# HEMATOLOGICAL AND PLASMA BIOCHEMICAL VALUES IN FOUR SPECIFIC PATHOGEN-FREE MICE STRAINS PRODUCED IN THE ANIMAL FACILITY AT CANTACUZINO INSTITUTE, BUCHAREST

# Cristin COMAN<sup>1</sup>, Ene VLASE<sup>1</sup>, Angelica DINU<sup>1</sup>

<sup>1</sup>, Cantacuzino" National Medical-Military Institute for Research and Development, Animal Facility, 103 Splaiul Independentei, District 5, 050096, Bucharest, Romania

Corresponding author email: comancristin@yahoo.com

#### Abstract

Laboratory mice are still the most used animal in research and animal experiments. In most experiments, knowledge of hematology and biochemistry data base is essential to achieving quality results and used the research results. Cantacuzino Institute from Bucharest is the most important provider of laboratory mice in Romania and therefore making it available to the users the hematological and biochemical physiological data of the mice strains from his animal facility was an ethical obligation. Hematologic analysis and biochemical from blood were performed in 4 strains of mice (2 inbred and 2 outbred) in both sexes and age groups, in dynamics. Hematologic analyses were made from whole blood and biochemical ones from blood plasma. The results showed differences between the lines, sexes and age categories. The results obtained can be used by the researchers on selecting the mice strains according to the purpose of the research and according to the values of the hematological and biochemical parameters. The results thereby helping to reduce stress in animals.

Key words: mice, hematological values, biochemical values.

# INTRODUCTION

Laboratory mouse is the most common mammals for scientific purposes, whether we are talking about research, monoclonal antibody production or drug and medical devices testing. This small mammal has several qualities that make it be so used: multiplying fast, short duration of generations, the existence of many inbred lines, knowledge of the genome and extensive knowledge of its physiological and immunological characteristics (Moore et al., 2000).

The determination of the hematological and biochemical profile of mice is necessary because it is possible to characterize the animal populations used in the research, to establish research results, to anticipate pathological conditions of the animal colonies and to monitor the effectiveness of a possible treatment (Mazzacara et al., 2008).

Each institution must establish the hematological and biochemical profile of its colonies because this profile is influenced by the strain of mice, age, health, nutrition, environmental conditions, etc. Reduction of variability is a standardization condition, and if

environmental conditions (air changes, temperature, relative humidity, noise and illumination) are set by regulations, other variability factors can be avoided by providing stable livestock strains with a constant profile (O'Connell et al, 2015). Reference values may be for a researcher an important research tool to initiate studies, especially if the lab does not have enough historical data on the hematological and biochemical profile.

Cantacuzino Institute from Bucharest is the largest producer and supplier of laboratory animals in Romania. With the 4 strains of mice (2 inbred and 2 outbred) we provide the same feed that we give to the animals in our facility and the same type of bedding. Our main users of mouse strains are research institutes that used mice for immunological, metabolic, oncological studies, etc., using inbred mice (BALB/c and C57Bl/6 strain) and human and veterinary medicines manufacturing plants for product safety testing using outbred lines (CD1 and NMRI). The purpose of this study was to determine hematological values (22 determined and calculated parameters) and biochemical (6 metabolic profile parameters) in 4 strains of mice, 2 inbred (BALB/c and C57BL/6) and 2 outbred (CD1 and NMRI) in both sexes at the age of 8-9 weeks and 20-21 weeks, strain breaded at specific pathogen free (SPF) animal facility of Cantacuzino Institute, Bucharest.

Establishing the hematological and biochemical profile will help researchers to choose their mice according to their intended profile, ensure the validity and reproducibility of experiments and tests, and help implement 3R by avoiding repeat analyses at the start of studies and implicitly reducing stress in animals (Schnell et al., 2002). Establishment of the basic data line is very important in the evaluation of the result of nonclinical experiments, in the confidence of test results.

## MATERIALS AND METHODS

Mice

The initial colonies with which our SPF animal facility was populated were bought by the Cantacuzino Institute in 2014 from Charles River Germany. The animals used in these studies were derived from 10-generation colonies. Mice were maintained under strict barrier conditions at animals without specific pathogenic germs, confirmed status quarterly control performed according to the recommendations of FELASA (Mahler et al., 2014). There were four groups of animals, one for each strain analysed (CD1, NMRI, BALB/c and C57BL/6). Each group was composed of 40 animals, (20 male and 20 female) that provided the statistically significant number of the study. The groups were formed at the age of 8-9 weeks of the animals, when we did the first analysis. The second was done in the same animals at the age of 20-21 weeks. The mice from these lots were the negative control groups from 4 different experiments, studies approved by the Ethics Commission of Cantacuzino Institute and authorized Veterinary Authority from Bucharest.

Housing and husbandry

The animals were kept in polysulfones cages, 10 mice in the cage, and were individually identified by ear punch. Food from the Cantacuzino Institute was administered *ad libitum*, as well as water that is acidified and is administered in bottles. Lighting system is 12 hours light/12 hours dark, temperature 20±2° C, relative humidity 55 +/- 10%. Mice were daily

evaluated for sanitary condition and weighed monthly.

Blood collection

Sample blood collection was done after a 12-14 hour diet. The harvesting method was the retroorbital one. After total anesthesia with an equal combination of ketamine and acepromazine, in a final volume of 0.1 ml. a total approximate amount of 200 microliters was harvested, sufficient for both types of hematological and biochemical analysis.

Hematological parameters

For the hematological determinations the blood sample were harvested on EDTA. Analyses have been made on an IDEXX Procyte 5 Diff on the same day as the harvest.

The samples were analysed hematological, being determined direct or calculated values for the following parameters: red blood cells count (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), hemoglobin corpuscular concentration (MCHC), red cell distribution width (RDW), platelets count (PLT), mean platelet volume (MPV), white blood cells count (WBC), neutrophils (Ne), lymphocytes (Ly), monocytes (Mo), eosinophils (Eo), basophils (Ba), reticulocyte (RETIC), platelet distribution width (PDW), platelet hematocrit (PCT).

Biochemical parameters

For biochemical analysis, blood was collected in vacutainers with lithium-heparin. The blood was centrifuged at 12.000 rpm for 2 minutes at 40C and then plasma was extracted. The determinations were made on an IDEXX VetTest Chemistry Analyzer apparatus. The parameters analysed are: Glucose (GLU), Uric Acid (URIC), Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST), Cholesterol (CHOL), Triglyceride (TRIG). The analyses were done on the same day as the harvest. *Statistics* 

Statistical analyses were performed with Microsoft Excel, current version. In each mouse strain, the determination of the hematological and biochemical value were

expressed as mean  $\pm$  standard deviation (Mean  $\pm$  SD) and also minimum and maximum values.

# RESULTS AND DISCUSSIONS

The results of the analyses are highlighted in Tables 1-8.

Table 1. CD1 strain – hematology value

Parameter		8 – 9 v	veeks		20-21 weeks			
	Male	;	Fema	le	Ma	le	Female	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
RBC 10^12/L	$9.13\pm0.44$	7.11-13.2	8.91±0.29	7.67-11.6	9.52±0.24	7.91-13.97	9.12±0.29	7.42-13.4
HCT %	51.22±0.23	35.2-68.4	$48.76\pm0.44$	37.7-72.5	$56.68\pm0.78$	39.9-74.6	53.44±0.87	41.5-76.4
HGB g/dL	$13.84\pm0.6$	11.1-17.3	$14.11\pm0.32$	11.6-18.1	$14.34\pm0.56$	11.8-17.8	$15.01\pm0.11$	12.1-18.7
MCV fL	52.83±0.12	46.5-55	54.52±0.28	44.3-60.1	56.2±0.49	46.2-61.3	57.11±0.59	46.1-64.2
MCH pg	14.95±0.37	12.9-15.5	$15.22\pm0.27$	13.2-15.9	$15.36\pm0.76$	13.6-17.1	15.85±1.33	13.9-17.4
MCHC g/dL	23.47±1.21	19.1-26.3	24.17±1.82	20.2-27.9	24.53±0.87	20.4-28.1	25.11±0.91	22.6-29.7
RDW %	$15.2\pm0.22$	14.3-17.8	16.1±1.45	15.1-19.3	$16.22\pm0.67$	14.5-18.3	$16.7\pm0.45$	15.8-20.1
RETIC %	$3.75\pm0.31$	3.2-4.2	$3.47\pm0.21$	3.1-4.3	$4.72\pm0.21$	3.6-4.91	4.47±0.67	3.7-4.9
RETIC K/μL	316.85±17.39	288.4-351	349.23±11.21	291-366	$347.8 \pm 15.3$	298.1-371.3	363.2±22.11	311-384
WBC 10^9/L	$9.16\pm0.79$	3.96-13.1	8.13±2.15	4.1-13.6	10.89±1.44	5.7-14.2	11.22±0.35	5.9-15.2
NEU %	25.11±3.32	16.2-31.2	22.39±1.87	17.3-28.7	23.97±2.76	17.9-32.5	20.76±1.96	14.5-25.4
LYM %	65.23±1.65	43.1-76.1	67.44±2.34	44.8-78.3	$66.07\pm2.82$	47.8-79.2	68.2±3.12	48.3-85.4
MONO %	$8.36\pm1.42$	3.9-12.7	$9.02\pm0.62$	4.2-12.8	$8.11\pm0.56$	4.9-13.9	$9.61\pm2.42$	5.8-14.5
EOS %	$0.82\pm0.64$	0.3-3.1	$0.64\pm0.54$	0.2-3.4	$1.11\pm0.54$	0.2-2.5	$0.97\pm0.67$	0.2-2.8
BASO %	$0.48\pm0.72$	0.01-1.3	$0.51\pm0.64$	0-1.26	$0.74\pm1.11$	0-1.2	$0.78\pm1.02$	0-1.03
NEU 10^9/L	$2.56\pm0.28$	0.24-4.87	2.41±1.16	0.36-5.12	$3.45\pm0.87$	0.45-5.23	3.65±1.22	0.65-5.38
LYM 10^9/L	$4.97\pm0.95$	2.43-9.87	5.27±1.46	2.11-10.2	6.61±1.78	2.97-10.4	5.76±1.65	2.65-10.4
MONO10^9/L	$0.53\pm0.11$	0.02-0.66	$0.67\pm0.54$	0.11-0.94	$0.98\pm0.65$	0.34-1.56	$1.16\pm0.43$	0.36-1.23
EOS 10^9/L	$0.09\pm0.06$	0.01-0.43	$0.12\pm0.46$	0.01- 0.6	$0.15\pm0.67$	0.01-0.79	$0.17\pm0.65$	0.03-0.82
BASO 10^9/L	$0.02\pm0.003$	0-0.09	$0.03\pm0.01$	0-0.12	$0.02\pm0.02$	0-1.4	$0.04\pm0.01$	0-0.09
PLT K/μL	1381.7±118.36	912-1891	1411±145.46	956-1986	1572±132.12	854-1954	1480±166.32	865-2164
MPV fL	$5.11\pm0.11$	4.9-5.68	5.27±0.26	4.3-6.3	$4.87\pm0.58$	4.2-6.1	$4.71\pm0.64$	4.09-6.22
PDW fL	7.19±0.41	6-8.1	$7.54\pm0.43$	5.9-8.4	$7.45\pm0.65$	6.01-8.23	$7.12\pm0.42$	5.91-8.11
PCT %	$0.65\pm0.33$	0.53-0.87	$0.51\pm0.43$	0.42-0.76	0.61±0.76	0.48-0.88	0.54±0.54	0.41-0.91

Table 2. CD1 strain – biochemical value

Parameter	8-9 weeks			20-21 weeks				
	Male		Female		Male		Female	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
GLU mmol/L	$8.64\pm0.45$	8.11-9.89	$8.46\pm0.21$	8.08-9.17	$9.84\pm0.67$	8.71-10.29	$9.12\pm0.34$	8.67-9.76
URIC µmol/L	121.21±0,3	118-135	$110\pm0.76$	97-123	134±0.94	119-142	129±1.54	107-145
ALT U/L	41.32±0.76	30-61	38±1.12	28-65	57±1.39	35-78	49.24±0.98	36-72
AST U/L	82.21±0.56	69-98	86.11±0.86	71-93	$95\pm0.82$	61-102	93±0.45	76-112
CHOL mmol/L	$1.49\pm0,85$	1.32-1.65	$1.87\pm0.31$	1.23-1.96	$2.19\pm0,32$	1.76-2.21	$2.21\pm0.81$	1.56-2.36
TRIG mmol/L	$0.76\pm0.73$	0.61-0.91	$0.85\pm0.45$	0.68-1.16	$0.96\pm0.98$	0.78-1.35	$1.15\pm0.23$	1.02-1.43

Table 3. NMRI strain - hematology value

Parameter		20-21 weeks						
	Male	e	Fema	le	Ma	le	Female	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
RBC 10^12/L	$7.89\pm0.44$	6.93-8.66	7.94±0.53	6.97-8.64	$8.72\pm0.24$	7.11-9.12	$9.23\pm0.19$	8.12-11.01
HCT %	37.23±1.98	33.1-40.1	$36.93\pm2.67$	31.1-40.8	46.66±1.48	36.2-56.9	49.87±1.35	39.6-64.3
HGB g/dL	11.87±0.54	10.6-12.6	$12\pm0.76$	10.8-13.3	13.24±0.61	11.1-16.9	15.1±0.5	11.9-18.4
MCV fL	47.22±1.65	44.2-50.4	46.5±1.2	44.3-49	56.1±1.23	46.8-63.7	55.34±0.82	49.2-67.1
MCH pg	$15.06\pm0.52$	14.2-15.9	15.11±0.38	14.8-15.6	$15.9\pm0.36$	14.6-17.4	16.25±.034	15.21-18.1
MCHC g/dL	$31.90\pm0.67$	31-33.2	32.53±0.79	30.9-33.5	34.13±0.37	32.8-39.7	34.12±0.45	32.1-40.2
RDW %	23.49±1.94	29.9-26.4	23.48±1.44	19.9-25.2	27.87±0.92	18.7-29.9	$24.6\pm0.87$	21.8-26.8
RETIC %	5.46±1.57	2.8-7.8	4.92±1.49	2.9-8.9	$5.32\pm1.34$	2.8-8.2	$5.05\pm0.24$	3.2-5.8
RETIC K/μL	432.42±29.84	224.9-632	391.75±27.82	194-569	$443.32\pm28$	312498	423.2±24.5	376-497
WBC 10^9/L	7.77±2.08	4.53-13.8	7.33±1.97	4.63-10	$9.12\pm1.53$	4.97-12.9	$9.12\pm0.95$	5.65-11.87
NEU %	27.87±6.7	17.5-46	21.03±6.45	12-33.5	27.36±4.87	19.23-35	24.22±5.76	17.23-32.3
LYM %	67.02±7.44	45.6-77.5	$73.70\pm6.87$	59.6-84.9	67.77±7.33	59.12-79.	70.55±5.98	47.23-82
MONO %	2.33±1.31	1.2-7.1	$1.8\pm0.81$	1-4.3	$2.12\pm0.54$	1.8-8.2	$2.19\pm1.87$	1.2-9.7
EOS %	2.35±0.80	1.2-4.7	$3.06\pm0.96$	1.3-4.9	$2.32\pm0.65$	0.8-2.9	$2.66\pm0.23$	1.1-3.2
BASO %	$0.42\pm0.31$	0-0.9	$0.4\pm0.21$	0-0.8	$0.44\pm0.23$	0-1	$0.38\pm0.26$	0-0.8
NEU 10^9/L	2.14±0.73	1.15-4.09	$1.46\pm0.37$	0.9-2.06	$2.45\pm0.65$	0.76-5.76	$1.87\pm0.65$	1.15-3.4
LYM 10^9/L	5.22±1.58	2.76-9.58	5.5±1.8	1.98-7.49	$5.61\pm1.01$	2.17-10.3	5.93±1.11	2.11-10.65
MONO10^9/L	$0.18\pm0.12$	0.06-0.26	$0.12\pm0.03$	0.06-0.19	$0.14\pm0.65$	0.04-0.8	$0.17\pm0.27$	0.09-0.9
EOS 10^9/L	$0.18\pm0.09$	0.1-0.46	$0.22\pm0.08$	0.1-0.37	$0.17\pm0.07$	0.08-0.53	$0.25\pm0.21$	0.08-0.52
BASO 10^9/L	$0.03\pm0.02$	0-0.09	$0.03\pm0.01$	0-0.06	$0.02\pm0.03$	0-0.1	$0.04\pm0.04$	0-0.1
PLT K/μL	440±186.65	171-819	452±155.26	213-707	932±152	345-1176	980±152	413-1236
MPV fL	$7.28\pm0.30$	6.8-7.8	$7.04\pm0.48$	6.5-8.4	$7.11\pm0.71$	5.2-8.9	$7.23\pm0.87$	4.75-9.3
PDW fL	8.43±0.88	7.1-10.1	$7.83\pm0.95$	6.5-10.1	$8.22\pm0.15$	7.23-9.9	$7.96\pm0.39$	6.26-9.12
PCT %	$0.32\pm0.13$	0.13 - 0.47	$0.33\pm0.09$	0.2-0.47	$0.39\pm0.87$	0.21-0.76	$0.43\pm0.58$	0.3-0.82

Table 4. NMRI strain – biochemical value

Parameters	8-9 weeks				20-21 weeks				
	Male		Female		Male		Female		
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	
GLU mmol/L	11.24±3.34	6.84-14.06	11.88±3.6	7.75-14.66	12.24±1.32	7.13-15.7	12.68±2.6	8.12-15.2	
URIC µmol/L	66.88±33.93	12-122	49.73±31.19	19-164	98.12±23.93	65-187	87.73±26.65	65-191	
ALT U/L	45±13.05	23-87	44±20.75	24-91	45±3.23	32-78	44±13.75	24-89	
AST U/L	108.75±4.65	66-223	87.18±3.6	60-169	123.32±4.62	82-197	107.21±2.56	80-160	
CHOL mmol/L	$2.80\pm0.47$	1.99-3.61	2.44±0.23	2.1-2.82	2.91±0.65	1.72-2.18	2.57±0.29	2.21-2.76	
TRIG mmol/L	$0.65\pm0.16$	0.35-0.95	$0.96\pm0.22$	0.5-1.26	$0.85\pm0.34$	0.64-1.32	$0.98\pm0.27$	0.65-1.58	

Table 5. BALB/c strain - hematology value

Parameter		20-21 weeks						
	Ma	le	Fema	ile	Ma	le	Female	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
RBC 10^12/L	$9.01\pm0.64$	7.87-11.96	10.12±0.59	7.11-11.8	$9.89\pm0.74$	7.21-12.7	10.54±0.28	7.42-11.4
HCT %	54.67±0.28	31.8-69.1	52.49±0.71	30.1-71.2	55.11±0.19	33.7-70.3	54.69±1.24	34.5-73.4
HGB g/dL	$14.98\pm0.75$	11.8-17.66	15.65±0.65	10.6-17.5	15.04±0.36	10.9-17.1	14.45±0.41	10.7-17.8
MCV fL	51.67±0.22	45.3-56.1	51.43±0.42	42.4-62.3	53.9±0.56	41.9-62.8	53.47±0.67	44.7-65.8
MCH pg	14.57±0.43	12.1-16.2	$14.32\pm0.66$	13.1-16.7	15.45±0.53	12.9-17.1	14.67±0.83	12.3-17.1
MCHC g/dL	24.87±1.68	19.6-29.88	24.48±2.45	19.4-33.1	25.44±1.83	20.9-31.2	25.78±1.44	21.2-30.5
RDW %	$17.8\pm0.77$	13.8-19.1	17.9±0.66	15.1-19.3	18.12±0.33	15.2-19.5	17.7±0.52	15.2-19.6
RETIC %	$2.85\pm0.64$	2.2-4.5	2.17±0.66	1.7-3.9	$3.32\pm0.31$	2.11-4.23	$3.47\pm0.66$	2.08-4.49
RETIC K/μL	310.42±36.76	257.2-366.8	328.76±28.55	278.9-378	$328.64 \pm 25$	281-381	343.8±32.56	321-396
WBC 10^9/L	$7.81\pm0.67$	3.87-12.65	$6.73\pm0.89$	3.3-11.9	$8.81\pm0.66$	4.9-12.3	$7.61\pm0.69$	4.2-12.5
NEU %	26.71±4.51	18.7-34.8	24.13±2.33	16.7-24.6	26.22±1.87	18.6-29.7	23.66±1.11	16.9-27.3
LYM %	66.12±0.8	49.7-68.7	66.23±1.34	51.7-75.3	66.11±1.74	51.2-78.3	66.5±2.05	52.8-81.4
MONO %	6.18±1.89	4.2-9.4	$8.45\pm0.87$	5.3-10.2	$6.28\pm0.49$	5.3-9.3	$8.66\pm0.87$	4.8-10.2
EOS %	$0.76\pm0.65$	0.3-2.2	$0.87\pm0.54$	0.3-2.8	$1.15\pm0.99$	0.2-3.2	$0.9\pm0.52$	0.4-2.9
BASO %	$0.22\pm0.89$	0.01-1.4	$0.31\pm0.47$	0.01-1.11	$0.24\pm0.11$	0-0.9	$0.28\pm0.38$	0-1.02
NEU 10^9/L	2.12±0.26	0.34-3.17	1.91±1.52	0.25-4.71	$2.35\pm0.93$	0.41-5.11	1.57±1.78	0.41-5.34
LYM 10^9/L	5.28±2.67	1.98-6.17	4.77±3.83	1.67-9.02	$6.02\pm1.65$	3.18-10.2	4.75±1.32	2.11-9.42
MONO10^9/L	$0.44\pm0.31$	0.02-0.87	$0.35\pm0.18$	0.05-0.63	$0.78\pm0.25$	0.31-1.2	$0.56\pm0.54$	0.23-0.98
EOS 10^9/L	$0.04\pm0.09$	0.00-0.3	$0.06\pm0.11$	0.01- 0.43	$0.08\pm0.17$	0.00-0.42	$0.09\pm0.55$	0.02-0.76
BASO 10^9/L	$0.01\pm0.004$	0-0.08	$0.02\pm0.01$	0-0.9	$0.03\pm0.01$	0-0.87	$0.02\pm0.01$	0-0.87
PLT K/μL	1118±89.65	872-1532	1265±78.94	911-1652	1342±92.34	893-1756	1143±88.82	781-1846
MPV fL	4.65±0.09	4.2-5.11	$4.87\pm0.22$	4.2-5.67	$4.94\pm0.78$	3.9-6.3	$4.99\pm0.83$	3.87-5.76
PDW fL	$6.88\pm0.38$	5.7-7.9	$7.13\pm0.67$	5.8-8.7	$7.13\pm0.98$	5.67-8.26	7.87±1.1	5.82-8.67
PCT %	$0.83\pm0.84$	0.45-0.98	$0.67\pm0.65$	0.32-0.91	$0.87\pm0.26$	0.48-1.13	$0.69\pm0.38$	0.33-0.93

Table 6. BALB/c strain – biochemical value

Parameter		8 - 9  w	veeks		20-21 weeks			
	Male		Female		Male		Female	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
GLU mmol/L	8.11±0.25	7.34-9.66	$8.23\pm0.28$	7.93-9.01	$9.12\pm0.72$	8.21-9.76	$9.32\pm0.56$	8.73-9.87
URIC µmol/L	$111.64\pm0,45$	93-127	115±0.96	101-129	$128\pm0.41$	104-138	131±0.39	115-141
ALT U/L	44.19±1.36	33-72	$42\pm1.88$	32-68	$49\pm3.43$	29-81	48.14±2.18	33-78
AST U/L	90±1.55	76-104	$86\pm0.57$	76-101	$95\pm3.72$	69-111	$95\pm0.76$	81-118
CHOL mmol/L	$1.28\pm1,84$	1.12-1.76	1.37±1.68	1.11-2.54	$1.99\pm2,67$	1.14-2.98	$1.85\pm0.67$	1.35-2.41
TRIG mmol/L	$0.97\pm0.74$	0.85-1.98	$0.95\pm0.87$	0.75-1.76	1.46±1.78	0.82-1.87	$1.26\pm0.84$	1.06-1.87

Table 7. C57BL/6 strain – hematology value

Parameters		8 – 9 v	veeks		20-21 weeks				
	Male	Male		Female		Male		Female	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	
RBC 10^12/L	$8.72\pm0.24$	8.34-9.17	8.81±0.27	8.46-9.39	$9.94\pm0.49$	9.31-10.8	10.37±0.99	8.04-11.34	
HCT %	37.22±1.23	35.4-39.6	38.12±1.23	36.8-40.8	42.1±1.92	39.9-46.1	44.41±4.84	33.4-50	
HGB g/dL	12.44±0.4	11.8-13.1	12.88±0.37	12.5-13.7	14.37±0.67	13.4-15.5	15.43±1.38	12.2-16.9	
MCV fL	42.64±0.52	42-43.8	43.23±0.38	42.6-43.8	42.36±0.6	41.9-43.8	42.77±0.87	41.5-44	
MCH pg	14.25±0.27	13.9-14.5	14.61±0.11	14.5-14.8	$14.46\pm0.1$	14.3-14.7	$14.89\pm0.14$	14.8-15.2	
MCHC g/dL	$33.42\pm0.36$	32.8-34	$33.8 \pm 0.23$	33.5-34.3	34.12±0.39	33.6-34.6	$34.82\pm0.84$	33.7-36.5	
RDW %	22.79±0.67	21.9-24.3	$22.42\pm0.42$	21.7-23.3	26.03±0.91	24.7-27	25.15±2.09	20.1-27.1	
RETIC %	$3.75\pm0.31$	3.2-4.2	$3.62\pm0.25$	3-3.9	$2.79\pm0.26$	2.6-3.3	2.91±0.31	2.2-3.4	
RETIC K/μL	326.85±27.19	283.4-358	318.19±22.1	261-341	250.22±82	231.2-338	301.76±44.5	213.1-340	
WBC 10^9/L	$3.56\pm0.77$	1.96-4.41	$3.07\pm0.68$	1.88-4.05	$6.62\pm2.59$	2.38-9.61	5.99±1.89	3.55-9.03	
NEU %	21.61±3.82	14.2-28	22.61±5.11	15.7-28.9	$20\pm10.07$	9.9-39.5	22.22±9.42	9.3-38.1	
LYM %	$76.23\pm3.71$	69.9-83.1	74.87±4.97	66.9-81.7	76.66±10.5	57.6-88.4	74.71±10.23	55.4-82.8	
MONO %	$0.87\pm0.4$	0.5-1.9	$0.95\pm0.21$	0.6-1.3	$0.89\pm0.33$	0.4-1.6	$0.87\pm0.39$	0.3-1.6	
EOS %	$0.85\pm0.6$	0.3-2.4	$1.28\pm0.87$	0.3-3.4	2.22±1.65	0.6-3	$1.96\pm1.1$	0.6-4.5	
BASO %	$0.44\pm0.32$	0-1	$0.29\pm0.24$	0-0.7	$0.23\pm0.16$	0-0.5	$0.23\pm0.14$	0-0.5	
NEU 10^9/L	$0.77\pm0.25$	0.44-1.17	$0.71\pm0.28$	0.46-1.26	$1.14\pm0.37$	0.62-1.72	1.23±0.41	057-1.86	
LYM 10^9/L	$2.71\pm0.55$	1.47-3.33	2.28±0.45	1.34-2.73	5.26±2.44	1.37-8.19	$4.57\pm1.8$	2.71-7.66	
MONO10^9/L	$0.03\pm0.01$	0.02-0.06	$0.02\pm0.008$	0.02-0.04	$0.06\pm0.04$	0.01-0.11	$0.05\pm0.03$	0.02-0.1	
EOS 10^9/L	$0.03\pm0.02$	0.01-0.1	$0.04\pm0.02$	0.01-0.09	$0.14\pm0.11$	0.03-0.39	$0.19\pm0.06$	0.02-0.27	
BASO 10^9/L	$0.01\pm0.008$	0-0.03	$0.009\pm0.00$	0-0.02	$0.01\pm0.007$	0-0.03	$0.01\pm0.01$	0-0.04	

PLT K/μL	1191.7±208	902-1535	895.6±102.7	717-1050	970±126	778-1129	634.55±179	336-829
MPV fL	$5.89\pm0.12$	5.7-6.1	$6.02\pm0.11$	5.8-6.1	$6.08\pm0.25$	5.8-6.5	6.48±0.26	6.1-6.8
PDW fL	$6.09\pm0.11$	6-6.3	6.19±0.12	6-6.4	$6.74\pm0.33$	6.4-7.3	$7.44\pm0.51$	6.5-7.9
PCT %	$0.70 \pm 0.12$	0.53-0.91	0.54+0.06	0 44-0 63	0.59+0.07	0.48-0.71	0.41+0.11	0.22-0.54

Table 8. C57BL/6 strain - biochemical value

Parameter	8-9 weeks				20-21 weeks				
	Male		Female		Ma	Male		Female	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	
GLU mmol/L	$7.04\pm0.71$	6.62-8.57	$8.46\pm1.83$	4.59-9.89	8.93±1.49	6.49-10.5	$9.78\pm2.4$	8.04-10.24	
URIC µmol/L	59.36±17.48	33-87	74.1 <sup>b</sup> ±35.1	41-140	92.2°±38.39	31-139	82.8±45.66	27-159	
ALT U/L	40.18±3.78	27-44	47.1±4.2	37-53	48.8±2.3	36-52	52.7±3.6	38-57	
AST U/L	50±9.99	32-67	44.7±3.9	37-56	55.28±11.5	35-61	49.4±4.33	41-54	
CHOL mmol/L	$1.19\pm0.19$	0.91-1.46	$0.86\pm0.27$	0.36-1.31	$1.78\pm0.25$	1.51-2.37	$1.67\pm0.12$	1.47-1.85	
TRIG mmol/L	$1.04\pm0.31$	0.8-1.15	$0.98\pm0.24$	0.53-1.36	$1.26\pm0.24$	0.87-1.68	$1.22\pm0.19$	0.86-1.52	

In CD 1 mice strain the red blood cells values are relatively similar, having small variations with sex and age. White blood cells parameters are higher in males, regardless of age. The values are higher at 20 weeks. Values in all biochemical parameters analysed are generally higher in males than females and higher with increasing age.

In NMRI mice strain red blood cells values are approximately the same. Also, white blood parameters are relatively cells similar, regardless of age. The blood platelets are higher at 20 weeks. Except glucose and triglycerides values, in all biochemical parameters analysed are generally higher in males than females and higher with increasing age. Standard deviation and the range are large in URIC, AST and ALT, which means either that the harvesting method is not the optimal one, or that these analyses should be done by other methods at this strain.

In BALB/c mice strain red blood cells values are relatively similar, regardless of age and sex. White blood cells parameters are higher in males, but lymphocytes are higher in female. The values are little modified with age. Biochemical values are generally higher in males than females and higher with increasing age.

In C57BL/6 mice strain red blood cells values are higher in female and grow with age. White blood cells values are higher in male and increased significant in age in both sex. The other parameters of white blood cells have similar value at different age. Blood platelets range is very large in both sex and ages. At this strain biochemical values are generally higher in females than males and higher with increasing age.

In all strain blood reticulocytes and platelets have a large standard deviation and very large limits which means that there is a variability in this study, variability determined either by the blood collection method or by the analytical method.

Based on the results of this studies it was conclude that our data could be comparable with those of literature sources (Loeb et al., 1999; Mitruka et al., 1997).

### **CONCLUSIONS**

The main goal of these studies was to provide to the users of these mice strain with the range and the average of the hematological parameters and the main biochemical metabolic parameters. The reference value presented could be used as baseline in research, testing, health evaluation etc. Because microclimate, breeding and experimentation conditions differ from unit to unit, it would be ideal for each animal facility unit that provide laboratory mice to set its own values.

# **ACKNOWLEDGEMENTS**

This study was supported by the Ministry of Education and Scientific Research and was funded through the Project PN 16 39 01/2016.

#### REFERENCES

Loeb W.F., Quimby F.W., 1999. Clinical chemistry of laboratory animals, 2nd ed. Taylor & Francis, Philadelphia, 5-176.

Mähler M., Berard M., Feinstein R., Gallagher A., Illgen-Wilcke B., Pritchett-Corning K., Raspa M., 2014. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim., 48(3):178-192.

 Mazzaccara, C, Labruna G, Cito G, Scarfo M, De Felice
M. 2008, Age related reference intervals of the main biochemical and hematological parameters in

- C57BL/6J, 129SV/EV and C3H/HeJ Mouse Strains. PLoS ONE. 3:e3772., doi:10.1371/journal.pone. 0003772.
- Mitruka B. M.; Rawnsley H. M., 1997. Clinical biochemical and hematological reference values in normal experimental animals. New York: Masson Publishing, 5 272.
- Moore, D. M. 2000. Hematology of the mouse (Mus musculus), In B. F. Feldman, J. G. Zinkl, and N. C. Jain (ed.), Schalm's veterinary hematology, 5th ed. Lippincott Williams & Wilkins, Philadelphia, 1219-1224.
- O'Connell K.E., Mikkola A.M, Stepanek, A.M., Vernet A., Hall C., Sun C.C., Yildirim E., Staropoli J.F., Lee J.T., Brown D.E., 2015. Practical murine hematopathology: a comparative review and implications for research. Comparative Medicine, 65 (2):96-113.
- Schnell M.A., Hardy C., Hawley M., Propert K.J., Wilson J.M., 2002. Effect of blood collection technique in mice on clinical pathology parameters. Hum Gene Ther 13: 155–161.

# **MISCELLANEOUS**