Clinical hematological and biochemical parameters in Swiss, BALB/c, C57BL/6 and B6D2F1 Mus musculus

Giorgio Silva-Santana1,2,3 | Juliet Cunha Bax4 | Débora Cristina Silva Fernandes2,3 | Daniela Tendler Leibel Bacellar5 | Cleber Hooper6 | Alexandre Alves Souza Oliveira Dias5 | Cristina Barbosa Silva7 | Aline Moreira de Souza4 | Simone Ramos5 | Ricardo Alexandre Santos5 | Thainara Ramos Pinto5 | Mariana Antunes Ramão5 | Ana Luíza Mattos-Guaraldi1,2,3,8

1Health Sciences Center, Institute of Microbiology Paulo de Góes, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil  
2Laboratory of Diphtheria and Corynebacteria of Clinical Relevance, Faculty of Medical Sciences, University of the State of Rio de Janeiro, Rio de Janeiro, RJ, Brazil  
3The Collaborating Centre for Reference and Research on Diphtheria/National Health Foundation/Ministry of Health, Rio de Janeiro, RJ, Brazil  
4Laboratory of Clinical Research and Molecular Diagnostic Prof. Marcilio Dias do Nascimento, Department of Clinical Practice and Pathology, Fluminense Federal University, Niterói, RJ, Brazil  
5Laboratory Bacterial Vaccines, Department of Immunology, National Institute for Quality Control in Health, Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil  
6Institute of Science and Technology in Biomodels, Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil  
7Institute of Biology, Federal Fluminense University, Niterói, RJ, Brazil  
8Biomedical Center, College of Medical Sciences, University of the State of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Abstract

Background: Animal models are widely used in scientific research in order to obtain information from a whole organism under a specific set of experimental conditions. Various lineages of mice have been used to investigate diseases and new therapeutic strategies, and, consequently, hematological and biochemical tests in these laboratory animals are essential to validate scientific studies. Our study seeks to establish reference values for hematological and biochemical parameters of four lineages of mice.

Methods: We evaluated the hematological and biochemical profiles of 20 males and 20 females from the lineages Swiss (heterogeneous), BALB/c and C57BL/6 (isogenic), and B6D2F1 (hybrid), totaling 160 mice. Analysis were standardized using the systems pocH-100iV Diff™ for 19 hematological parameters and VITROS® 350 for 12 biochemical parameters.

Results: Results are shown as means and standard deviation, grouped by lineage and genre. Comparing the values obtained in this study with the values from previous...
studies, some variations were detected, which could be explained by differences in methodologies or individual variability. 

**Conclusion:** Thus our study shows that knowledge and disclosure of the values of physiological parameters of laboratory animals is necessary, and emphasises the importance of considering variations influenced by gender, lineage and genotype in the choice of the best experimental model. 

**KEYWORDS**
B6D2F1, BALB/c, biochemical, C57BL/6, hematological, Swiss

## 1 INTRODUCTION

Laboratory mice are the most commonly used animal model for biological studies of human health leading to the establishment of new diagnostic and therapeutic strategies.1,2 The demand for rat models has also increased in pharmacological, oncological and toxicological research, as well as in studies on drug efficacy,2 due to their easy creation, short generation time and the availability of inbred lineages (at least 20 generations of brother-sister mating).3,4 The extensive mapping of the mouse's genome and the detailed understanding of its immunological properties5 have improved the standardization of experimental models, increasing the reproducibility of studies and the comparison of results by researchers worldwide, thereby minimizing the need to repeat experiments.2 Advances in transgenic research and genetic targeting in models that use laboratory animals have led to a deeper understanding of the mechanisms of many diseases, and revealed new possibilities for treatment in human and veterinary medicine.1,5

Swiss heterogenous mice (non-consanguineous, heterogamous, outbred) present 99% heterozygosity between allele genes and therefore they have been used as a source of consanguineous animals representing natural populations and to obtain hybrid and transgenic descendants.6 Their aggressive behavior makes them good models in studies related to causes and/or mechanisms of aggression.7 This lineage is widely used for scientific purposes such as biomedical research on metabolic and autoimmune diseases, complement fixation, and mammary gland or lung tumors, as well as in pharmacology as stock in security testing for drugs.6 Due to their superior maternal abilities, Swiss females are used as ideal pseudogestants in the transfer of transgenic embryos and embryos of other lineages of mice.9

Both BALB/c and C57BL/6 lineages are isogenic (consanguineous, isogamous, inbred), obtained by crossing brothers and sisters for 20 consecutive generations, which allows the production of genetically homogeneous populations and, consequently, the need for fewer samples per experimental group.6 BALB/c mice have 99% homozygosity among allele genes, and are used for the production of monoclonal antibodies from plasmocytes.6 They have a low incidence of mammary tumors; however, they may develop other types of cancer, such as reticular neoplasias, and primary tumors in the lung and kidneys over their lifetime. This lineage has been frequently used in studies of infection with Listeria monocytogenes due to their great susceptibility: the mean time to death for females is three days.10 The C57BL/6 lineage has 6.5% of its genome originating from the Mus spretus species,11 and is widely used for conducting spontaneous and induced mutations, since it can totally express such mutations. Its main characteristics are low susceptibility to tumors and hearing loss induced by noise, and it is commonly used in studies on cardiovascular biology, development and genetics, and immunological, neurological and sensory stimuli. More specific characteristics are expressed in sublineages such as C57BL/6J, which is widely used in the production of transgenic mice. C57BL/sublineages are genetically prone to develop obesity induced by diet, diabetes type 2, atherosclerosis, high incidence of microphthalmia, reduced bone density and hereditary hydrocephalus.11

Unlike the other lineages, the B6D2F1 lineage is a hybrid (originally from crossing C57BL/6 [B6] females with DBA/2 [D2] males), and is heterozygous for the B6 and D2 alleles at all locations in the genome (genetic and phenotypic uniformity). The females have been used as embryo donors for microinjection in the creation of transgenic/knockout and deletion mutations, and in behavioral research, radiation and nutrient bioassays, drug and hormone research, and transplant (tumor, ovaries and skin) research using other lineages.12 Variations in the hematological and biochemical parameters of mice may be related to lineage, genotype, and genre, and are influenced by age, diet, environment, place of collection, and other factors.11,12 Therefore, knowledge of the physiological parameters and appropriate interpretation of hematological and biochemical serum rates are relevant to an evaluation of homeostasis and the alterations induced by pathological processes in different organs,15,16 since they provide information about the animal’s clinical conditions, nutritional balance, iron deficiency in hemoglobin and presence of infections, and are useful in monitoring the efficacy and the prognosis of treatments.17

Our study sought to establish values to serve as reference values in various hematological and biochemical of serum parameters in mice of the Swiss (heterogeneous), BALB/c and C57BL/6 (isogenic) and B6D2F1 (hybrid) lineages, to guide researchers in the best choice of an experimental model.
2 | MATERIALS AND METHODS

2.1 | Animal experimentation

All experimental procedures described in this study were approved by the Animal Experimentation Ethics Committee of the Institute of Biology Roberto Alcântara Gomes of the University of the State of Rio de Janeiro (UERJ) in accordance with Law 11.794/2008 (Arouca Law) approved in 2018 under protocol 055/2018. All procedures used are in accordance with the Brazilian Directive for the Care and Use of Animals for Scientific and Didactic Purposes of National Council for Animal Experimentation Control (CONCEA).

This study used 20 male and 20 female mice from each of the Swiss (heterogeneous), BALB/c and C57BL/6 (isogenic) and B6D2F1 (hybrid) lineages, totaling 160 individuals; with 60 days of life mice were 60 days old (age). The mice were examined and showed no lesions or inflammation of the skin and no ectoparasites at the time of collection. The animals were kept in the biotower of the School of Medical Sciences of the UERJ, in rigorous sanitary conditions with quarter-hourly monitoring. They were free of specific pathogens (SPF), and were housed in racks of mini isolators (5 mice/cage; cage dimensions 45 × 31 × 19 cm) lined with sterile pine wood shavings of (ASPEN – LIGNOCEL®), which were periodically renewed. The mice were kept in filtered air at positive pressure, with a 12 hours light/dark cycle, starting at 6:30 am, temperature ~21 to 22°C (~2°C) and humidity ~50 to 55%. All animals received sterile water (autoclaved) and appropriate rations (Nuvilab® CR-1) ad libitum, meeting the nutritional requirements for the species. The animals were not exposed to chemical or drug treatments that could change their natural physiological state.

2.2 | Blood sampling method and sample handling

All biological material was collected by the same qualified professional. Material was collected after an 8-hour fast, in the morning, to avoid variations in the parameters. Initially, the absolute weights of the animals were measured using a precision balance (Marte – AD 2000, maximum load capacity 210 g; sensitivity of 0.01 g). Subsequently, they were sedated by intraperitoneal injection (lower right quadrant) of 16 mg/kg of xylazine hydrochloride® as a muscle relaxant and 120 mg/kg of ketamine hydrochloride® for deep sedation, using BD Ultra-Fine TM syringes (needle 25 × 5 mm). After deep anesthesia was confirmed by the absence of a foot reflex, the animals were positioned in dorsal decubitus for decontamination of the collection site with 70% ethanol. Blood was collected (intracardiac puncture – cardiocentesis) via a needle (27 × 8 mm), inserted into the abdominal wall, under the xiphoid process, according to the method of Silva-Santana et al (2020).18 This procedure resulted in the death of the animals, certified by the absence of vital signs (heartbeat and respiratory movements).

Aliquots of 300 µL of blood were collected in tubes (10 × 45 mm, maximum volume 500 µL – VACUPLAST) containing ethylenediamine tetra-acetic acid (EDTA-K2) and carefully mixed by inversion in a homogenizer (Electra – Homolab 22T) for a complete blood count (CBC). For biochemical analysis of serum, aliquots of blood were deposited in tubes (10 × 45 mm, maximum volume 500 µL – VACUPLAST) containing separator gel and coagulation activators, to the maximum volume indicated by the manufacturer, waiting 30 minutes to blood clot retraction. The aliquots were then centrifuged for 5 minutes at 2500 rpm (Eppendorf® Minispin® model SPIN 1.000, Hamburg, Germany) to separate the serum. The biological samples were then sent to the Institute of Science and Technology in Biomodels, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

2.3 | Hematological parameters

CBC was performed in an automated veterinary hematology counter poCH-100IV Diff™ (Sysmex® - Roche) to establish the following parameters: red blood cell (RBC) count, hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width-coefficient of variation (RDW-CV), red blood cell dimension width-standard deviation (RDW-SD), number of white blood cells (WBC), percentage ratio of small white blood cells (W-SCR), percentage ratio of middle white blood cells (W-MCR), percentage ratio of large white blood cells (W-LCR), number of small white blood cells, lymphocytes (W-SCC), number of large white blood cells, monocytes (W-MCC), number of large white blood cells, gametocytes (W-GLC), creatinine (CR) and urea (UR). The biochemical kits and calibrators (BAS)), evaluation of blood cell morphology and photographic documentation. The equipment was calibrated and the precision of results determined before each analysis using control blood ABX Minotrol 16, and an external quality evaluation program was implemented.

2.4 | Clinical biochemistry parameters

The blood serum was processed in an automated spectrophotometer VITROS® 350 Chemistry System (Ortho-Clinical Diagnostics, Johnson-Johnson Co., Rochester, NY) for analysis of the following parameters: total protein (TP), triglycerides (TG), cholesterol (CHL), alkaline phosphatase (AP), uric acid (UA), alanine transaminase (ALT), aspartate transaminase (AST), albumin (ALB), globulin (GLB), glucose (GLC), creatinine (CR) and urea (UR). The biochemical kits and calibration controls used were acquired from Labtest Diagnosis (Lagoa...
**TABLE 1** Reference intervals for erythrocyte indices in healthy mice of mixed breeds

| Erythrogram | SWISS (mean ± SD) | BALB/c (mean ± SD) | C57BL/6 (mean ± SD) | B6D2F1 (mean ± SD) |
|-------------|-------------------|--------------------|---------------------|--------------------|
| Parameters (Unit) | Male | Female | Male | Female | Male | Female | Male | Female |
| BW (g) | 35.55 ± 2.66 | 29.35 ± 1.19 | 27.03 ± 1.39 | 20.80 ± 0.67 | 27.02 ± 1.22 | 20.01 ± 2.20 | 37.43 ± 1.36 | 32.75 ± 1.51 |
| RBC (×10^6/µL) | 7.42 ± 0.85 | 7.74 ± 0.69 | 9.00 ± 0.44 | 9.08 ± 0.62 | 8.45 ± 1.15 | 8.86 ± 1.01 | 8.70 ± 1.17 | 7.99 ± 0.69 |
| HCT (%) | 39.81 ± 1.64 | 40.74 ± 1.71 | 43.52 ± 2.89 | 42.57 ± 3.68 | 41.91 ± 3.41 | 37.14 ± 3.46 | 46.19 ± 5.67 | 42.73 ± 2.20 |
| HGB (g/dL) | 12.92 ± 0.34 | 13.45 ± 0.71 | 13.64 ± 0.74 | 13.82 ± 1.07 | 13.13 ± 0.97 | 12.01 ± 1.02 | 13.89 ± 0.99 | 13.03 ± 0.15 |
| MCV (fL) | 57.81 ± 7.79 | 54.56 ± 5.17 | 49.23 ± 1.98 | 47.73 ± 4.26 | 50.74 ± 3.18 | 42.69 ± 8.51 | 53.09 ± 3.46 | 54.09 ± 1.94 |
| MCH (pg) | 18.58 ± 2.03 | 17.52 ± 2.21 | 15.56 ± 1.01 | 14.48 ± 1.04 | 16.23 ± 1.38 | 14.10 ± 2.56 | 16.19 ± 1.06 | 16.59 ± 1.28 |
| MCHC (g/dL) | 31.78 ± 0.97 | 32.57 ± 1.02 | 31.77 ± 1.50 | 33.00 ± 2.60 | 31.03 ± 2.04 | 28.94 ± 1.24 | 30.43 ± 1.61 | 30.59 ± 1.19 |
| RDW-CV (%) | 15.64 ± 1.47 | 16.55 ± 1.72 | 16.34 ± 1.22 | 14.47 ± 0.88 | 15.33 ± 0.71 | 14.33 ± 1.60 | 20.80 ± 4.10 | 21.99 ± 5.29 |

Note: CBCs were performed in an automated veterinary hematology counter poc'H-100IV Diff™ (Sysmex® - Roche). Data show means ± standard deviation (SD), calculated with GraphPad Prism version 6.01.

Abbreviations: BW, body weight; HCT, hematocrit; HGB, hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; RDW-CV, red cell distribution width - coefficient of variation; RDW-SD, red blood cells dimension width - standard deviation.

**TABLE 2** Reference intervals for platelets indices in healthy mice of mixed breeds

| Plaquetogram | SWISS (mean ± SD) | BALB/c (mean ± SD) | C57BL/6 (mean ± SD) | B6D2F1 (mean ± SD) |
|--------------|-------------------|--------------------|---------------------|--------------------|
| Parameters (Unit) | Male | Female | Male | Female | Male | Female | Male | Female |
| PLT (×10^3/µL) | 0.418 ± 0.080 | 0.626 ± 0.087 | 0.560 ± 0.358 | 0.560 ± 0.119 | 0.171 ± 0.056 | 0.120 ± 0.034 | 1.249 ± 0.013 | 1.056 ± 0.080 |
| MPV (fL) | 6.16 ± 0.14 | 9.79 ± 2.43 | 7.38 ± 0.79 | 5.70 ± 1.64 | 8.05 ± 1.74 | 6.50 ± 0.89 | 4.83 ± 1.32 | 6.79 ± 0.19 |
| PDW (fL) | 7.61 ± 0.58 | 10.96 ± 0.71 | 7.58 ± 0.57 | 6.23 ± 1.72 | 9.79 ± 2.11 | 7.67 ± 1.08 | 6.23 ± 1.32 | 8.44 ± 0.52 |
| P-LCR (%) | — | — | — | — | — | — | — | — |

Note: CBCs were performed in an automated veterinary hematology counter poc'H-100IV Diff™ (Sysmex® - Roche). Data show means ± standard deviation (SD), calculated with GraphPad Prism version 6.01.

Abbreviations: —, absence of data; MPV, mean platelet volume; PDW, platelet dimensions width; P-LCR, percentage of giant platelets; PLT, number of platelets.
3 | RESULTS

Male mice presented higher values of total body weight compared to females of the same age. The animals tested showed no evidence of hemoparasites in the cytological evaluation of blood smears by microscopy. The analysis of erythrocytes revealed a greater number of RBC in females; the other parameters varied according to lineage and gender (Table 1). Higher PLT counts were found in C57BL/6 and B6D2F1 males, while the opposite was observed in Swiss. Male and female mean PLT counts were similar in BALB/c mice (Table 2).

The automated leukocyte count showed that BALB/c females have a greater number of immune cells. However, the differential leukometry data showed that the male Swiss mice had higher rates of W-SCC (LYN) compared to other lineage of mice and the females had higher rates of W-MCC (MON) and W-LCC (NEU). In the cytological evaluation of blood smears by microscopy, the highest numbers of LYN and MON were found in females and males, respectively, in the B6D2F1 lineage, the highest numbers of EOS were found in C57BL/6 females, and NEU were highest in BALB/c males (Table 3).

Blood cell morphology did not differ among the lineages studied. Erythrocytes presented as anucleate biconcave discs with central pallor. A few polychromatophilic erythrocytes and some cells containing Howell-Jolly corpuscles, which are common aspects in some species, were observed. Lymphocytes and neutrophils were the most commonly observed leukocytes. Regarding morphology, lymphocytes showed a rounded nucleus with condensed chromatin and reduced cytoplasm, monocytes were kidney-shaped and ovoid, with clearer chromatin and almost imperceptible granulation in the cytoplasm, eosinophils presented with a bilobulated nucleus and cytoplasm filled with orange granules, and neutrophils presented with a polylobulated nucleus and discrete fine basophilic granules dispersed in abundant cytoplasm. Immature forms of neutrophils were not observed. Anucleate and slightly flattened platelets were observed, as well as few platelet aggregates (Figures 1 and 2).

4 | DISCUSSION

The blood serum was limpid and semi-transparent in aspect. The analyses of biochemical parameters demonstrated higher ALB and UR levels in Swiss males; higher CHL levels in BALB/c males, and higher AP and ALT in females; higher TP and UA levels in C57BL/6 males, and higher AST and GLB in females; and finally, higher TG levels in B6D2F1 males and higher GLC levels in females (Table 4).

The evaluation of hematological and biochemical phenotypes is relevant to determining the physiological profile of different lineages and/or populations of mice, as well as allowing the creation of reference values that will form the basis of the interpretation of variations caused by diseases.

Mice are the most commonly used animals in experiments. However, there are controversies over the use of species and subspecies created in laboratories due to the presence of special intersections, in which the animals have some genes or even chromosomes of other species. Therefore, knowledge of the hematological and biochemical phenotypes of the lineages produced in the laboratory and used in research is important.

Hematological parameters are used as biomarkers in the diagnosis of organ or tissue injuries and as an aid in animal reproduction, and in diagnosing infections, parasitosis and other pathologies. In clinical practice, the parameters commonly used to evaluate the erythrogram are the RBC, HCT, HGB, MCV, MCH and MCHC counts, and less frequently RDW counts (CV and SD). HGB is of extreme importance in electron transport, and reduction and transfer of oxygen for hydroxylation reactions; it can be measured as the total volume in the erythrocytes, and has a value of ~10 to 17 g/dL in mice. The dataset obtained for RBC, MCV, MCH and MCHC enables an analysis of the correlation between the size of erythrocytes and the HGB concentration in its interior, which is important for characterizing different degrees of anemia. MCV is used to evaluate the degree of anisocytosis (size of erythrocytes) and in the classification of anemia among normocytic, microcytic or macrocytic erythrocytes. MCH can be estimated from the relationship between the mean concentration of hemoglobin inside of the cell and the number of erythrocytes, which may vary between ~13 and 17 pg in mice. MCH measures hemoglobin concentration in erythrocytes, and values range between ~30 and 38 g/dL in mice. RDW evaluates the range of variation in size of erythrocytes in the same sample of blood, through a histogram of the distribution of erythrocytes according to volume. Different disorders may either increase or decrease this parameter.

RBC and MCHC were highest in BALB/c females among the groups studied. Similar RBC data were presented by Santos et al (2016) (8.90 ± 0.90 × 10⁶/µL) for collections by puncture in the axial plexus, and similar MCHC data were reported by Spinelli et al (2012) (32.24 ± 0.58 g/dL) for collections by puncture in the
### Table 3
Reference intervals for leukocyte indices in healthy mice of mixed breeds

| Leukogram | Parameters (Unit) | SWISS (mean ± SD) | BALB/c (mean ± SD) | C57BL/6 (mean ± SD) | B6D2F1 (mean ± SD) |
|-----------|-------------------|-------------------|-------------------|-------------------|-------------------|
|           | Male              | Female            | Male              | Female            | Male              | Female            |
|           | SWISS (mean ± SD) | BALB/c (mean ± SD)| C57BL/6 (mean ± SD)| B6D2F1 (mean ± SD)|                   |                   |
|           |                   | Male              | Female            | Male              | Female            | Male              | Female            |
|           |                   |                   |                   |                   |                   |                   |                   |
|           |       |                   |                   |                   |                   |                   |                   |
|           |       |                   |                   |                   |                   |                   |                   |
| Global leucometry |           |                   |                   |                   |                   |                   |                   |
| WBC (x10^3/µL) | 4.98 ± 1.26      | 5.16 ± 1.02       | 5.36 ± 0.96       | 6.23 ± 2.57       | 5.80 ± 0.34       | 4.24 ± 0.74       | 3.84 ± 0.23       | 5.99 ± 0.48       |
| W-SCR (%)     | 83.43 ± 1.28     | 92.46 ± 6.22      | 75.66 ± 6.37      | 66.58 ± 26.19     | 84.95 ± 7.04      | 80.01 ± 5.79      | 81.59 ± 6.04      | 85.89 ± 4.21      |
| W-MCR (%)     | 12.46 ± 1.12     | 13.15 ± 1.88      | 17.33 ± 1.99      | 13.29 ± 6.69      | 13.25 ± 1.16      | 13.94 ± 1.56      | 14.30 ± 3.81      | 10.26 ± 2.61      |
| W-LCR (%)     | 4.45 ± 1.04      | 4.82 ± 1.41       | 7.37 ± 3.24       | 5.55 ± 1.55       | 5.08 ± 0.44       | 6.74 ± 1.05       | 4.14 ± 2.27       | 3.73 ± 1.61       |
| W-SCC (x10^3/µL) | 6.57 ± 0.97    | 5.59 ± 0.75       | 3.88 ± 1.02       | 3.59 ± 0.76       | 5.25 ± 0.50       | 4.16 ± 0.39       | 3.19 ± 0.40       | 5.13 ± 0.15       |
| W-MCC (x10^5/µL) | 1.01 ± 0.18   | 1.24 ± 0.37       | 1.01 ± 0.35       | 0.85 ± 0.19       | 0.75 ± 0.15       | 0.80 ± 0.10       | 0.59 ± 0.11       | 0.69 ± 0.19       |
| W-LCC (x10^3/µL) | 0.39 ± 0.11   | 0.50 ± 0.19       | 0.46 ± 0.21       | 0.43 ± 0.11       | 0.29 ± 0.08       | 0.40 ± 0.09       | —                 | —                 |
| Differential leucometry |           |                   |                   |                   |                   |                   |                   |
| LYM (x10^5/µL) | 7.65 ± 0.63      | 6.86 ± 0.97       | 6.60 ± 0.64       | 7.27 ± 0.85       | 7.28 ± 0.55       | 5.02 ± 0.33       | 7.45 ± 0.63       | 7.99 ± 0.19       |
| MON (x10^5/µL) | 2.72 ± 0.73      | 3.10 ± 0.87       | 2.52 ± 1.29       | 2.98 ± 1.79       | 2.30 ± 0.57       | 1.99 ± 0.50       | 3.29 ± 0.31       | 3.10 ± 0.29       |
| EOS (x10^5/µL) | 1.15 ± 0.55      | 1.39 ± 0.34       | 1.49 ± 0.94       | 1.88 ± 0.98       | 2.18 ± 0.70       | 4.19 ± 0.83       | 1.99 ± 0.31       | 1.30 ± 0.29       |
| NEU/SEG (x10^5/µL) | 1.39 ± 0.34    | 1.69 ± 0.27       | 3.25 ± 0.57       | 2.16 ± 0.64       | 2.73 ± 0.41       | 2.12 ± 0.45       | 1.62 ± 0.40       | 1.59 ± 0.11       |

Note: CBCs were performed in an automated veterinary hematology counter pocH-100IV Diff™ (Sysmex® - Roche). Data show means ± standard deviation (SD), calculated with GraphPad Prism version 6.01.

Abbreviations: —, absence of data; BAS, basophiles cells; EOS, eosinophils; LYM, lymphocytes; MON, monocytes; NEU/BAN, absence of immature neutrophils (bands); NEU/SEG, neutrophils/Segmented; WBC, number of white blood cells; W-LCC, NEU, number of large white blood cells, gametocyte; W-LCR, percent ratio of large white blood cell; W-MCC, MON, number of middle white blood cells, monocyte; W-MCR, percent ratio of middle white blood cell; W-SCC, LYN, number of small white blood cells, lymphocyte; W-SCR, percent ratio of small white blood cell.
submandibular vein. B6D2F1 males presented higher indices of HCT and HGB, and B6D2F1 females of RDW (CV and SD). No comparable studies in hematology have used mice of this lineage. Swiss males presented the highest MCV and MCH values, which were similar to those presented by Diniz et al (2006) for mice produced by TACONIC, an animal model company (MCV, 58.5 ± 0.65 fl and MCH, 18.58 ± 2.03 pg). No other studies have used RDW as a parameter for profiling lineages of mice, which is strange, since the RDW index changes early, even before reductions in HGB and MCV, demonstrating its great importance in identification of anisopoikilocytosis.24,25 The morphology of heritocytes was similar among the lineages studied, as reported in previous studies.26 The morphological changes in the erythrocytes were not observed in our study, that is, the cells were normal and had no changes.

**FIGURE 1** Photomicrographs of bloods smears (Diff-Quick staining; 1000×; bar = 500 µm) from healthy mice: Swiss female (A and B) and male (C); BALB/c male (D and E) and female (F). The smears show red blood cells present in large quantities with uniform distribution, and evidence of leukocytes: lymphocyte (1), monocyte (2), eosinophil (3), neutrophil (4) and platelets (5)

**FIGURE 2** Photomicrographs of bloods smears (Diff-Quick staining; 1000×; bar = 500 µm) from healthy mice: C57BL/6 male (A and B) and female (C); B6D2F1 female (D) and male (E and F). The smears show red blood cells present in large quantities with uniform distribution, and evidence of leukocytes: lymphocyte (1), monocyte (2), eosinophil (3), neutrophil (4)
# Reference intervals for serum biochemical indices in healthy mice of mixed breeds

| Parameters (Unit) | SWISS (mean ± SD) | BALB/c (mean ± SD) | C57BL/6 (mean ± SD) | B6D2F1 (mean ± SD) |
|-------------------|-------------------|-------------------|-------------------|-------------------|
|                   | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| UR (mg/dL)        | 60.54 ± 6.46 | 40.69 ± 6.63 | 40.48 ± 3.91 | 36.24 ± 3.42 | 57.66 ± 3.16 | 60.15 ± 6.95 | 58.51 ± 10.18 | 39.29 ± 1.48 |
| ALT (UI/L)        | 46.15 ± 5.62 | 59.03 ± 9.58 | 99.44 ± 39.61 | 239.50 ± 141.20 | 76.37 ± 20.26 | 110.00 ± 16.83 | 65.72 ± 6.95 | 73.79 ± 9.46 |
| AST (UI/L)        | 121.60 ± 35.93 | 147.30 ± 23.58 | 135.20 ± 26.53 | 209.10 ± 36.21 | 136.70 ± 34.86 | 293.40 ± 62.65 | 90.09 ± 8.12 | 95.23 ± 16.25 |
| AP (UI/L)         | 169.20 ± 32.56 | 163.60 ± 48.13 | 135.20 ± 26.53 | 362.90 ± 226.60 | 97.29 ± 9.58 | 106.00 ± 16.83 | 249.80 ± 6.28 | 119.00 ± 8.27 |
| TP (g/dL)         | 5.31 ± 0.31 | 5.14 ± 0.47 | 5.21 ± 0.46 | 5.23 ± 1.68 | 8.03 ± 0.34 | 7.85 ± 0.62 | 4.42 ± 0.17 | 5.60 ± 0.53 |
| ALB (g/dL)        | 2.77 ± 0.17 | 1.84 ± 0.37 | 2.40 ± 0.47 | 1.74 ± 0.50 | 2.35 ± 0.12 | 2.10 ± 0.47 | 2.29 ± 0.12 | 2.33 ± 0.06 |
| GLB (g/dL)        | 2.60 ± 0.23 | 3.34 ± 0.28 | 2.77 ± 0.23 | 3.50 ± 1.89 | 5.80 ± 0.31 | 5.94 ± 0.40 | 2.19 ± 0.08 | 3.30 ± 0.56 |
| UA (mg/dL)        | 2.32 ± 0.28 | 3.32 ± 0.20 | 2.19 ± 0.24 | 2.55 ± 0.24 | 2.67 ± 0.61 | 1.09 ± 0.10 | 1.89 ± 0.21 | 1.43 ± 0.16 |
| CHL (mg/dL)       | 90.20 ± 6.13 | 91.81 ± 8.51 | 129.10 ± 11.82 | 77.72 ± 30.33 | 77.73 ± 3.22 | 44.92 ± 6.18 | 102.00 ± 6.64 | 66.03 ± 5.32 |
| TG (mg/dL)        | 140.40 ± 15.36 | 94.02 ± 3.29 | 76.89 ± 22.42 | 68.11 ± 34.57 | 31.56 ± 2.12 | 31.56 ± 2.42 | 214.40 ± 12.49 | 187.80 ± 11.52 |
| GLC (mg/dL)       | 147.60 ± 31.76 | 214.40 ± 47.29 | 160.40 ± 26.21 | 141.90 ± 30.47 | 233.20 ± 4.65 | 208.80 ± 6.95 | 239.80 ± 22.13 | 242.40 ± 9.62 |

Note: The Creatinine (CR) showed values below the detection limits (<0.2 mg/dL). Blood sera were processed at VITROS® 350 (Ortho Clinical Diagnostics - Johnson & Johnson) automated spectrophotometer. The Mean and Standard Deviation (SD), calculated through GraphPad Prism version 6.01.

Abbreviations: ALB, albumin; ALT, alanine transaminase; AP, alkaline phosphatase; AST, aspartate transaminase; CHL, cholesterol; GLB, globulin; GLC, glucose; TG, triglycerides; TP, total protein; UA, uric acid; UR, urea.
Currently, many automated hematological counters provide different parameters for platelet analysis. Despite still being little understood, they are used in medical and laboratory practice, particularly because of the difficulty in standardization. In analogy to the erythrogram and leukogram, the parameters that analyze the platelets composed of PLT, PDW, MPV and P-LCR came to be called plateletogram.27

The platelets are anucleate fragmented cells derived from megakaryocytes. They have hemostatic (buffer) function, and maintain endothelium integrity by releasing pro-angiogenic cytokines.28 These cells can be activated spontaneously or in response to stimuli, depending on lineage activation stage.21 In mice, platelet count is ~900 to 1600 x 10^3/mm^3.29 Analysis of the association between MPV and PDW is used to determine the distribution, width and size of platelets. Disturbances may result in variations in pre-established parameters.27,29 The P-LCR measurement is clinically important in identifying rare hereditary diseases such as macrothrombocytopenia (abnormally large platelets).27 B6D2F1 males showed a higher PLT level among the groups studied. No previous studies have used this lineage. Swiss females presented the highest MPV and PDW values. Despite their greater sensitivity as a clinical marker of platelet function and activity compared with PLT, we could not find any comparable studies containing analyses of these parameters.29 High levels of these indices indicate the existence of large platelets, which are more metabolically and enzymatically active. On the other hand, large platelets are more aggregative, facilitating thrombus formation, and are related to vascular diseases, including of the coronary artery.27,29 We could not measure P-LCR in the lineages studied, possibly due to the small sample volume (<12 fl), indicating that animals in the selected lineages had no macroplatelets. Evaluation of platelet morphology by blood smears is also part of the complete blood count, and this showed a normal aspect in our study, as well as a normal count.

Understanding immunological characteristics is essential to evaluating inherited or acquired disorders.30 Leukocytes participate in immune and inflammatory processes, being responsible for mediating the innate and adaptive immune response. In mice, the total WBC count is ~2 to 10 x 10^3/mm^3 and, together with the parameters W-SCR, W-MCR and W-LCR, it can be quantified automatically or manually. The differential leukocyte count provided by automated hematological counters is based solely in the size of these cells and must be always confirmed by microscopic analysis of a blood smear.21,31

BALB/c females showed higher WBC rates than other lineages. Similar values were obtained by Spinelli et al (2014) (5.88 ± 1.40 x 10^3/µL), who collected the blood by puncture of the retro-orbital vein plexus; males of the same lineage showed high W-MCR and W-LCR values. Swiss females showed higher W-SCR rates. We could not find any comparable studies using automated assessments of small, medium and large white blood cells.

LYNs are mostly mediators of adaptive immunity,27 and comprise ~70% to 80% of leukocyte counts in mice, which can increase to above 80% in young animals.21,31 MON comprise ~0% to 2% of the total leukocyte count in mice 21,31 and, along with free macrophages and those fixed in the tissues, are responsible for initiating the immune response, and phagocytizing and destroying microorganisms such as bacteria.32 Our automated and microscopic evaluation of lymphocytes and monocytes showed higher W-SCC and MON values in males of the Swiss and B6D2F1 lineages and higher W-MCC and MON values in females. EOS represent ~0% to 7% of the total count of leukocytes in mice.21,31 and, together with BAS, are mediators of the pathogenesis of many inflammatory processes against helminthic infections and allergic diseases.33

The determination of biochemical parameters provides important information on clinical status, nutritional balance, and the metabolic functioning of the organs and tissue, as well as evidence of occult diseases, enabling the monitoring of treatment and prognosis.36,37

Clinical evaluation of renal function is based on measurement of UR, together with CR.38,39 Generated in the liver, UR is the main nitrogen metabolite derived from protein degradation; 90% is excreted by the kidneys, (~40% to 70%) returns to plasma.38 Mice at eight weeks of age have, on average, 0.28 mg/dL CR,39 derived from creatine catabolism. CR is present in large amounts in the skeletal and cardiac muscles, liver and kidneys, and is excreted by the kidneys, especially by glomerular filtration.31 Swiss males presented the highest UR indices. Branco et al (2011) reported similar values (53.00 ± 1.90 mg/dL) using brachial plexus bleeding. However, we could not measure CR in the animals tested, possibly because its concentration was below the minimum (~<0.2 mg/dL) required for detection by the automated system. Hypothetically, the highest CR rates should be expressed in males of the B6D2F1 and Swiss lineages, since they have higher body weights than the other lineages and variations in serum concentration are intimately related to body weight and mass and muscle metabolism.43

The assessment of hepatic function in response to anatomical or biochemical alterations42 is commonly subjected to dosage testing of ALT, AST, AP and TP.43 The origin of ALT is predominantly plasmatic; it is found abundantly in the liver, in moderate amounts in the
kidneys and in small quantities in the heart and skeletal muscula-
ture.\textsuperscript{43,44} AST is an intracellular enzyme present in the cytoplasm and
mitochondria\textsuperscript{44} in various organs and tissues, including liver, kidneys,
heart, brain, skeletal muscle and erythrocytes.\textsuperscript{45} In seven-week-old
mice, ALT and AST concentrations are, on average, of 41 U/L and
152 U/L, respectively.\textsuperscript{152} AP is present in the liver (epithelial cells of
bile duct), bones (osteoblasts), intestines, kidneys and placenta, and
is composed of a group of membrane-associated isoenzymes located
in various tissues; however, only AP present in bone and hepatobi-
liary tissue are important for diagnosis.\textsuperscript{14,36,44} The evaluation of TP
is an important determinant of metabolism homeostasis.\textsuperscript{47} Proteins
are found in all components of cells, being fundamental to their
structures and functions\textsuperscript{48} and are commonly evaluated in conjunc-
tion with ALB and GLB.\textsuperscript{47} In six-week-old mice, the concentrations
of AP and TP are, on average, 86 U/L and 5.22 g/dL, respectively.\textsuperscript{49}
In our evaluation of the parameters related to the liver, BALB/c
females presented higher ALT and AP indices than other lineages.
Barbosa et al (2017) obtained lower values (29.72 ± 4.40 U/L and
2.32 ± 0.85 U/L, respectively) in samples collected by cardio-
centesis.\textsuperscript{57} C57BL/6 females presented higher indices of AST and C57BL/6
males of TP, did not obtain data for AST in C57BL/6 female mice in
the literature. However, Almeida et al (2008) obtained lower results
for TP in males of the same lineage (243.08 ± 51.13 g/dL) in samples
collected by puncture of the retro-orbital vein plexus.

Changes to the ALT, AST, AP and TP can be symptomatic of some
diseases. The rapid elevation of ALT indicates hepatic lesion.\textsuperscript{43,44}
When associated with an increase in AST concentration, it indicates
a profound damage from hepatocytes.\textsuperscript{43} In small animals, increased
AP is observed in inflammatory and degenerative disorders of the
skeletal musculature, hepatocellular dysfunction, and heart diseases
(eg ischemia, congestion, necrosis, neoplasia, trauma).\textsuperscript{45,50}

ALB and GLB are important in maintaining osmotic pressure,
to avoid blood extravasation, and are commonly evaluated in con-
junction with TP as determinants of metabolism homeostasis.\textsuperscript{47} The
plasma reduction of both may have similar causes to a decrease in
TP.\textsuperscript{45,51} ALB is synthesized in the liver and comprises 50% of total
protein in the serum. It is responsible for 80% of colloidal osmotic
pressure and acid-base equilibrium (in metabolic acidosis).\textsuperscript{52} GLB in-
dices are obtained from the difference between TP and ALB; they
are divided in alpha, beta and gamma globulins, and are present in
inflammatory and/or infectious processes.\textsuperscript{51} Swiss males showed
higher ALB indices than other lineages. Diniz et al (2006) reported
similar values (2.78 ± 0.12 g/dL). C57BL/6 females presented higher
GLB indices. We could not find comparable studies in this lineage.

Despite being rarely used in hepatic evaluation, UA is formed
mainly in the liver as a final product of purine catabolism.\textsuperscript{53}
C57BL/6 males presented greater UA indices than other lineages.
Almeida et al (2008) reported lower UA indices (1.54 ± 0.68 mg/
dL). High levels of UA (hyperuricemia) can result from genetically
determined metabolic imbalances, such as enzyme activity and de-

ciciency in renal excretion, leading to deposition of urate crystals
in the articulation (gout), and cardiovascular diseases such as ar-
terial hypertension.\textsuperscript{53}

CHL and TG lipid indices can vary due to their origins, which
can be exogenous (consequence of the diet) or endogenous (pro-
duced by the liver). CHL produced by the liver acts as a precursor
to steroid hormones.\textsuperscript{51} TG can be formed in the cells of the intesti-
nal mucosa, from monoglycerides and long-chain fatty acids ab-
sorbed in alimentary ingestion (exogenous); they can also be formed
in the liver, and transported by blood as low-density lipoproteins
(LDLP) (endogenous).\textsuperscript{54} BALB/c males presented higher CHL indi-
ces than other lineages. Santos et al (2016) described similar values
(135.00 ± 6.00 mg/dL). B6D2F1 males presented higher TG indices.
We could not find comparable studies mentioning parameters for
this index using this lineage.

GLC present in the serum is obtained mainly through the diet,
and to a lesser extent from hepatic glycogen. Its main function is to
generate energy (adenosine triphosphate – ATP) for biological func-
tions.\textsuperscript{55,56} B6D2F1 females showed higher GLC indices than other
lineages. Similar studies reporting GLC values using this lineage
were not found.

Our results and the comparisons with previous studies provide
evidence of the existence of variations among lineages and be-
tween genres of mice. These variations must be considered during
the selection of animals for experimentation, in the evaluation
and observation of the results obtained, and in the analysis of the
modifications resulting from induced or spontaneous pathological
processes.

5 | CONCLUSION

The determination of values for hematological and biochemical pa-
rameters in Swiss, BALB/c, C57BL/6 and B6D2F1 mice is important
in the choice of experimental model and study design because of
the significant differences in these parameters between lineages,
genders and routes of blood collection. Automated analyses should
always be confirmed by microscopy evaluation and compared with
the data from the literature for optimal interpretation. Each biote-
rion should establish its own reference values, since a wide range of
variations in physiological parameters can be due to the conditions
to which the animals are subjected, and this can affect the results
of research. It is important to consider the many variables that di-
rectly interfere with metabolism and, consequently, hematological
and biochemical values: species, age, genetic variation and the en-
vironmental conditions to which the animals are subjected, namely
temperature, relative humidity, ventilation, lighting, noise, manipula-
tion, feeding, water, microbiota, presence of pathogens and contact
with other animals.

ACKNOWLEDGEMENTS

The authors thank the Institute of Microbiology Paulo de Góes –
Universidade Federal do Rio de Janeiro (UFRJ); the Laboratory
of Diphtheria and Corynebacteria of Clinical Relevance –
Universidade do Estado do Rio de Janeiro (UERJ); the Laboratory
of Clinical Research and Molecular Diagnostic; Prof. Marcílio Dias
do Nascimento, Department of Clinical Practice and Pathology, Universidade Federal Fluminense (UFF); Institute of Science and Technology in Biomodels; and National Institute for Quality Control in Health, Fundação Oswaldo Cruz (Fiocruz). This study was financed in part by the National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Level Personnel, Brazil (CAPES), Finance Code 001 and Research Support Foundation for the State of Rio de Janeiro, Brazil (FAPERJ).

CONFLICT OF INTERESTS
The authors declare that there are no conflicts of interests.

ORCID
Giorgio Silva-Santana https://orcid.org/0000-0002-3000-6513

REFERENCES
1. Branco ACS, Diniz MFFM, Almeida RN, et al. Biochemical and hematological parameters of Wistar rats and Swiss mice in the Professor Thomas George Animal Laboratory. Rev Bras Ciênc Saúde. 2011;15(2):209-214.
2. Otto GP, Rathkolb B, Oestereicher MA, et al. Clinical chemistry reference intervals for C57BL/6J, C57BL/6N, and C3HeB/FeJ mice (Mus musculus). J Am Assoc Lab Anim Sci. 2016;55(4):375-386.
3. Diniz MFFM, Medeiros IA, Santos HB, et al. Haematological and biochemical parameter standardization of Swiss mice and Wistar rats. Rev Bras Ciênc Saúde. 2006;10(2):171-176.
4. Santos EW, Oliveira DC, Hastreiter A, et al. Potency assay of epoetin alpha: relationship to mean corpuscular volume among clinical patients of the Oswaldo Cruz Teaching Hospital, Recife. Brazil. Rev Bras Hematol Hemoter. 2010;32(1):34-39.
5. Zou H, Hansson GK. Effect of sex and age on serum biochemical reference ranges in C57BL/6J mice. Comp Med. 2004;54(2):176-178.
6. Ferreira LM, Hochman B, Barbosa MVJ. Experimental models in research. Acta Cir Bras. 2005;20:28-34.
7. Ferrari PF, Palanza P, Rodgers RJ, Mainardi M, Parmigiani S. Comparing different forms of male and female aggression in wild and laboratory mice: an ethopharmacological study. Physiol Behav. 1996;60(2):549-553.
8. Koike H, Ibi D, Mizoguchi H, et al. Behavioral abnormality and pharmacologic response in social isolation-reared mice. Behav Brain Res. 2009;201(1):114-121.
9. Cossins RA, Teixeira DNS, Cota UA, Chica JEL. Reference values for blood-based biochemical parameters in BALB/c and C57BL/6 wild-type mice. J Bras Patol Med Lab. 2008;44(6):429-432.
10. Restell TI, Porfirio LC, Souza AS, Silva IS. Hematology of Swiss mice (Mus musculus) of both genders and different ages. Acta Cir Bras. 2014;29(5):306-312.
11. Spinelli MO, Motta MC, Cruz RJ, Godoy CMSC. 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. Acta Sci Health Sci. 2014;36(1):1-4.
12. Silva-Santana G, Aguiar-Alves F, Lenzi-Almeida KC, et al. Pathological profiles of systemic infections by Panton-Valentine leukocidin-positive, methicillin-resistant Staphylococcus aureus strains in a murine model. J Appl Microbiol. 2020;128(6):1820-1842.
13. Chorilli M, Michelin DC, Salgado HRN. Animações de laboratório: o camundongo. Ver Ciênc Farm Básica Apl. 2007;28(1):11-23.
14. Shahsavani D, Kazerani HR, Kaveh S, Gholi-pour-Kanani H. Determination of some normal serum parameters in starry sturgeon (Acipenser stellatus Pallas, 1771) during spring season. Comp Clin Pathol. 2010;19:57-61.
15. Weiss DJ, Wardrop KJ. Schalm’s Veterinary Hematology. 6th ed. Ames, IA: Wiley Blackwell; 2010.
16. Ribeiro-Alves MA, Gordan PA. Diagnosis of anemia in patients with chronic kidney disease. J Bras Nefrol. 2014;36(1 Suppl 1):9-12.
17. Carvalho RS, Macedo LP, Teixeira FA, Binda MB, Coelho CS. Mean corpuscular volume (MCV) and red blood cell distribution width (RDW) in quarter horses used for barrel racing. Cienc Anim Bras. 2016;17(3):411-417.
18. Monteiro L. Reference values for RDW-CV and RDW-SD and their relationship to mean corpuscular volume among clinical patients of the Oswald Cruz Teaching Hospital, Recife, Brazil. Rev Bras Hematol Hemoter. 2010;32(1):34-39.
19. Silva M, Couto NMR. Evaluation of RDW as a indicator of the iron deficiency in patients with chronic renal failure under hemodialysis. RBAC. 2016;48:211-215.
20. Shalm OW. Differential diagnosis of anemia in cattle. J Am Vet Med Assoc. 1972;161(11):1269-1275.
21. Monteiro L. Reference values for the platelet indices and algorithm for plateletgram evaluation. RBAC. 2017;49(3):263-267.
22. Kaplan ZS, Jackson SP. The role of platelets in atherothrombosis. Hematology Am Soc Hematol Educ Program. 2011;2011:51-61.
23. Wendland AE, Farias MG, Manfroi WC. Mean platelet volume and cardiovascular disease. J Bras Patol Med Lab. 2009;45(5):371-378.
24. Alamooti AA, Ardalan FA, Abboudi A, Firoozjaie F. Determination of lymphocyte subsets reference values in healthy Iranian men by a single platform flow cytometric method. Cytometry A. 2012;77(9):890-894.
25. Burtis CA, Ashwood ER, Bruns DE. Tietz Fundamentals of Clinical Chemistry. 6th ed. Philadelphia, PA: W.B. Saunders Company; 1978;1979:690-694.
26. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol. 2006;24:147-174.
27. Souza C, Lopes STA, Batina PN, et al. Parasitic stress in Saanen goats: hematologic evaluation and netrophilic function. J Am Vet Med Assoc. 2006;12(2):17-23.
28. Feldman BF, Zinkl JG, Jain NC. Schalm’s Veterinary Hematology. 5th ed. Baltimore, MA: Lippincott Williams & Wilkins; 2000.
29. González FHD, Carvalho V, Moller VA, Duarte FR. Perfil bioquímico sanguíneo de cães e gatos na cidade de Porto Alegre, Rio Grande do Sul, Brasil. Arq Fac Vet UFRGS. 2001;29:1-6.
30. Sodré FL, Costa JCB, Lima JCC. Evaluation of renal function and damage: a laboratorial challenge. J Bras Patol Med Lab. 2007;43(5):329-337.
31. Burtis CA, Ashwood ER, Burns DE. Tietz Fundamentos de Química Clínica. 6th ed. Rio de Janeiro, Brazil: Elsevier; 2008.
40. Riken BioResource Center/Experimental Animal Division. Blood chemistry, hematology, and body size for 33 RBRC wild-derived mouse strains. MPD: 37811. Mouse Phenome Database web site, The Jackson Laboratory, Bar Harbor, Maine USA; 2008. http://phenome.jax.org. Accessed April 8, 2020.

41. Guyton SC, Hall JE. Tratado de Fisiologia Médica. 11th ed. Rio de Janeiro, Brazil: Elsevier; 2006.

42. Ming-Hong Y, Ting-Chun W, Sin-Yie L, Chee-Yin C, Chun-Ching L. The hepatoprotective effect of Bupleurum kaoi, an endemic plant to Taiwan, against dimethylnitrosamine-induced hepatic fibrosis in rats. Biol Pharm Bull. 2005;28(3):442-448.

43. Schumann G, Bonora R, Ceriotti F, et al. International Federation of Clinical Chemistry and Laboratory Medicine. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 Degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 6. Reference procedure for the measurement of catalytic concentration of Gamma-Glutamyltransferase. Clin Chem Lab Med. 2002;40(7):734-738.

44. Couto JLA, Vieira RCS, Barbosa JM, Machado SS, Ferreira HS. Liver function abnormalities in undernourished and Schistosoma mansoni-infected mice. Rev Soc Bras Med Trop. 2008;41(4):390-393.

45. Harris DJ. Clinical tests. In: Tully TN, Dorrestein GM, Jones AK, eds. Handbook of avian medicine. 2nd ed. Philadelphia, PA: Saunders Elsevier; 2009:79-82.

46. Rusyn I, Threadgill DW. Effects of trichloroethylene in males of 17 inbred mouse strains. MPD: Rusyn 2. Mouse phenome database web site, The Jackson Laboratory, Bar Harbor, Maine USA; 2010. http://phenome.jax.org. Accessed April 11, 2020.

47. Fudge AM. Blood testing artifacts: interpretation and prevention. Seminars. In: Avian and exotic pet medicine. Vol. 3. 1994:2-4.

48. Ravel R. Clinical Laboratory Medicine: Clinical Application of Laboratory Data. 6th ed. St Louis, MO: Mosby; 1995.

49. Yuan R, Korstanje R. Aging study: Blood chemistry for 32 inbred strains of mice. MPD: Yuan 3. Mouse Phenome Database web site, The Jackson Laboratory, Bar Harbor, Maine USA; 2008. http://phenome.jax.org. Accessed April 11, 2020.

50. Coles EH. Veterinary Clinical Pathology. 4th ed. Philadelphia, PA: W. B. Saunders; 1986.

51. Junghanns MK. Aids to diagnosis. In: Coles BH, ed. Essentials of Avian Medicine and Surgery. 3rd ed. Iowa: Blackwell Science; 2007:56-71.

52. Santos NSJ, Draibe AS, Kamimura MA, Cuppari L. Serum albumin as nutritional marker of hemodialysis patients. Rev Nutr. 2004;17(3):339-349.

53. Marion M, Carvalho JAM, Bochi GV, Sangoi MB, Moresco RN. Uric acid as a risk factor for cardiovascular diseases and metabolic syndrome. Rev Bras Farm. 2011;92(1):3-8.

54. Schiavo M, Lunardelli A, Oliveira JR. The influence of diet on the triglycerides serum concentration. J Bras Patol Med Lab. 2003;39(4):283-288.

55. Pegoraro NCC, Gascón TM, Sant’Anna AVL, et al. Comparative evaluation of glucose measurements in serum and plasma of patients from the ABC School of Medicine laboratory. Rev Bras Farm. 2011;92(1):9-12.

56. Cardoso GP, Silva Junior CT, Cardoso RBB. Acute hyper and hypoglycemic states: current conduct. J Bras Med. 2013;101:41-45.