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Cross-Comparison of Immunophenotyping Assay Performance in Naïve Filipino, Mauritian, and Cambodian Nonhuman Primates

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INTRODUCTION

Nonhuman primates (NHPs) are indispensable for nonclinical safety assessment in the drug development process. However, increased demand for Cambodian NHPs and accompanying supply constraints mean that the scientific community needs to consider alternate sources.

Flow cytometry analysis is a well-established platform in preclinical toxicology and pharmacology testing and this study aimed to compare immunophenotyping of leukocyte populations using two of Altasciences validated analytical methods in cynomolgus macaques sourced from the Philippines and Mauritius as alternatives to those sourced from Cambodia.

MATERIALS AND METHOD

Blood from naïve cynomolgus macaques aged one to four years from one of the Philippines (n=34; n=14 males and n=20 females), Mauritius (n=40; n=20 males and n=20 females), or Cambodia (see below) was aliquoted and stained using two of Altasciences validated analytical methods (Panels 1 and 2) used to phenotype leukocyte populations using flow cytometry. Panel 1 phenotyped the cell populations listed in Table 1 using a FACSCanto II (Becton Dickinson, USA); Panel 2 phenotyped the cell populations listed in Table 2 using a BD LSRFortessa (Becton Dickinson, USA).

Historical data from naïve Cambodian macaques aged 1.5 to four years (n=42; n=21 male and n=21 female) assayed with Panel 1 were used as reference controls for analysis; historical data from a separate set of naïve Cambodian macaques also aged 1.5 to four years (n=32; n=16 males and n=16 females) assayed with Panel 2 were also used as reference controls.

Flow cytometry data was analyzed in BD FACSDiva (Panel 1, v6.1.3; Panel 2, v9.02), and lymphocyte and monocyte counts from Advia120 hematology analyzer (Siemens, USA) were used in tandem with flow cytometry relative percentages to calculate absolute counts for all cell populations. Statistical analyses were performed in GraphPad Prism to compare male and female NHPs from the Philippines and Mauritius to those from Cambodia as reference controls ($p < 0.05$).

Figure 1. Representative cytograms for Panel 1 gating of T cells, B cells, and NK cells

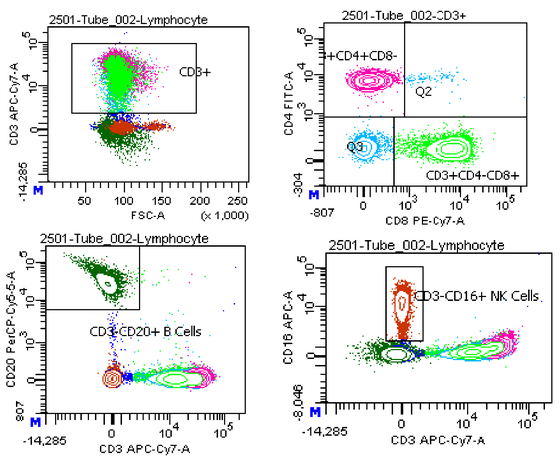


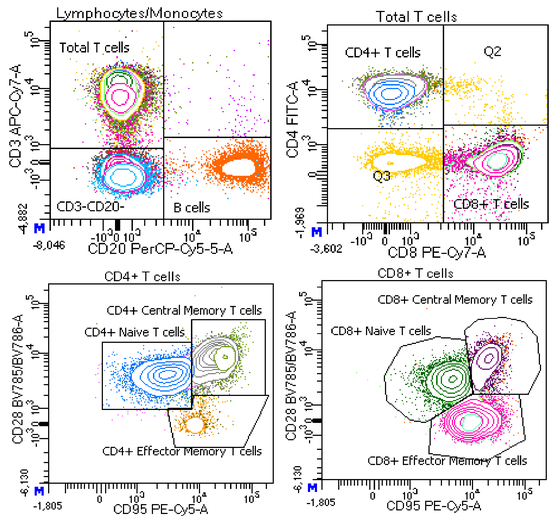
Table 1. Panel 1 cell populations presented

Cell Type	Phenotype
Total T cells	CD3+
CD4+ T cells	CD3+CD4+CD8-
CD8+ T cells	CD3+CD4-CD8+
B cells	CD45+CD3-CD20+
Natural Killer (NK) cells	CD3-CD16+
Monocytes	CD3-CD14+

Table 2. Panel 2 cell populations presented

Cell Type	Phenotype
Total T cells	CD45+CD3+CD20-
CD4+ T cells	CD45+CD3+CD20-CD4+CD8-
CD8+ T cells	CD45+CD3+CD20-CD4-CD8+
B cells	CD45+CD3-CD20+
Natural Killer (NK) cells	CD45+CD3-CD159a+
Natural Killer (NK) T cells	CD45+CD3+CD159a+
Monocytes	CD45+CD3-CD20-CD159a-CD14+
Dendritic cells (DCs)	CD45+CD3-CD20-CD159a-CD14-HLADR+CD11c+
CD4+ Naïve T cells	CD45+CD3+CD20-CD4+CD8-CD28+CD95-
CD4+ Effector Memory T cells	CD45+CD3+CD20-CD4+CD8-CD28-CD95+
CD4+ Central Memory T cells	CD45+CD3+CD20-CD4+CD8-CD28+CD95+
CD8+ Naïve T cells	CD45+CD3+CD20-CD4-CD8+CD28+CD95-
CD8+ Effector Memory T cells	CD45+CD3+CD20-CD4-CD8+CD28-CD95+
CD8+ Central Memory T cells	CD45+CD3+CD20-CD4-CD8+CD28+CD95+

Figure 2. Representative cytograms for Panel 2 gating of T cells and selected T cell subsets



RESULTS

Panel 1 showed differences in absolute counts of total T cells, helper T cells, cytotoxic T cells, and B cells between macaques of different origin (Figure 3). However, relative percentages were comparable in all analyzed cell populations except for monocytes across macaques of different origin (Figure 4).

Panel 2 showed that absolute counts of total T cells, helper T cells, cytotoxic T cells, B cells, and monocytes phenotyped were different between macaques of different origin (not shown, as results were similar to data presented for Panel 1 in Figures 3 and 4). Differences in cell counts between macaques of different origins were also observed in regulatory T cells, CD69+ activated NK cells, and NK T cells (not shown), as well as dendritic cells, naïve T cells, and memory T cell populations (Figure 5). Relative percentages of dendritic cells, naïve T cells, and memory T cell populations were also comparable across macaques of different origins (Figure 6).

Glossary: NK, natural killer; EM, effector memory; CM, central memory; DC, dendritic cell

Figure 3. Panel 1, absolute counts of monocytes and T, B, and NK cells

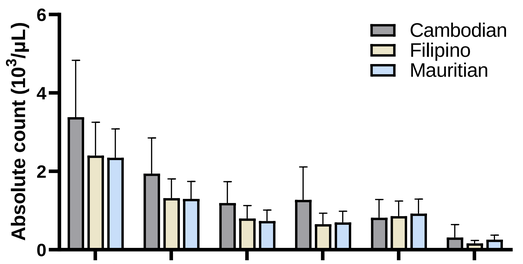


Figure 4. Panel 1, relative percentages of monocytes and T, B, and NK cells

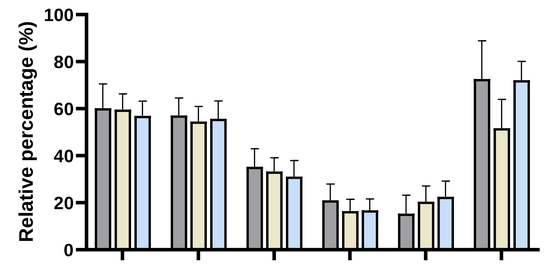


Figure 5. Panel 2, absolute counts of DCs and memory T cells

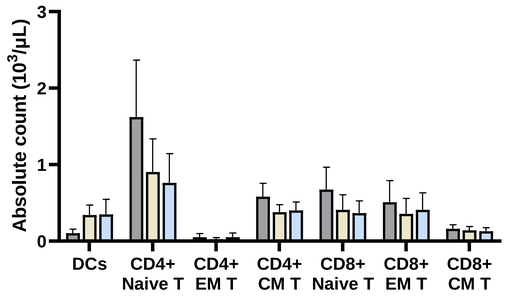
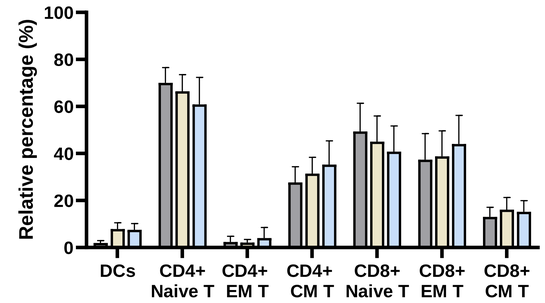


Figure 6. Panel 2, relative percentages of DCs and memory T cells



CONCLUSION

Differences were noted in the absolute counts for several cell populations in cynomolgus macaques from different countries of origin. Despite these observed differences in cell counts, the assays showed robust cell staining in macaques from all countries of origin, and relative percentages of most cell populations were comparable across macaques of different origins. This led to the following conclusions:

- Flow cytometry assays already validated in cynomolgus macaques in one country of origin remain viable for use in cynomolgus macaques from another country of origin, eliminating the need for expensive and time-consuming assay re-validation.
- Composition of leukocytes (i.e., relative percentages) were similar between macaques of different origins, indicating that macaques of different origins have similar immunological makeup.
- Absolute counts for several analyzed populations were different between macaques of different origins. It is recommended that only macaques from a single country of origin be used in a single study and, ideally, across multiple studies for continuity of data throughout the development of a new product. It is also recommended that sponsors consider the specific goals of their studies if macaques of different origins must be used in different studies throughout a product's development, particularly if quantification of immune populations is a key endpoint.