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Reference intervals for hematological parameters of animals bred and kept at the vivarium of the Faculty of Medicine of the State University of São Paulo

Marilda Osti Spinelli*, Milena Coutinho Motta, Robson José Cruz and Cyntia Marques Santos Corrêa Godoy

*Centro de Bioterismo, Faculdade de Medicina, Universidade de São Paulo, Av. Dr. Arnaldo, 455, 01246-903, São Paulo, São Paulo, Brazil. *Author for correspondence. E-mail: marilda@biot.fm.usp.br*

ABSTRACT. Knowledge of specific hematological parameters of laboratory animals used in experiments is highly relevant to evaluate functional alterations of their vital organs. Hematological reference rates for rats, mice, rabbits and hamsters, bred in conventional healthy conditions and derived from the Vivarium of the FMUSP's Vivarium Center are established. One hundred and twenty mice of the strains BALB/c, C57BL / 6 and Swiss, 80 Wistar rats, 80 Golden hamsters and 80 New Zealand rabbits, males and females, apparently normal and healthy, over 10-weeks-old, were employed in current experiment. The analysis was determined by flow cytometry process (CELM CC510) involving the separation of erythrocytes and leukocytes, and cell identification by the suspension method and quantification. Hematological derivatives such as hematometric indexes (application of equations) and morphological quantification of leukocytes and erythrocytes were determined by scanning/differential (Panótico-commercial), whereas hemoglobin dosage was determined by spectrophotometry (CELM E210D). Results are given as means and standard deviants of hematological profiles in the blood of the animals grouped by species, strain and sex. Data are an important asset for researchers in their analysis of experimental results. CEAU Certificate n.° 251/11 HCFMUSP.

Keywords: hematological rates, blood, sanitary conditions, animals.

Intervalos de referência para alguns parâmetros hematológicos de animais criados e mantidos pelo Biotério da Faculdade de Medicina da Universidade de São Paulo

RESUMO. Conhecer os parâmetros hematológicos individuais de animais de laboratório utilizados na experimentação é importante para avaliar as alterações funcionais dos seus órgãos vitais. Este trabalho estabelece valores de referência hematológicos de roedores e lagomorfos criados em condições sanitárias convencionais, provenientes do Biotério do Centro de Bioterismo da FMUSP. Foram utilizados 120 camundongos das linhagens BALB/c, C57BL/6 e Swiss, 80 ratos Wistar, 80 hamster Golden e 80 coelhos New Zealand, machos e fêmeas, aparentemente normais e saudáveis, todos acima de 10 semanas. A análise foi determinada pelo processo de citometria de fluxo (CELM CC510) para a separação de eritrócitos e leucócitos, e a identificação celular pelo método em suspensão e quantificação. Os derivados hematológicos como índices hematimétricos (aplicação de equações), a quantificação morfológica dos leucócitos e eritrócitos foram determinados por varredura/diferencial (Panótico-comercial) e a dosagem de hemoglobina por espectrofotometria (Celm E210D). Os resultados estão apresentados pelas médias e pelos erros padrões dos perfis hematológicos encontrados no sangue dos animais, os quais estão agrupados por espécie, linhagem e sexo. Os perfis servirão como valiosa ferramenta aos pesquisadores na análise de seus resultados experimentais. Certificado CEAU n° 251/11 HCFMUSP.

Palavras-chave: valores hematológicos, sangue, condições sanitárias, animais.

Introduction

Interest in the development of research in veterinary hematology has recently been on the increase. This is mainly due to improvements in techniques and to laboratory support to seek solutions related to clinical problems proper to the animals' different species and strains (CCAC, 1984; MENENDEZ, 1985; JUNIOR et al., 2006).

According to Krache (1943), routine exams in hematology determine a set of hematometric parameters followed by erytrogram mm^{-3}, leukogram mm^{-3}, concentration of hemoglobin in g dL^{-1}, hematometric indexes (MCV, MCH and MCHC) correlating the liquid to the solid part of blood elements, followed by percentages of morphological scanning of mono- and poly-morphonuclear cells

(SANDERSON, 1981; MITRUKA; RAWNSLEY, 1977; SILVA et al., 1990).

Hematological profiles are being used extensively in human and veterinary medicine not merely for individual clinical evaluation but also for the evaluation of animal populations, especially those used in research projects (TONKS, 1970; YOSHIDA et al., 2000).

When hematological rates are interpreted properly, they express important information on the animal's clinical conditions, nutritional balance, deficit conditions with regard to hemoglobin iron and the presence or absence of infections from adverse factors, monitoring on which type of treatment and prognostics (BERNSTEIN, 1975; HRAPKIEWICZ et al., 1998).

So that a correct interpretation of results could be achieved, several researchers have demanded reference rates for laboratory animals at the sites in which they are kept. Therefore, every Institution must determine its research animals' hematological profile. Each site is actually affected by race, sex and age. Actually it is possible to have animal species, sanitary conditions, pregnancy and other factors that would vary from place to place (JAIN, 1993; LAKOWISCZ, 1996; YOSHIDA et al., 2000; SACHER, 2000).

Current analysis demonstrates to researchers using rodents in their research that the minimum usage of sanitary barriers with regard to the rearing of the animals greatly affects the animal's good development. In fact, it results in an important variable for the development of excellent results in research projects (RODRIGUES, 2003; SANTOS, 2004). Since much variability in techniques used by different laboratories for the analysis of blood parameters is not extant, current essay established rates that may be used as reference within the context of laboratories in Brazil, since most known rates are available in international literature.

Material and methods

Experimental animals

Animals of different species and strains, estimated standard age 8 – 10 weeks, from the Vivarium Center of the Faculty of Medicine of the University of São Paulo, were used, following references by the Federation of European Animal Science Association (FELASA). Animals were separated in groups according to sex (males and females). Procedures were undertaken according to international ethical and welfare principles for animals. Experiment was approved by the Ethics Committee of the HC/FMUSP (Certificate 251/2011). There were 360 animals in all, comprising 120 mice with 40 animals per sex and strains BALB/c, C57BL/6 and Swiss; 80 Wistar rats, 80 Golden hamsters and 80 New Zealand rabbits. All animals were apparently normal and healthy when blood was collected.

Animals received commercial balanced diet *ad libitum* in quantities recommended for each species and water. They were kept in polypropylene cages lined with sterile wood shavings. Since the animals were conventional, sanitary barriers were less strict. They were kept in exclusive rooms for each strain and genus, at a temperature between 22°C ± 2°C. Further, all people concerned complied with hygienization and uniform-wearing rules on entering the premises (TONKS, 1970).

Blood parameters

After the animals were anaesthetized in CO_2 chamber without previous fasting, samples with 100 μ total blood from the orbital retro plexus of mice and from heart puncture of the other animal species were harvested. Blood was immediately conditioned in microtubes with coagulant tetra-acetic acid (EDTA) at 10 mg dL^{-1} concentration for hematological tests (YOSHIDA et al., 2000; NICOLOSI, 2002; NICHOLS, 2003).

Blood samples were analyzed on the same day of collection by flow cytometry (Celm CC510) to determine erytrogram (red series-RDC) and leukogram (white series-WBC).

Hematocrit (Ht) was determined by micro-centrifugation at 2000 rpm (Fanen 211); hemoglobin (Hb) dose was performed by spectrophotometry at 540 nm (Celm E210D); mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and concentration of mean corpuscular hemoglobin (CMCH) were calculated by equation relationships (Ht/RDC, Hb/RDC, Hb/Ht).

Morphological count of leukocytes and erythrocytes was determined by scanning/differential (Panótico-commercial), analyzed under microscope Zeiss Hall 100 and determined by relative counting which provided cell percentage.

Results and discussion

Tables 1, 2 and 3 show results for the different species and strains, coupled to descriptive statistical rates (means and standard deviation) of the blood tests under analysis (BEIGUELMEN, 1991).

Table 1. Hematological rates of C57Bl6 and Swiss mice from the Conventional Vivarium of the Vivarium Center of the FMUSP.

	Strain.	Swiss		C57Bl/6	
	Sex	♂	♀	♂	♀
Parameters	Unit.	Results			
Erythrogram	10^6 mm^{-3}	5.56±1.03	5.81±1.08	5.31±2.5	6.33±0.49
Hematocrit	%	34.60±5.5	41.80±4.9	38.80±16.0	40.20±3.49
Hemoglobin	g dL^{-1}	13.19±1.5	14.25±1.7	13.44±1.1	13.70±1.35
MCV	fm^3	72.59±6.9	73.00±6.5	58.83±1.7	63.48±4.25
MCH	Pg	24.15±3.3	24.89±2.2	20.01±0.5	21.44±1.45
CMCH	g dL^{-1}	33.13±1.9	34.10±0.1	34.11±0.01	34.09±0.05
Leukogram	10^3 mm^{-3}	3.38±1.07	4.30±1.51	5.74±0.5	3.96±1.7
Eosinophils	%	2.80±0.75	2.00±1.41	1.40±1.3	0.60±0.49
Monocytes	%	4.60±1.0	5.20±1.47	2.40±1.0	1.20±0.4
Lymphocytes	%	52.60±2.1	51.20±1.6	47.20±2.7	52.40±3.55
Basophils	%	0.00±0	0.00±0	0.00±0	0.00±0
Band cells	%	10.40±1.4	9.20±1.47	6.0±0.9	5.80±1.60
Segmented	%	19.40±2.8	23.20±3.2	37.00±5.7	34.20±1.17
Neutrophils	%	29.80±2.4	34.20±2.2	43.00±4.9	40.00±1.90

*Tests carried out by the Animal Health Quality Control Laboratory of the C.B - FMUSP. * STRAIN; UNIT.

Table 2. Hematological rates of Balb/c mice and Wistar rats from the Conventional Vivarium of the Vivarium Center of FMUSP.

	Strain	Balb/c		Wistar	
	Sex	♂	♀	♂	♀
Parameters	Unit.	Results			
Erythrogram	10^6 mm^{-3}	8.586±0.05	8.828±0.63	6.06±0.88	5.53±1.48
Hematocrit	%	43.60±5.08	42.60±5.74	40.6±3.26	32.80±9.3
Hemoglobin	g dL^{-1}	14.86±1.73	14.52±1.96	13.84±1.11	11.14±2.8
MCV	fm^3	50.28±4.03	48.26±5.00	67.76±5.38	60.13±3.7
MCH	Pg	17.21±1.34	16.45±1.87	23.10±1.83	20.97±2.0
MCHC	g dL^{-1}	34.07±0.04	41.20±8.70	33.94±0.30	33.94±0.3
Leukogram	10^3 mm^{-3}	5.90±1.02	5.88±1.40	8.00±2.53	9.60±2.06
Eosinophils	%	1.40±1.02	1.60±0.49	2.00±1.09	1.80±1.12
Monocytes	%	4.00±1.06	5.40±19.01	4.20±1.17	3.60±1.49
Lymphocytes	%	51.41±2.73	46.00±4.24	43.80±3.82	45.00±3.5
Basophils	%	0.00±0	0.00±0	0.00±0	0.00±0
Band cells	%	8.00±2.23	10.6±1.74	11.20±1.72	8.60±1.62
Segments	%	29.40±4.93	27.20±41.1	27.60±4.93	32.60±3.1
Neutrophils	%	37.40±4.31	37.80±39.6	38.80±3.43	41.00±1.8

*Tests carried out by the Animal Health Quality Control Laboratory of the C.B - FMUSP.

Table 3. Hematological rates of Golden hamsters and New Zealand rabbits from the Conventional Vivarium of the Vivarium Center of FMUSP.

	Strain.	Golden		New Zealand	
	Sex	♂	♀	♂	♀
Parameters	Unit.	Results			
Erythrogram	10^6 mm^{-3}	7.27±0.9	7.57±0.1	5.94±1.0	4.86±0.6
Hematocrit (HT)	%	44.40±4.1	42.20±1.9	31.20±1.8	26.40±4.5
Hemoglobin	g dL^{-1}	15.14±1.4	16.47±0.7	10.58±0.7	8.94±1.6
MCV	fm^3	61.67±1.6	62.6±2.9	52.58±3.9	4.02±2.9
MCH	pg	21.05±0.6	1.26±1.0	1.83±1.4	36.22±7.3
MCHC	g dL^{-1}	34.09±0	4.09±0.01	33.89±0.4	34.10±0.02
Leukogram	10^3 mm^{-3}	12.52±3.5	11.82±1.0	9.98±2.4	6.66±2.8
Eosinophils	%	1.40±1.0	1.40±1.0	5.40±1.1	5.20±1.2
Monocytes	%	3.20±1.7	2.00±1.9	2.40±0.9	2.00±0.6
Lymphocytes	%	45.80±3.3	46.00±3.0	39.00±4.5	40.02±20.9
Basophil	%	0.00±0	0.00±0	0.00±0	0.00±0
Band cells	%	10.40±1.5	9.00±2.2	11.40±1.3	12.60±3.5
Segments	%	27.80±5.4	32.60±4.0	37.00±5.2	40.20±1.6
Neutrophils	%	38.40±3	41.60±2.6	48.40±4.9	52.80±3.6

*Tests carried out by the Animal Health Quality Control Laboratory of the C.B - FMUSP.

Data showed slight individual variations when each parameter was evaluated, according to the specificity of each strain. When they were compared with the International System, with monitoring and categories different from the Brazilian ones, discrepancies were detected due to different sanitary conditions and genetic characteristics (LAKOWISCZ, 1996; FRICH et al., 1980; JUNIOR et al., 2006).

When the above results were compared with those of other Brazilian institutions, some parameters had similar reference rates whereas others were different, according to each strain and species. Variations may occur due to differences in sanitary category (conventional, with or without sanitary barriers), climate (sample harvest period), type of handling (with protecting equipments, sanitary controls), variables such as age (FELASA standard), diet (specific to each species and commercial standard), sex, season in which rate results should be considered within the variation amplitude for the colony under analysis, and other

factors (YOSHIDA et al., 2000; NICOLOSI, 2000; SANTOS et al., 2004). Care should therefore be taken when assigning data of one experiment to a certain species or race. It may justify the variability of results among the data in current research when compared to that of other authors.

When animals of the same conventional category and different species and strains are compared, several hematological and biochemical variations are present in all analyses. In animals of the same species and different strains, data given in the tables show that each strain has a specific and determining characteristic for each type of animal.

An adequate vivarium with trained officers and efficient administration may facilitate the control of variables so that homogeneity alterations of the sample or of research conditions could be avoided

Conclusion

Data analysis showed that rates in current assay differed from those of the literature. Reference rates for each animal according to each type of vivarium are therefore required. The standardization of these rates will surely help researchers in their different research work not only in the Universidade de São Paulo, Brazil, but also in other higher institutions.

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