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Clinical Pathology Reference Data for the Philippine Cynomolgus Monkey for Preclinical Toxicology Studies

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BACKGROUND INFORMATION

Demand for the use of cynomolgus macaque (CM; *Macaca fascicularis*) in biotherapeutic development has greatly increased. Due to the demand itself and other global factors (e.g., the SARS-CoV-2 pandemic, and exportation challenges), continued use of CMs has necessitated exploring the use of underutilized CM origins in preclinical toxicology programs[1]. However, genetic and environmental variability amongst CM origins may add complexity to data interpretation and robust reference data should be available to aid in recognizing test article/test item-related effects[2]. Cynomolgus macaques of insular Philippine-origin is the focus of this current study because this origin is not routinely used versus Mauritius or Mainland/Continental origin CM and there is a recognized lack of reference data (i.e., standard hematology, coagulation, serum biochemistry, urinalysis, and urine chemistry test results) for this origin. In addition to the main emphasis of tabulating and comparing standard clinical pathology test results from Philippine-origin CM's, clinical observations, body weight, and rectal temperatures were assessed during the blood and urine collection period to monitor animal health status.

OBJECTIVES

To investigate selected hematology, serum biochemistry, coagulation, and urine parameters (measurands) for Philippine-origin macaques and compare results to published Mauritius, Chinese, and Cambodian-origin CM reference data.

METHODS

Data was collected from 20 male and 20 female naïve Philippine-origin CM with ages ranging from 1.2 to 2.2 years and body weight from 1.73 to 2.25 kg. Animals were socially housed in enclosures complying with the Animal Welfare Act and recommendations set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council 2011). Animals were serologically negative for simian immunodeficiency virus (SIV), *Cercopithecine herpesvirus* 1 (B virus), simian retroviruses type D, rabies, simian T-cell leukemia virus (STLV), and filoviruses. Additional screening tests included tuberculin, bacterial, and parasitological assessment. Blood for hematology tests was collected into K_2EDTA tubes and analyzed on an Advia 120 hematology analyzer (Siemens, USA). Blood for coagulation tests was collected into 3.2% sodium citrate tubes and analyzed on an STA Compact Max (Stago, France). Blood for serum chemistry was collected into non-additive tubes and analyzed on a Beckman AU680 (Beckman Coulter Life Sciences, USA). Urine for urinalysis and urine chemistry tests was collected into non-additive containers by urine chemistry dipstick method on a Clinitek Advantus (Siemens, USA) or on a Beckman AU680 (Beckman Coulter Life Sciences, USA). Clinical pathology data were analyzed by Provantis software (Version 10.4, Instem, UK), and representative results were compared to previously reported results for Mauritius, Chinese, and Cambodian-origin CMs[3].

RESULTS

Physical Assessments

Rectal temperatures were assessed within 3 days of blood and urine sample collections and ranged from 38.3 to 39.7°C. No clinical observations to suggest an abnormal health condition were observed.

Hematology Assessments

Standard hematology measurands for the Philippine-origin CM are presented in Table 1, and the number of animals represented was 20 each for males and females. The red blood cell count, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, reticulocyte count (male only), and white blood cell count were most comparable to published results for Mauritius-origin CM[3]. Hematocrit and red cell distribution widths were most comparable to the Chinese and Cambodian-origin CM. Platelet counts for males were higher than the mean result for Mauritius-origin NHP yet lower than both Chinese and Cambodian-origin NHP, and the platelet concentration for Philippine-origin CMs was below all other results. Absolute neutrophil count (both sexes) and absolute lymphocyte count (males) were within the range of results for all other origin CM, and the lymphocyte count for female Philippine-origin CMs was mildly lower than other origin counts. The remaining leukocyte counts were not directly compared but are expected to be comparable.

Table 1. Hematology (group mean ±2SD)

Measurand	Male	Female
Red Blood Cell (10^6/µl)	6.66 ± 0.33	6.93 ± 0.36
Hemoglobin (g/dl)	12.3 ± 0.6	12.7 ± 0.5
Hematocrit (%)	43.2 ± 1.5	44.7 ± 2.5
Mean Corpuscular Volume (fl)	65.0 ± 2.9	64.5 ± 2.7
Mean Corpuscular Hemoglobin (pg)	18.5 ± 1.0	18.4 ± 1.0
Mean Corpuscular Hemoglobin Concentration (g/dl)	28.4 ± 1.0	28.5 ± 1.2
Red Blood Cell Distribution Width (%)	12.7 ± 0.3	12.8 ± 0.9
Reticulocyte (Absolute;10^3/µl)	38 ± 14	31 ± 9
Platelet (10^3/µl)	400 ± 83	331 ± 82
Mean Platelet Volume (fl)	9.4 ± 0.8	9.9 ± 1.1
White Blood Cell (10^3/µl)	9.65 ± 3.63	9.27 ± 2.76
Neutrophil (Absolute; 10^3/µl)	4.43 ± 3.16	5.39 ± 2.60
Lymphocyte (Absolute; 10^3/µl)	4.65 ± 1.47	3.45 ± 0.79
Monocyte (Absolute; 10^3/µl)	0.32 ± 0.13	0.27 ± 0.07
Eosinophil (Absolute; 10^3/µl)	0.17 ± 0.17	0.08 ± 0.12
Basophil (Absolute; 10^3/µl)	0.03 ± 0.03	0.02 ± 0.01
Large Unstained Cells (Absolute; 10^3/µl)	0.07 ± 0.04	0.06 ± 0.02

Coagulation Assessments

Standard coagulation measurands for the Philippine-origin CM are presented in Table 2, and the number of animals represented was 20 each for males and females. The activated partial thromboplastin time was comparable to Mauritius, Chinese, and Cambodian-origin CMs whereas the prothrombin time was slightly longer than reported for other origin CM. Fibrinogen concentration was mildly higher for Philippine-origin CMs when compared with reported results for the other origin CMs[3].

Table 2. Coagulation (group mean ±2SD)

Measurand	Male	Female
Prothrombin Time (sec)	11.2 ± 0.4	11.2 ± 0.4
Activated Partial Thromboplastin Time (sec)	18.7 ± 1.0	19.3 ± 1.1
Fibrinogen (mg/dl)	231 ± 29	231 ± 56

Serum Biochemistry Assessments

Standard serum biochemistry measurands for the Philippine-origin CM are presented in Table 2, and the number of animals represented was 20 each for males and females. Albumin concentration was comparable to Mauritius, Chinese, and Cambodian-origin CMs, and total protein and globulin concentrations in Philippine-origin CM were most comparable to Chinese-origin CMs. Alanine aminotransferase, aspartate aminotransferase, and creatine kinase (males only) activities and blood urea nitrogen concentration were comparable to the Mauritius, Chinese, and Cambodian-origin CMs, with minor variations noted in other representative serum biochemistry parameters[3]. C-reactive protein concentration for all animals was below assay linearity (lower limit of quantitation [LLOQ] 0.5 mg/dL) however is expected to behave similarly to other origin CMs (increase to detectable levels in the presence of an inflammatory stimuli[4]).

Table 3. Serum Biochemistry (group mean ±2SD)

Measurand	Male	Female
Total Protein (g/dl)	7.2 ± 0.3	7.6 ± 0.2
Albumin (g/dl)	4.6 ± 0.2	5.0 ± 0.2
Globulin (g/dl)	2.6 ± 0.2	2.6 ± 0.2
Albumin/Globulin Ratio	1.8 ± 0.2	1.9 ± 0.1
Alanine Aminotransferase (U/l)	42 ± 14	35 ± 9
Aspartate Aminotransferase (U/l)	39 ± 8	39 ± 7
Creatine Kinase (U/l)	216 ± 140	162 ± 154
Alkaline Phosphatase (U/l)	690 ± 138	744 ± 157
γ-glutamyl Transferase (U/l)	84 ± 28	98 ± 23
Total Bilirubin (mg/dl)	0.3 ± 0.1	0.4 ± 0.2
Glucose (mg/dl)	51 ± 13	47 ± 10
Total Cholesterol (mg/dl)	144 ± 21	151 ± 18
Triglyceride (mg/dl)	50 ± 10	54 ± 9
Urea Nitrogen (Serum; mg/dl)	19 ± 3	20 ± 2
Creatinine (mg/dl)	0.4 ± 0.1	0.5 ± 0.1
Total Calcium (mg/dl)	9.9 ± 0.3	10.4 ± 0.4
Inorganic Phosphate (mg/dl)	6.7 ± 0.5	7.5 ± 0.7
Potassium (mEq/l)	4.5 ± 0.3	4.5 ± 0.3
Sodium (mEq/l)	149 ± 2	151 ± 3
Chloride (mEq/l)	108 ± 2	108 ± 2

Urine Assessments

No reported results were available for data comparison. Selected urine measurands for the Philippine-origin CM are presented in Table 4, and the number of animals represented was 20 each for males and females except for female urine chloride/creatinine ratio which was from 19 females. No results for urine microalbumin, β-macroglobulin, glucose, and phosphate were obtained due to concentrations being below the level of quantitation (LLOQ: 0.5 mg/dL, 0.5 mg/dL, 10 mg/dL, and 10 mg/dL, respectively) and therefore ratios were not calculated. Given this lack of robust data for comparison, it is imperative to collect samples for analysis in the acclimation/pre-study phase for an appropriate comparator. Urine chemistry ratios reported to one decimal place in this text due to system limitation, however, two decimal places is preferred.

Table 4. Urine Parameters (group mean ±2SD)

Measurand	Male	Female
Urine Specific Gravity	1.021 ± 0.006	1.018 ± 0.006
Urine Volume (ml)	54.5 ± 26.5	62.5 ± 26.1
Urine Protein/Creatinine Ratio	0.28 ± 0.8	0.34 ± 0.12
Urine γ-glutamyl Transferase/Creatinine Ratio	0.7 ± 0.2	0.9 ± 0.2
Urine Total Calcium/Creatinine Ratio	1.1 ± 0.4	1.2 ± 0.5
Urine Urea Nitrogen/Creatinine Ratio	24.2 ± 4.6	24.8 ± 3.6
Urine Sodium/Creatinine Ratio	1.4 ± 0.4	1.4 ± 0.4
Urine Chloride/Creatinine Ratio	1.1 ± 0.5	1.2 ± 0.4
Urine Potassium/Creatinine Ratio	1.5 ± 0.4	1.6 ± 0.4

CONCLUSION

To the authors' knowledge, this is the first robust assessment of standard clinical pathology endpoints for Philippine-origin CM which were found to be generally comparable to published data for Mauritius and Mainland/Continental CMs. Similar to the Mauritius-origin CMs, the Philippine-origin CM mean corpuscular volume (MCV) was lower and red blood cell count (RBC) higher than reported Chinese and Cambodian CMs results which allows for comparable hemoglobin concentration and thus red cell mass across these origins. Although different origin CM have similar clinical pathology results, there are subtle differences that can be recognized using robust reference data. An additional consideration is the age difference between Philippine-origin CM (1.2-2.2 years) in this study and the ages of the previously reported Mauritius, Chinese, and Cambodian-origin CMs animals (2-5 years). The lack of pronounced or impactful differences supports the use of Philippine-origin CMs in preclinical toxicology assessments and drug development programs and similar to other CM-origin reference data investigations, CMs of Philippine-origin are considered a valuable alternative CM origin.

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