty-five central squares of the two sides of the chamber, similar to the method used for the erythrocyte count and multiplying them by 1.000.

For automated determination were used the hematological analyzers Cell- Dyn 3200 (Abbot®) and ABX Pentra 60 C + (Horiba®) in accordance to the manufacturer's recommendations, in a municipal clinical pathology laboratory of Recife - PE. With these equipment it was possible to obtain total count of red blood cells, hemoglobin, hematocrit, hematological indexes (MCV, MCH and MHCM), total leukocyte count, relative and absolute count of leukocytes (neutrophils, eosinophils, basophils, lymphocytes and monocytes) and platelet count.

The data was expressed as mean ± mean standard error (MSE). Statistical significance between the groups were tested using one-way ANOVA and then by Tukey Test, a p less than 0.5 would be considered significant.

Results

By the hemogram was obtained average values for 10 samples of the erythrocyte series, leukocytes and platelets, accompanied by the corresponding standard error of the mean (S.E.M.). Table 1 is a summary of the means and standard deviations of the erythrocyte. It was observed that there are no significant differences between hematological variables and the red series.

In Table 2 are the mean and standard deviations of total leucocyte count and differential count of leukocytes. The values obtained by hemocytometer compared to Cell-dyn and Pentra apparatus revealed significant differences ($p < 0.05$) for granulocytes (neutrophils, eosinophils and basophils).

In Table 3 are the means and standard deviations of platelets observed. None of the used methods represented significant difference ($p < 0.05$).

Table 1: Erythrogram evaluation through hematologic counters Celldyn 3200 and Pentra 60C + compared to the hemocytometer and other non-automated methods in adult Wistar rats (*Rattus norvegicus*)

Counters	Erythrocyte (x10^6 mm3)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fL)	MCH (Pg)	MHCM (g/dL)
Cell-dyn	6,93 ± 0,361^a	13,45 ± 0,478^b	39,32 ± 1,768^c	56,87 ± 1,453^d	19,49 ± 0,794^e	34,25 ± 0,721^f
Pentra	7,01 ± 0,302^a	13,31 ± 0,465^b	39,31 ± 1,645^c	56,21 ± 1,425^d	19,03 ± 0,582^e	33,86 ± 0,446^f
Hemocytometer	7,27 ± 0,342^a	13,42 ± 0,387^b	39,00 ± 1,505^c	53,93 ± 2,319^d	18,57 ± 0,703^e	34,57 ± 1,293^f

Values represent mean ± M.S.E of 10 animals.
*Means with the same letters are not significantly different. (ANOVA test - one way) followed by Tukey Test ($p < 0.05$).

Table 2: Leukogram assessment through hematologic counters Celldyn 3200 and Pentra 60C + compared to the hemocytometer and differential counts by light microscopy in Adult male Wistar rats (*Rattus norvegicus*)

Counters	Leukocytes (mm^3)	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Lymphocytes (%)	Monocytes (%)
Cell-dyn	2490,00 ± 570,183^a	6,66 ± 6,822^b	0,11 ± 0,039^d	18,25 ± 8,309^f	70,92 ± 7,850^h	4,06 ± 1,613^i
Pentra	3200,00 ± 1143,130^a	9,16 ± 6,851^{bc}	0,10 ± 0,078^d	6,42 ± 6,201^{fg}	76,60 ± 9,271^h	7,72 ± 4,905^i
Hemocytometer	3136,50 ± 807,509^a	22,40 ± 2,414^c	0,70 ± 0,216^e	0,00 ± 0,000^g	74,20 ± 2,258^h	2,50 ± 0,316^i

Values represent mean ± M.S.E of 10 animals.
*Means with the same letters are not significantly different. (ANOVA test - one way) followed by Tukey Test ($p < 0.05$).

Table 3: Platelet count of evaluation through hematologic counters Celldyn 3200 and Pentra 60C + compared to hemocytometer in Adult male Wistar rats (*Rattus norvegicus*)

	Cell-dyn (mm^3)	Pentra (mm^3)	Hemocytometer (mm^3)
Platelets /mm^3	657.300,0 ± 27.864,04^a	610.400,0 ± 43.019,75^a	584.100,0 ± 82.962,34^a

Values represent mean ± M.S.E of 10 animals.
*Means with the same letters are not significantly different. (ANOVA test - one way) followed by Tukey Test ($p < 0.05$).

Discussion

The results for the erythrocyte count showed that there are no differences between the automated and non-automated methods, indicating that for all parameters evaluated the readings made by automated counters were similar to those performed by the Neubauer chamber. Similar results were found by Messias et al. (2009).

The total leukocyte count also denoted no significant differences between the methodologies used, however, variations were noticed in the differential count, showing discrepancy between granulocytes. For basophils in automated methodology, the values found were not consistent with the existing literature, and when evaluated by hemocytometer, the value was zero. Basophils in rats and other mammals are rare or uncommon in the bloodstream (Messias et al., 2009). Probably these results have occurred due to the inability of the used equipment to differ other cellular elements from similar sizes like the neutrophils. The differences shown in neutrophils and eosinophils reflect deviations in basophil count by using automated methods. Messias et al. (2009) also reported these differences when rat blood was analyzed using a hematology analyzer for humans compared to the hemocytometer. The values obtained in both automated methods compared with the manual count, even with low values, are consistent with values usually found for rats (Harkness; Wagner., 1993; Cubas et al., 2007; Messias et al., 2009; 2010; Melo et al., 2012; Lima et al., 2014).

The total platelet count showed no significant differences when compared to automated methodologies with hemocytometer. Similar findings were reported by Messias et al. (2009). Nevertheless, the automatic analyzers can overestimate the platelet count, what may be related to poor sensitivity at levels below the normal range (Segal et al., 2005).

Conclusions

The results provide the conclusion that although the hematology analyzers used in this work are not suitable for rodents, the absolute counts (total of red blood cells, white blood cells, platelets and RBC indices) performed are reliable and fast. The hematology counters can be used with caution and is not recommended for differential leukocyte count and the analysis of the morphology of blood cells.

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