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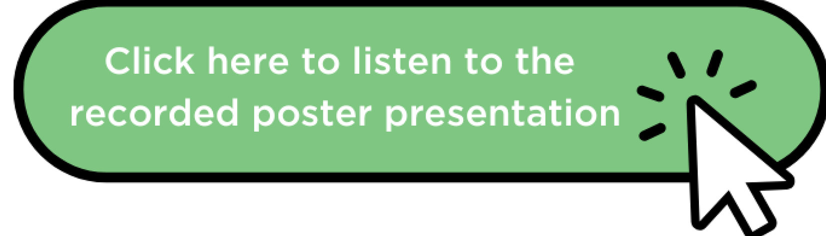
Comparison of Baseline Levels of Biomarkers Commonly Used to Assess Drug Candidate Safety, Efficacy, and Mechanism of Action in Cynomolgus Monkeys of Cambodian, Chinese, or Cambodian-Chinese Mixed Origin Bred in a U.S.-Based Research Facility

Ashley Kay Mahoney[1], Megan Strenge[2], Tara Arndt[2], Rosalia Matteo[1], Francesca Barone[1], Norbert Makori[2]
[1]Altasciences Preclinical Sacramento, CA, United States, [2]Altasciences Preclinical Seattle LLC, Seattle, WA, United States



BACKGROUND

Cynomolgus monkeys (*Macaca fascicularis*) are a commonly used nonhuman primate model in preclinical toxicology research. Preclinical drug programs predominantly utilize cynomolgus monkeys of a single origin (e.g., Cambodian or Chinese) to reduce experimental variability; however, a restriction in origin poses supply challenges. To address supply shortages, a US-based facility is breeding cynomolgus monkeys of Southeast Asian origin.

OBJECTIVE

This study aims to compare baseline levels of clinical pathology, peripheral blood lymphocytes (by immunophenotyping), complement protein fragments, and cytokines in cynomolgus monkeys of Cambodian, Chinese, or Cambodian-Chinese mixed origin.

METHOD

Test System

Thirty-one research naïve cynomolgus monkeys of Cambodian, Chinese, or Cambodian-Chinese origin were sourced and maintained in a US breeding facility (Valley Biosystems; West Sacramento, CA, USA). 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 Cercopithecine herpesvirus 1 (B virus), simian immunodeficiency virus (SIV), simian T-cell leukemia virus 1 (STLV-1), and simian retrovirus 1-5, 8 (SRV-1, SRV-2, SRV-3, SRV-4, SRV-5, SRV-8).

Table 1. Age and Bodyweight (group mean ±1SD)

Origin	Cambodian		Chinese		Cambodian-Chinese	
Sex	Male	Female	Male	Female	Male	Female
Sample Size	5	6	5	5	5	5
Age (years)	1.32 ± 0.16	1.60 ± 0.21	1.94 ± 0.20	1.89 ± 0.20	1.74 ± 0.47	1.96 ± 0.05
Body Weight (kg)	1.76 ± 0.31	1.80 ± 0.31	2.12 ± 0.17	1.91 ± 0.19	2.00 ± 0.43	2.08 ± 0.25

Sample Collection and Analysis

Animals were fasted overnight, sedated with Ketamine (10 mg/kg, IM), and blood was collected from the femoral vein for serum chemistry, coagulation, hematology, peripheral blood lymphocytes, complement protein fragments, and cytokines analysis.

Thirty-one animals were sampled for clinical pathology assessments. Blood for serum chemistry tests was collected into serum separator tubes and analyzed using a DxC700AU analyzer (Beckman Coulter), blood for coagulation tests was collected into 3.2% sodium citrate tubes and analyzed using a STA Compact (Stago, France), and blood for hematology tests was collected into K2EDTA tubes and analyzed using an Advia 120 hematology analyzer (Siemens, USA). All blood samples for serum chemistry, coagulation, and hematology were processed and analyzed by Quality Veterinary Laboratory (Davis, CA, USA) on the day of collection.

Thirty animals were sampled for immunophenotyping. Blood for immunophenotyping was collected into K2EDTA tubes and analyzed using a BD LSRFortessa (Becton Dickinson, USA). Flow cytometry data was analyzed in BD FACSDiva v9.0.1. Lymphocyte and monocyte counts obtained from hematology tests were used in conjunction with flow cytometry relative percentages to calculate absolute counts for all cell populations (refer to **Table 2**).

Thirty animals were sampled for complement protein fragments. Blood for complement C3a protein was collected into K2EDTA tubes, luminescence was detected using a MicroVue Bb Plus EIA (Quidel Product No. A027) and BioTek Synergy H1 microplate reader, and data was acquired using BioTek Gen5 (v2.09).

Thirty animals were sampled for cytokines. Blood for cytokines (IFN-y, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, MCP-1, and TNF-a) was collected into serum separator tubes, electrochemiluminescence was detected using an MSD U-PLEX Biomarker (NHP) Assay kit and MSD MESO Quickplex SQ 120 (USA), and data was acquired using MSD Discovery Workbench v4.0.12. Cytokine data that were below the limit of quantification were not statistically analyzed.

A two-way analysis of variance (ANOVA) was conducted to assess the effect of origin and sex on each clinical pathology parameter, complement protein C3a, and quantifiable levels of cytokines. Tukey's HSD test for multiple comparisons was conducted at a significance level of .05 to identify group means that statistically differed. A one-way ANOVA was conducted to assess the effect of origin on cell populations. All statistics were conducted in GraphPad Prism 10.6.1.

Table 2. Cell Populations

Cell Type	Phenotype
Total T Cells	CD45+ CD3+ CD20-
Helper T Cells	CD45+ CD3+ CD20- CD4+ CD8-
Cytotoxic T Cells	CD45+ CD3+ CD20- CD4- CD8+
B Cells	CD45+ CD3- CD20+
NK Cells	CD45+ CD3- CD159a+
NK T Cells	CD45+ CD3+ CD159a+
Monocytes	CD45+ CD3- CD20- CD159a- CD14+
Dendritic Cells	CD45+ CD3- CD20- CD159a- CD14- HLA DR+ CD11c+

Table 3. Serum Chemistry (group mean ±1SD)

Parameter	Cambodian	Chinese	Cambodian-Chinese
Total Protein (g/dL)	6.55 ± 0.31	6.59 ± 0.29	6.33 ± 0.43
Albumin (g/dL)	4.06 ± 0.35	4.03 ± 0.29	3.77 ± 0.45
Globulin (g/dL)	2.49 ± 0.36	2.56 ± 0.35	2.57 ± 0.23
Albumin/Globulin Ratio	1.69 ± 0.32	1.63 ± 0.33	1.47 ± 0.27
Alanine Aminotransferase (U/L)	37.64 ± 9.26	33.50 ± 6.06	37.70 ± 5.64
Aspartate Aminotransferase (U/L)	57.27 ± 23.08	68.90 ± 29.86	58.00 ± 17.24
Creatine Kinase (U/L)	1261.82 ± 2479.60	1176.30 ± 1749.50	1144.50 ± 990.78
Alkaline Phosphatase (U/L)	421.90 ± 76.67	440.10 ± 135.30	378.40 ± 85.81
Total Bilirubin (mg/dL)	0.18 ± 0.06	0.20 ± 0.09	0.21 ± 0.06
Glucose (mg/dL)	63.82 ± 17.96	49.50 ± 12.73	50.40 ± 15.08
Total Cholesterol (mg/dL)	153.55 ± 31.48	136.80 ± 14.32	150.70 ± 23.13
Urea Nitrogen (mg/dL)	21.09 ± 4.28	17.30 ± 3.40	18.00 ± 4.90
Creatinine (mg/dL)	0.49 ± 0.05	0.48± 0.04	0.48± 0.04
Calcium (mg/dL)	9.85 ± 0.40	9.64 ± 0.51	9.58 ± 0.30
Inorganic Phosphate (mg/dL)	6.49 ± 0.84	6.57 ± 0.93	6.05 ± 0.75
Potassium (mEq/L)	4.80 ± 0.74	4.67 ± 0.82	4.51 ± 0.66
Sodium (mEq/L)	140.50 ± 1.64	140.10 ± 1.20	142.10 ± 0.88
Chloride (mEq/L)	104.30 ± 1.49	103.80 ± 1.93	104.60 ± 2.22

Table 4. Coagulation (group mean ±1SD)

Parameter	Cambodian	Chinese	Cambodian-Chinese
Prothrombin Time (sec)	11.39 ± 0.50	11.46 ± 0.44	11.79 ± 0.60
Activated Partial Thromboplastin Time (sec)	20.35 ± 1.63	19.54 ± 1.71	20.38 ± 1.36
Fibrinogen (mg/dL)	288.27 ± 77.41	289.90 ± 56.37	261.00 ± 45.66

Table 5. Hematology (group mean ±1SD)

Parameter	Cambodian	Chinese	Cambodian-Chinese
Red Blood Cell (10^6/µL)	5.61 ± 0.39	5.57 ± 0.29	5.67 ± 0.32
Hemoglobin (g/dL)	13.21 ± 0.77	13.01 ± 0.48	13.11 ± 0.80
Hematocrit (%)	42.07± 2.66	41.32 ± 1.43	42.17± 3.03
Mean Corpuscular Volume (fl)	75.18 ± 4.88	74.37 ± 4.09	74.48 ± 2.45
Mean Corpuscular Hemoglobin Concentration (g/dL)	31.42 ± 0.94	31.48 ± 0.75	31.15 ± 0.92
Red Blood Cell Distribution Width (%)	12.89 ± 0.62	12.80 ± 0.79	12.59 ± 0.66
Reticulocyte (Absolute; 10^3/µL)	57.25 ± 25.96	45.70 ± 8.19	50.46 ± 17.39
Platelet (10^3/µL)	336.40 ± 76.75	329.50 ± 74.72	298.00 ± 68.50
Mean Platelet Volume (fl)	9.70 ± 1.35	8.57 ± 0.92	9.81 ± 1.96
White Blood Cell (10^3/µL)	10.61 ± 3.38	10.26 ± 2.32	10.63 ± 5.49
Neutrophil (Absolute; 10^3/µL)	4.21 ± 2.33	4.79 ± 2.57	4.87 ± 5.22
Lymphocyte (Absolute; 10^3/µL)	5.96 ± 3.09	5.13 ± 1.43	5.28 ± 1.95
Monocyte (Absolute; 10^3/µL)	0.33 ± 0.14	0.28 ± 0.10	0.37 ± 0.20
Eosinophil (Absolute; 10^3/µL)	0.07 ± 0.06	0.04 ± 0.07	0.06 ± 0.07
Basophil (Absolute; 10^3/µL)	0.03 ± 0.06	0.01 ± 0.03	0.02 ± 0.04

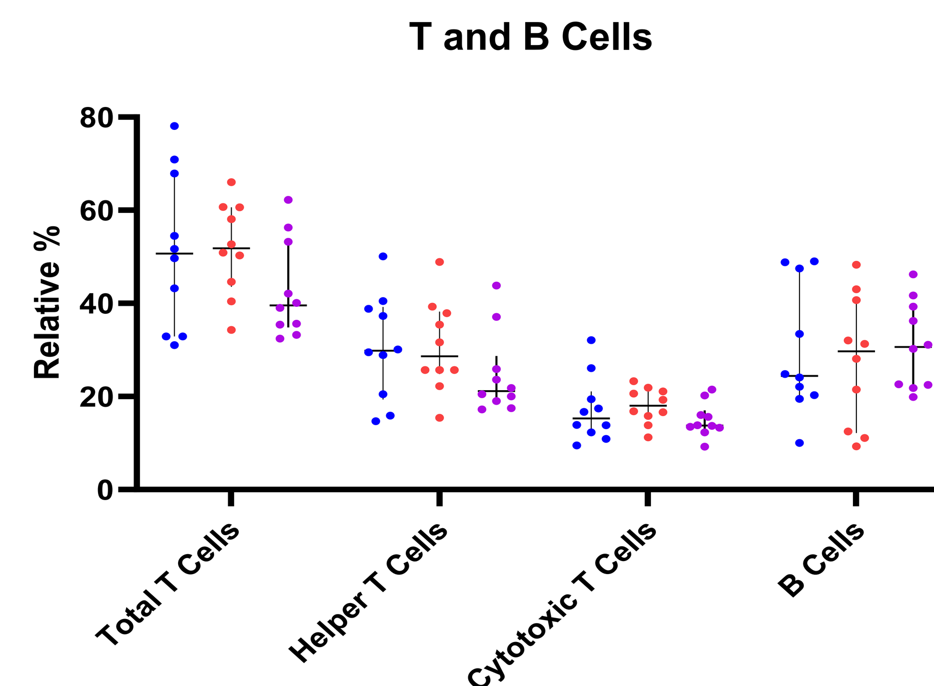


Figure 1. Relative Percentage of T and B Cell Populations

RESULTS

Serum Chemistry

Group means (±1 SD) for standard serum chemistry parameters are presented in **Table 3**.
An effect of origin was observed for sodium concentration (F [2,25] = 6.91, p=0.004); however, post hoc comparisons between origin groups did not reach statistical significance. There were no other statistically significant differences between origins.
Sex differences were observed for albumin/globulin ratio (F [1, 25] = 4.82, p = 0.0376), calcium (F [1,25] =10.49, p = 0.0034), inorganic phosphate (F [1,25] = 7.73, p = 0.0102), and chloride (F [1,25] =16.03, p = 0.0005). Post hoc comparisons revealed that the albumin/globulin ratio was greater in females of Cambodian origin (M = 1.90, SD = 0.18) than males of Cambodian origin (M = 1.44, SD = 0.29, p = 0.0114) and females of Cambodian-Chinese origin (M = 1.44, SD = 0.24, p = 0.0297). In addition, calcium concentration was greater in females of Chinese origin (M = 10.00, SD = 0.20) than males of Cambodian origin (M = 9.28, SD = 0.48, p = 0.0033), and inorganic phosphate concentration was greater in males of Chinese origin (M = 7.08, SD = 0.64) than females of Chinese origin (M = 6.06, SD = 0.94, p = 0.0446). Chloride concentration was greater in males of Cambodian-Chinese origin (M = 105.80, SD = 2.39) than females of Cambodian-Chinese origin (M = 103.40, SD = 1.34, p = 0.0194) and greater in males of Chinese origin (M =105.40, SD = 0.55) than females of Chinese origin (M = 102.20, SD = 1.30, p = 0.0027).

Sex differences were also observed for albumin (F [1,25] = 5.96, p = 0.0221), urea nitrogen (F [1,25] = 6.41, p = 0.0180), and potassium (F [1, 25] = 5.01, p = 0.0344); however, post hoc comparisons did not reach significance.

Coagulation

Group means (±1 SD) for standard coagulation parameters are presented in **Table 4**. No differences were observed between origins for any coagulation parameter; however, a sex difference was observed for fibrinogen (F [1,25] = 8.71, p = 0.0068). Post hoc comparisons revealed that fibrinogen concentration was greater in males of Cambodian origin (M = 343.00, SD = 68.70) than females of Cambodian origin (M = 242.67, SD = 52.14, p = 0.0038), fibrinogen concentration was greater in males of Chinese origin (M = 325.00, SD = 50.87) than females of Chinese origin (M = 254.60, SD = 38.02, p = 0.0413), and fibrinogen concentration were greater in males of Chinese origin (M = 325.00, SD = 50.87) than males of Cambodian-Chinese origin (M = 258.20, SD = 33.97, p = 0.0409).

Hematology

Group means (±1 SD) for standard hematology parameters are presented in **Table 5**.
There was a significant interaction effect of origin and sex on platelet count F [2,25] = 4.90, p = 0.0160). Post hoc comparisons revealed that platelet count was greater in females of Chinese origin (M = 278.00, SD = 42.83) than males of Chinese origin (M = 381.00, SD = 64.02, p = 0.0174), and greater in males of Cambodian origin (M = 370.00, SD = 308.33) than males of Cambodian-Chinese origin (M = 263.00, SD = 27.61, p = 0.0358).

Immunophenotyping

(**Figure 1** and **Figure 2**) present relative percentages of cell populations plotted over the interquartile range (IQR). A 1-way ANOVA revealed no significant differences between origins in absolute count of total T cells (F [2, 27] = 1.24, p = 0.31), helper T cells (F [2, 27] = 1.31, p = 0.29), cytotoxic T cells (F [2, 27] = 0.83, p = 0.45), B cells (F [2, 27] = 0.01, p = 0.99), natural killer cells (F [2, 27] = 1.09, p = 0.35), natural killer T cells (F [2, 27] = 0.39, p = 0.68), monocytes (F [2, 27] = 1.23, p = 0.31), or dendritic cells (F [2, 27] = 0.18, p = 0.84). Furthermore, there were no significant differences between origins relative percentage of total T cells (F [2, 27] = 1.51, p = 0.24), helper T cells (F [2, 27] = 1.21, p = 0.31), cytotoxic T cells (F [2, 27] = 1.01, p = 0.38), B cells (F [2, 27] = 0.18, p = 0.83), natural killer cells (F [2, 27] = 1.30, p = 0.29), natural killer T cells (F [2, 27] = 0.37, p = 0.69), monocytes (F [2, 27] = 1.22, p = 0.31), or dendritic cells (F [2, 27] = 0.16, p = 0.85).

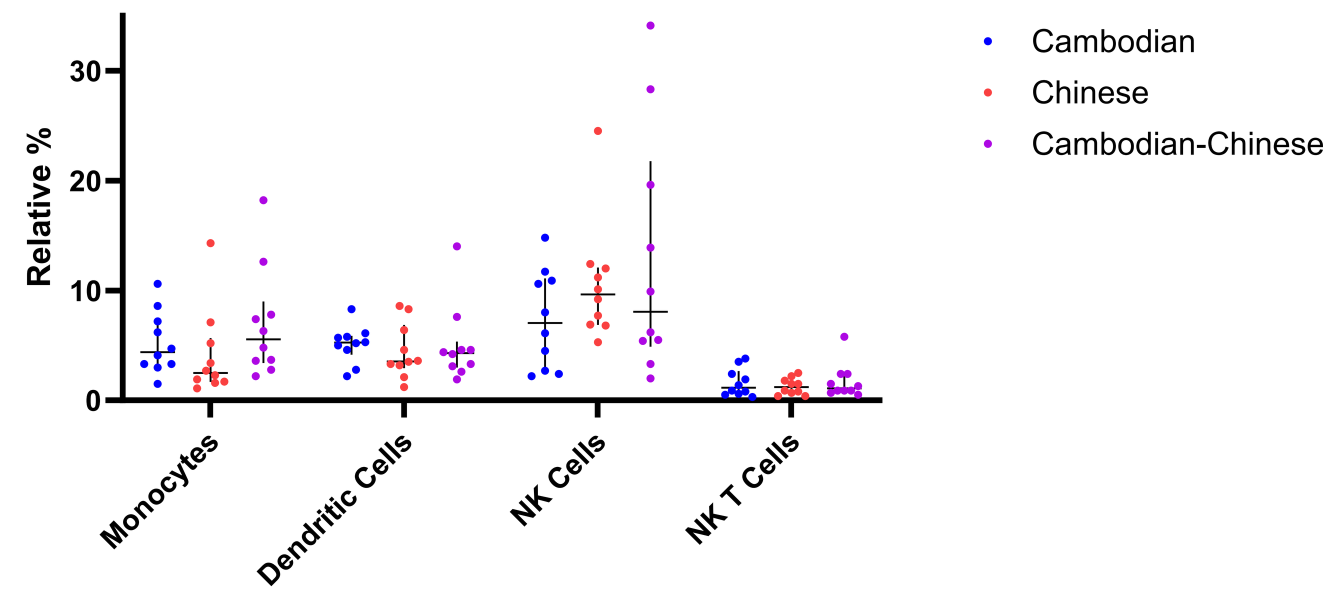


Figure 2. Relative Percentage of Monocyte, Dendritic Cell, and Natural Killer Cell Populations

Complement Protein C3a

Figure 3 presents individual C3a concentration values plotted over the IQR. Group means (±1 SD) for C3a concentration were 49.45 ± 26.37 (Cambodian), 22.36 ± 0.53 (Chinese), 34.31± 13.53 (Cambodian-Chinese).

A 2-way ANOVA revealed no significant interaction between origin and sex on C3a concentration (F [2,24] =0.25, p = 0.78). Moreover, there was no significant main effect of origin (F [2, 24] = 0.51, p = 0.61) or sex (F [1, 24] = 0.72, p = 0.40).

Cytokine Assessments

All samples were below the limit of quantification for IFN-y, IL-1b, IL-2, IL-4, IL-6, IL-10, and TNF-a. Twenty-nine of thirty samples were BLQ for IL-8 and IL-12. All samples were above the limit of quantification for MCP-1.

Figure 4 presents individual MCP-1 concentration values plotted over the IQR. Group means (±1 SD) for MCP-1 concentration were 121.10 ± 5.42 (Cambodian), 127.10 ± 13.22 (Chinese), 121.70 ± 12.51 (Cambodian-Chinese).

A 2-way ANOVA revealed no significant interaction between origin and sex on MCP-1 concentration (F [2,24] = 0.44, p = 0.65). Moreover, there was no significant main effect of origin (F [2, 24] = 0.05, p = 0.95) or sex (F [1, 24] = 0.03, = 0.86).

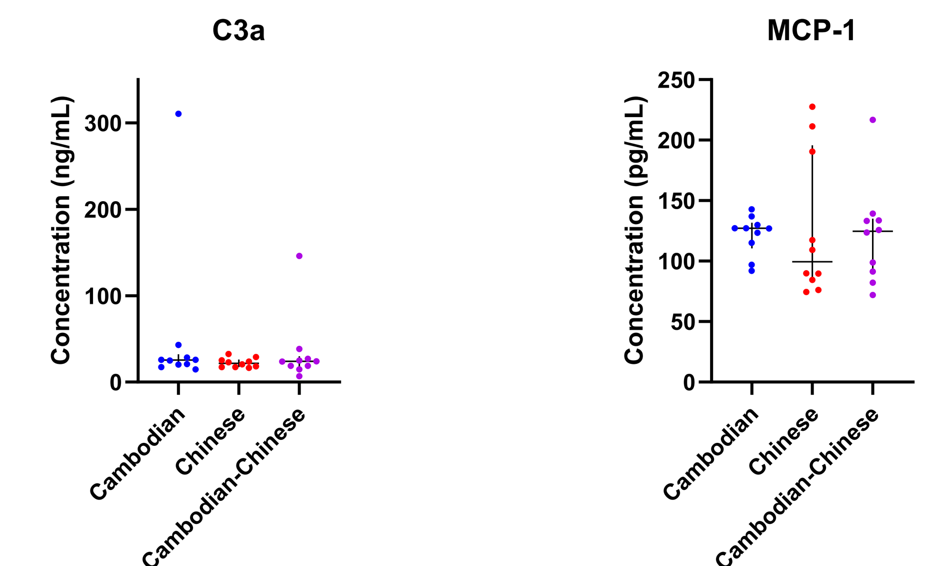


Figure 3. Concentration of C3a

Figure 4. Concentration of MCP-1

CONCLUSIONS

Cynomolgus monkeys sourced from a single origin (e.g., Cambodia or China) are commonly used in preclinical toxicology research; however, a U.S.-based facility is breeding cynomolgus monkeys of mixed Southeast Asian origin to address animal supply shortages. Here, we compared baseline levels of common toxicology biomarkers (clinical pathology parameters, peripheral blood lymphocytes, complement protein fragment C3a, cytokines) between animals of Cambodian origin, Chinese origin, and Cambodian-Chinese origin.

No meaningful differences were observed between origins for any biomarker evaluated. Statistically significant differences included platelet count, fibrinogen, albumin/globulin ratio, calcium, inorganic phosphate, and chloride. Consistent with findings by Arndt et al (2022), differences in electrolyte and mineral concentrations achieved statistical significance; however, the sex differences observed for calcium, inorganic phosphate, and chloride were not clinically meaningful based on the small physiological range of each biomarker. To overcome differences that may be attributed to inter-animal variability, additional research is needed. Greater sample sizes should be utilized to reliably characterize the average concentration of each biomarker and to accurately identify differences between groups.

In conclusion, although interpretation is limited by low sample size, cynomolgus monkeys of mixed Cambodian-Chinese origin were comparable to cynomolgus monkeys of Cambodian origin and Chinese origin, with no meaningful differences observed between origins. To address supply shortages, cynomolgus monkeys of mixed Cambodian-Chinese origin may be used within a single study, and cynomolgus monkeys of different origins may be used across a preclinical drug program.

REFERENCES

1. Arndt, T., Meindel, M., Clarke, J., Shaw, A., & Gregori, M. (2022). Comparison of Routine Hematology, Coagulation, and Clinical Chemistry Parameters of Cynomolgus Macaques of Mauritius Origin With Cynomolgus Macaques of Cambodia, China, and Vietnam Origin. *Toxicologic pathology*, *50*(5), 591–606.

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