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Cross-Comparison of Biomarker Assay Performance in Naïve Philippines, Cambodian, and Mauritius, and Non-Naïve Mauritius Cynomolgus Macaque Nonhuman Primates

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INTRODUCTION

Since the 2019 pandemic, Cambodian-bred cynomolgus NHPs have been the staple of preclinical research for Pharmaceutical Companies and Contract Research Organizations across the United States of America and Europe, due to the availability of the animals from large breeding facilities. Before the 2019 COVID-19 pandemic, China was the main supplier of cynomolgus NHPs, however, since the pandemic, no exports of the cynomolgus NHPs have been available. In November 2022, imports of Cambodian cynomolgus NHPs have halted. Due to this ongoing situation, Altasciences investigated alternative strains, including Philippine and Mauritius cynomolgus NHPs to determine if these strains would be suitable for use. This presentation covers a comparison of the standard panels (cytokines and complements) that are evaluated in toxicological studies.

MATERIAL AND METHODS

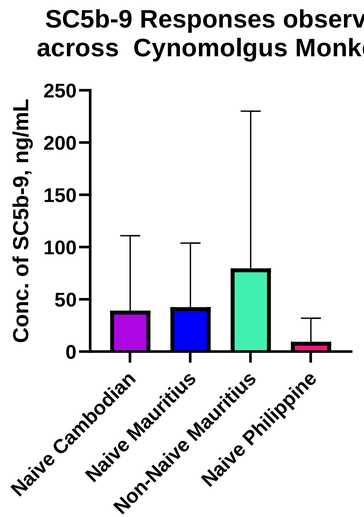
A total of 100 (40 Philippine, 50 Mauritius, 10 Cambodian) NHP samples were evaluated. All animals, except for 10 non-naïve Mauritius (12 to 21 years), were between the ages of 1 to 4 years old.

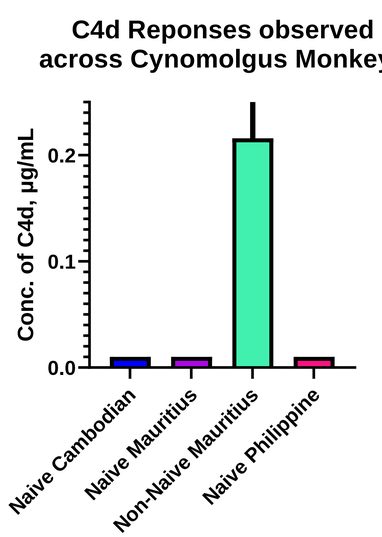
MSD U-plex 10-plex Kit for NHP (Cat. # K15068M-4):
Serum samples were thawed and diluted by a factor of 10, or as appropriate, in diluent 57. Standards, study samples, and quality controls (QCs) were added in duplicate to a microtiter plate containing pre-coated antibodies and then incubated for one hour while shaking. After a wash step, the SULFO TAG detection antibody solution was added to each well, and the plate was incubated for one hour while shaking. After a wash step, read buffer B was added to each well, and the plate was read in the MSD SQ 120 instrument.

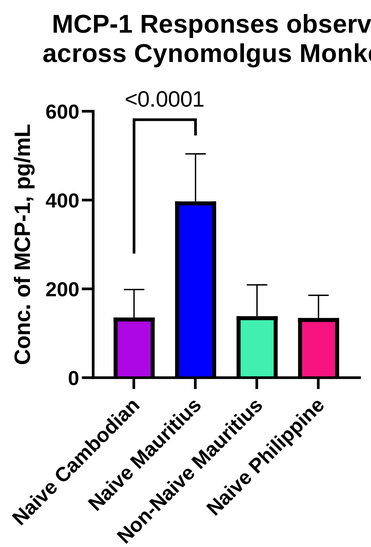
Complement Assays, Quidel Bb, C3a, C4d, SC5b-9 (Cat. #A027, A031, A009, and A020 respectively)
K2EDTA samples were thawed in a 37ºC water bath and diluted in complement specimen diluent. At room temperature, complement specimen diluent (blank), standards, quality controls, and samples were added in duplicates to microtiter plates, and then incubated for a specified amount of time. After a wash step, the conjugate (containing detection antibodies) was added for detection of bound complement sample, followed by another incubation. Additional washing steps were conducted, and Tetramethylbenzidine substrate solution was added to each well (for Bb, C3a, SC5b-9); or 0.7% 2-2'-Azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt was added to each well for C4d, and incubated at room temperature (protected from light), followed by the addition of stop solution. The plate was read at 450 nm (A450) for Bb, C3a, and SC5b-9, 405 nm (A405) for C4d, all with blank subtraction.

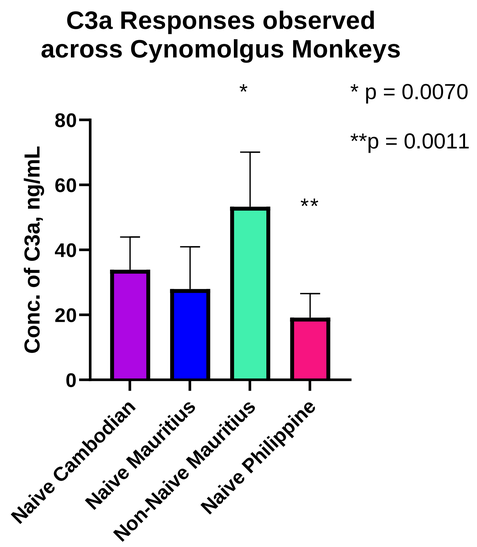
RESULTS

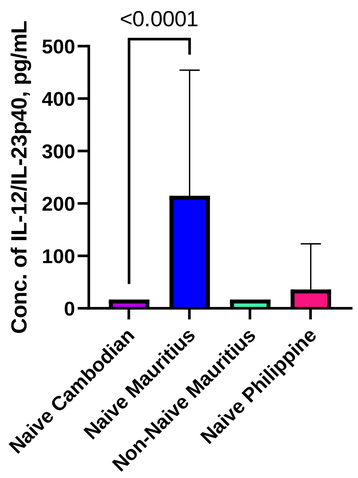
All samples from all strains were below the limit of quantitation (BLQ) for IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, and TNF-α. Mean and standard deviation are plotted for Bb, C3a, C4d, IL-6, IL-12/IL-23p40, and SC5b-9. For each Welch's t-test, the naïve Cambodian strains served as the control for the comparison.

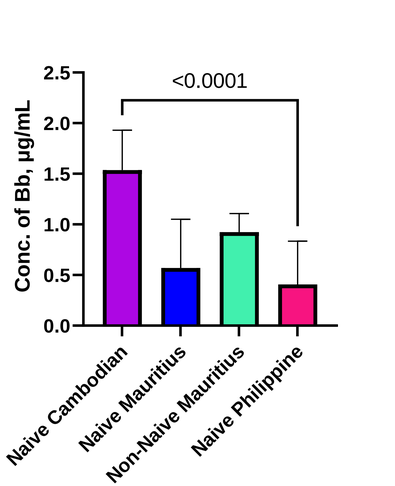












CONCLUSIONS

For C4d and SC5b-9, no statistical differences in responses were observed between any of the populations.

For Bb, the only statistical difference was observed between the naïve Cambodian and the naïve Philippine NHPs.

For C3a, statistical differences were observed when the naïve Philippine and non-naïve Mauritius NHPs were compared to the naïve Cambodian NHPs.

While statistical differences between naïve Cambodian and the naïve Mauritius NHPs were observed for IL-12/IL-23p40, it is noted that 18 of the 40 naïve Mauritius NHPs were above the limit of quantitation.

For MCP-1, the only statistical difference was observed between the naïve Cambodian and the naïve Mauritius NHPs, with the naïve Mauritius NHPs producing more MCP-1.

Despite the differences between some of the Cynomolgus Macaques strains, the analysis shows robustness of the assays to detect changes/fluctuations in cytokines and complement factors. This presentation demonstrates that based on the data, the Mauritius and Philippine strains are suitable for use in toxicological studies.