

CLINICAL RESEARCH IN INFECTIOUS DISEASES

IMMUNOGENICITY SUMMARY REPORT

for

DMID Protocol 20-0003:

Phase I, Open-Label, Dose-Ranging Study of the Safety and
Immunogenicity of 2019-nCoV Vaccine (mRNA-1273)
in Healthy Adults

29 OCTOBER 2020

Prepared and distributed by:
Statistical and Data Coordinating Center (SDCC)
Emmes
Rockville, Maryland

THIS COMMUNICATION IS PRIVILEGED AND CONFIDENTIAL

TABLE OF CONTENTS

1.	Summary	7
2.	ELISA Method Description	7
3.	Plaque-Reduction Neutralization Test (PRNT) Method Description	8
4.	Pseudovirus Neutralization Assay (PsVNA) Description.....	9
	Figure 1. Cells Stained with Fluorescently Labelled RBD to Reveal hACE2 Expression.....	10
	Table 1. ID ₅₀ and ID ₈₀ Values for Assay Controls in 9 Runs of SARS-CoV-2 Pseudovirus Neutralization Assay during Fit-for-Purpose Testing.....	11
	Table 2. ID ₅₀ and ID ₈₀ Values for Assay Controls in 12 Runs of SARS-CoV-2 Pseudovirus Neutralization Assay during Testing of Clinical Samples	12
5.	SARS-CoV-2 nLuc High Throughput Neutralization Assay Description	12
	Figure 2. Genome organization of the SARS-CoV-2-Seattle nLuc recombinant virus.....	13
	Figure 3. Human COVID-19 convalescent sera tested in the SARS-CoV-2 nLuc HTNA	14
6.	Focus-Reduction Neutralization Test (FRNT) Method Description.....	15
	Figure 4. Schematic of the FRNT assay	16
	Table 3. Viridot FRNT settings.....	17
7.	Focus-Reduction Neutralization Test mNeonGreen (FRNT-mNG) Method Description	19
	Figure 5. Schematic of icSARS-CoV-2-mNG	19
	Figure 6. Schematic of the FRNT-mNG assay.....	20
	Figure 7. Sample Curves	21
	Table 4. Viridot FRNT-mNG settings	22
	Table 5. Viridot background correction settings.....	23
8.	Convalescent Sera Description	23
	Table 6. Convalescent Sera Tested in Serological Assays.....	24
9.	T Cell Mediated Immune Response Analysis Description	25
	Table 7. 13-color ICS Antibody Panel.....	26
	Figure 8. Flow Cytometric Gating Strategy	27
	Figure 9. SARS-CoV-2-Specific T Cell Responses in COVID-19 Convalescent Patient Samples. .	28
10.	Tables and Figures.....	29
	Table 8. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age 18 -55	30
	Table 9. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age 56-70	32
	Table 10. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age ≥ 71	34
	Table 11. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - S-2P – Age 18-55.....	36
	Table 12. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - S-2P – Age 56-70.....	38
	Table 13. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - S-2P – Age ≥ 71	40

Figure 10. Reverse Cumulative Distribution of Serum IgG ELISA Area Under the Curve (AUC) Values by Time Point and Vaccination Group - S-2P	42
Figure 11. Serum IgG ELISA Area Under the Curve (AUC) Values by Time Point and Vaccination Group - S-2P	43
Figure 12. Geometric Mean Area Under the Curve (AUC) Values by Time Point and Vaccination Group - S-2P	44
Figure 13. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - S-2P – Age 18-55	45
Figure 14. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - S-2P – Age 56-70	46
Figure 15. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - S-2P – Age ≥ 71	47
Table 14. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age 18-55	48
Table 15. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age 56-70	50
Table 16. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age ≥ 71	52
Table 17. Serum IgG ELISA Area Under the Curve (AUC) Geometric Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age 18-55	54
Table 18. Serum IgG ELISA Area Under the Curve (AUC) Geometric Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age 56-70	56
Table 19. Serum IgG ELISA Area Under the Curve (AUC) Geometric Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age ≥ 71	58
Figure 16. Reverse Cumulative Distribution of Serum IgG ELISA Area Under the Curve (AUC) Values by Time Point and Vaccination Group – RBD	60
Figure 17. Serum IgG ELISA Area Under the Curve (AUC) Values by Time Point and Vaccination Group – RBD	61
Figure 18. Geometric Mean Area Under the Curve (AUC) Values by Time Point and Vaccination Group – RBD	62
Figure 19. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - RBD – Age 18-55	63
Figure 20. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - RBD – Age 56-70	64
Figure 21. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - RBD – Age ≥ 71	65
Table 20. Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age 18 -55	66
Table 21. Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age 56-70	68
Table 22. Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – ≥ 71 Years	70
Table 23. Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - Vaccination Group - S-2P – Age 18-55	72
Table 24. Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - Vaccination Group - S-2P – Age 56-70	74

Table 25.	Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - Vaccination Group - S-2P - ≥ 71 Years	76
Figure 22.	Reverse Cumulative Distribution of Serum IgG ELISA Endpoint Titer Values by Time Point and Vaccination Group - S-2P.....	78
Figure 23.	Serum IgG ELISA Endpoint Titer Values by Time Point and Vaccination Group - S-2P	79
Figure 24.	Geometric Mean Endpoint Titer Values by Time Point and Vaccination Group - S-2P ...	80
Figure 25.	Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - S-2P – Age 18-55	81
Figure 26.	Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - S-2P – Age 56-70	82
Figure 27.	Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - S-2P – ≥ 71 Years	83
Table 26.	Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age 18-55	84
Table 27.	Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age 56-70.....	86
Table 28.	Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – ≥ 71 Years	88
Table 29.	Serum IgG ELISA Endpoint Titer Geometric Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age 18-55.....	90
Table 30.	Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age 56-70	92
Table 31.	Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD - ≥ 71 Years	94
Figure 28.	Reverse Cumulative Distribution of Serum IgG ELISA Endpoint Titer Values by Time Point and Vaccination Group - RBD	96
Figure 29.	Serum IgG ELISA Endpoint Titer Values by Time Point and Vaccination Group – RBD	97
Figure 30.	Serum IgG ELISA Geometric Mean Endpoint Titer Values by Time Point and Vaccination Group – RBD.....	98
Figure 31.	Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - RBD – Age 18-55	99
Figure 32.	Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - RBD – Age 56-70	100
Figure 33.	Serum IgG Endpoint Titer Distribution by Time Point and Treatment Group - RBD - ≥ 71 Years	101
Table 32.	Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID ₅₀ – Age 18-55	102
Table 33.	Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID ₅₀ – Age 56-70	104
Table 34.	Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID ₅₀ – Age ≥ 71	106
Figure 34.	Pseudovirus Neutralization Assay Titers by Time Point and Vaccination Group - ID ₅₀ .	108
Figure 35.	Pseudovirus Neutralization Assay GM by Time Point and Treatment Group - ID ₅₀	109
Figure 36.	Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID ₅₀ – Age 18-55	110

Figure 37. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID ₅₀ – Age 56-70	111
Figure 38. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID ₅₀ - Age ≥ 71	112
Table 35. Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID ₈₀ – Age 18-55	113
Table 36. Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID ₈₀ – Age 56-70	115
Table 37. Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID ₈₀ – Age ≥ 71	117
Figure 39. Pseudovirus Neutralization Assay Titers by Time Point and Vaccination Group - ID ₈₀	119
Figure 40. Pseudovirus Neutralization Assay GM by Time Point and Treatment Group - ID ₈₀	120
Figure 41. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID ₈₀ – Age 18-55	121
Figure 42. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID ₈₀ – Age 56-70	122
Figure 43. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID ₈₀ – Age ≥ 71	123
Table 38. Plaque Reduction Neutralization Test Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group – PRNT ₈₀ - 18-55 Years	124
Table 39: Plaque Reduction Neutralization Test Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - PRNT ₈₀ – 56-70 Years	125
Table 40: Plaque Reduction Neutralization Test Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - PRNT ₈₀ – ≥71 Years	126
Figure 44. Plaque Reduction Neutralization Test Geometric Mean by Time Point and Vaccination Group - PRNT ₈₀	127
Figure 45: Plaque Reduction Neutralization Test Titers Distribution by Time Point and Treatment Group - PRNT ₈₀	128
Table 41. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID ₅₀ - 18-55 Years	129
Table 42. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID ₅₀ – 56-70 Years	130
Table 43. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID ₅₀ – ≥ 71 Years.....	131
Figure 46: FRNT-mNG Geometric Mean by Time Point and Vaccination Group - ID ₅₀	132
Figure 47: FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID ₅₀ - 18-55 Years	133
Figure 48. FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID ₅₀ – 56-70 Years	134
Figure 49. FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID ₅₀ – ≥ 71 Years	135
Table 44. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group – ID ₈₀ – Age 18-55	136

Table 45. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group – ID ₈₀ – Age 56-70.....	137
Table 46. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group – ID ₈₀ – Age ≥71.....	138
Figure 50. FRNT-mNG Geometric Mean by Time Point and Vaccination Group - ID ₈₀	139
Figure 51. FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID ₈₀ – Age 18-55	140
Figure 52: FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID ₈₀ – Age 56-70	141
Figure 53: FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID ₈₀ – Age ≥ 71	142
Figure 54. Binding to SARS-CoV-2 Spike Proteins in ELISA Expressed as Area-Under-the-Curve (AUC) is Highly Correlated with Binding Expressed as Endpoint Dilution Titer	143
Figure 55. Binding to S-2P or RBD Proteins are Highly Correlated.....	145
Figure 56. Pseudovirus Neutralization Correlates with Binding in ELISA.....	147
Figure 57. Live-Virus Neutralization (PRNT ₈₀) Correlates with Binding in ELISA	149
Figure 58. FRNT-mNG Correlates with Binding in ELISA	150
Figure 59. Correlation Heatmap	151

IMMUNOGENICITY SUMMARY REPORT DMID Protocol 20-0003

Phase I, Open-Label, Dose-Ranging Study of the Safety and Immunogenicity of 2019-nCoV Vaccine (mRNA-1273) in Healthy Adults

1. SUMMARY

DMID Protocol 20-0003 is designed to assess the safety, tolerability, and immunogenicity of 2 vaccinations of mRNA-1273 at 25 mcg, 50 mcg, 100 mcg and 250 mcg. The immunogenicity data presented in this report focus on cumulative immune responses elicited in subjects aged 18 - 55 years, 56 - 70 years, and ≥ 71 years, through Day 119 post vaccination, or through Day 57 post vaccination for subjects that received the 50 mcg dose.

2. ELISA METHOD DESCRIPTION

Testing was performed using the automated ELISA method as detailed in VRC-VIP SOP 5500 *Automated ELISA on Integrated Automation System*. Quantification of IgG concentrations in serum/plasma were performed with a Beckman Biomek based automation platform. The IgG ELISA testing of clinical samples has been expanded to evaluate the presence of binding antibodies to either of two SARS-CoV-2 antigens, S-2P and RBD, each in singleplex format. The SARS-CoV-2 S-2P antigen (VRC-SARS-CoV-2 S-2P (15-1208)-3C-His8-Strep2x2) was described previously in the Immunogenicity Summary Report, 15 May 2020, SN 0014 and in the Clinical Information amendment for BB-IND 019635 SN 0017. Similar to the S-2P antigen, the SARS-CoV spike receptor binding domain (RBD - Ragon-SARS-CoV-2 S-RBD (319-529)-His8-SBP) antigen was produced, purified and generously provided by Dr. Dominic Esposito (Frederick National Laboratory for Cancer Research, NCI (see attached Protein Expression Laboratory Production Report for RBD).

Briefly, in singleplex format either SARS-CoV-2 S-2P at a working concentration of 2 $\mu\text{g/mL}$ (125 $\mu\text{L/well}$), or SARS-CoV-2 RBD antigen at a working concentration of 4 $\mu\text{g/mL}$ (125 $\mu\text{L/well}$) were coated onto Immulon 4HBX flat bottom plates overnight for 16 hours at 4° C. Antigen concentrations were defined during assay development and antigen lot titration. Plates were washed and blocked (3% milk TPBS) for 1 hour at room temperature. Duplicate serial 4-fold dilutions covering the range of 1:100 – 1:1638400 (8-dilution series) of the test sample (diluted in 1% milk in TPBS) were incubated at room temperature for 2 hours followed by Horseradish Peroxidase - labeled goat anti-human antibody detection (1 hour at room temperature) (Thermo Fisher Catalogue # A1881), and TMB substrate addition (15 minutes at room temperature; DAKO Catalogue # S1599) addition. Color development was stopped by addition of sulfuric acid and plates were read within 30 minutes at 450 nm and 650 nm via the Molecular Devices Paradigm plate reader. Each plate harbored a negative control (assay diluent), positive control and batches of 5 specimens run in duplicate. For SARS-CoV-2 S-2P, SARS-CoV-2 S2-specific monoclonal antibody S-652-112 (provided by VRC-VPL) spiked in NHS and/or pool of COVID-19 convalescent sera (VRC 200, NCT00067054) were used as a positive control. For SARS-CoV-2 RBD a pool of COVID-19 convalescent sera (VRC 200, NCT00067054) was included on each plate as positive control. All controls are trended over time. Endpoint Titer dilution from raw OD data was interpolated using the plate background OD + 10 STDEV by asymmetric sigmoidal 5-pl curve fit of the test sample. In

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

the rare event, the asymmetric sigmoidal 5-pl curve failed to interpolate the endpoint titer, a sigmoidal 4-pl curve was used for the analysis. Where the binding responses reached the upper limit of quantitation, the test samples were repeated in an 8-point, 4-fold dilution series starting at 1:5000 and the final reciprocal endpoint titer were reported. Area under the curve (AUC) was calculated with baseline anchored by the plate background OD + 10 STDEV. Data analysis was performed using Microsoft Excel and GraphPad Prism Version 8.0.

3. PLAQUE-REDUCTION NEUTRALIZATION TEST (PRNT) METHOD DESCRIPTION

Vaccinee sera were incubated at 56°C for 45 min and manually diluted in gelatin saline (0.3% [wt/vol] gelatin in phosphate-buffered saline supplemented with CaCl₂ and MgCl₂) to generate a 1:4 dilution of the original specimen, which served as a starting concentration for further serial log₂ dilutions in gelatin saline using an automated liquid handling system. The terminal serum concentration corresponded to 1/131,072 of the original. Antisera were combined with an equal volume of SARS-CoV-2 clinical isolate, SARS-CoV-2/human/USA/USA-WA1/2020 (GenBank: MN985325.1), in gelatin saline, producing an average final virus concentration of 580 plaque-forming units (PFU) per mL in each serum dilution ranging from final concentrations of 1/8 to 1/262,144 of the original. Virus/serum mixtures were incubated for 20 min at 37°C, followed by adsorption of 0.1 mL aliquots to each of two confluent Vero E6 cell monolayers in 10-cm² wells for 30 min at 37°C. Four aliquots of untreated (i.e., no serum) control virus were subjected to identical conditions. Cell monolayers were overlaid with Dulbecco's modified Eagle's medium containing 1% agar and incubated for 3 days at 37°C in humidified 5% CO₂. Plaques were enumerated by direct visualization, and the average number of plaques in virus/serum (duplicate) and virus-only (quadruplicate) wells was used to calculate percent neutralization at each serum dilution according to the following formula: 1 - (ratio of mean number of plaques in the presence and absence of serum). Each specimen was tested in two independent assays performed at different times. Fractional neutralization from duplicate specimens was plotted as a function of log₂ serum dilution, and the dose-response relationship was fit to a five-parameter logistic regression model using the package nplr⁴ in R⁵. PRNT₈₀ titers, expressed as the reciprocal of the highest serum dilution reducing virus infectivity by 80%, were calculated from resulting curves.

Convalescent sera were tested as described above with the following modifications: 1) serial log₄ serum dilutions to achieve final serum concentrations ranging from 1/4 to 1/524288 or 1/8 to 1/131072 of the original; 2) final virus concentration of 670 PFU per mL; 3) mean number of plaques in virus-only wells calculated from counts of four or 16 wells; 4) fractional neutralization from duplicate specimens plotted as a function of log₄ serum dilution; 5) duplicate specimen testing during the same work session.

Four dilutions of a COVID-19 convalescent serum control, spanning a 256-fold concentration range, were included with each performance of PRNT for longitudinal monitoring of assay stability. In addition, duplicate neutralization curves were inspected for agreement relative to expected deviations naturally arising from numerous interacting biological as well as technical variables inherent to PRNT. Unusually large disagreement between duplicate curves was resolved by additional testing.

Specimens exhibiting less than 80% inhibitory activity at the lowest dilution tested, 1:8, were assigned a titer of 4.

PRNT Assay Optimization. The Vanderbilt laboratory has extensively explored conditions for assay optimization and reproducibility, including identification of top-performing cell type and plating density, defining the incubation period that maximizes accuracy of plaque enumeration, and incorporation of robotic liquid handling for accurate and precise serum dilution. SARS-CoV-2 PRNT has been optimized as a fit-for-purpose research assay, permitting discrimination of day 1 signal from the day 43 neutralizing antibody response to vaccination

4. PSEUDOVIRUS NEUTRALIZATION ASSAY (PsVNA) DESCRIPTION

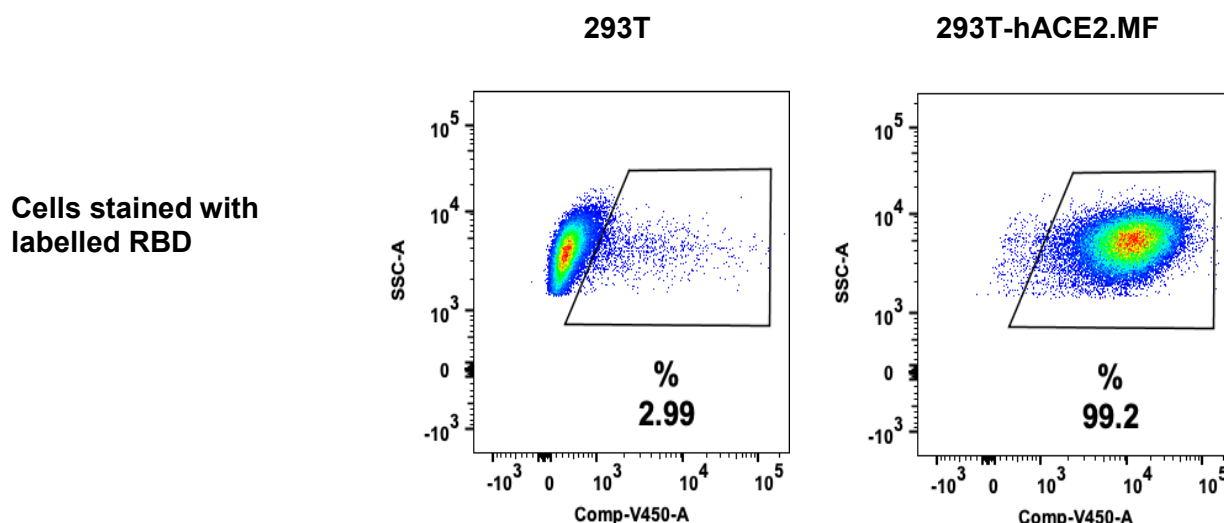
SARS-CoV-2 neutralizing antibodies in serum were measured in a pseudovirus neutralization assay (PsVNA), an exploratory readout. This is a research assay under development for the purpose of evaluating the samples in the clinical trial DMID Protocol number 20-0003.

This assay uses pseudotyped virus particles, also called pseudoviruses, in a single round of infection. It is performed according to the Human Immunology Core (HIC) SOP. Briefly: to produce SARS-CoV-2 pseudoviruses, an expression plasmid bearing codon-optimized CMV/R-SARS-CoV-2 full-length S (parental sequence Wuhan-1, Genbank #MN908947.3) is co-transfected into HEK293T/17 cells (ATCC # CRL-11268) with packaging plasmid pCMVDR8.2, luciferase reporter plasmid pHR' CMV-Luc, and a TMPRSS2 expression plasmid. Pseudoviruses are mixed with serial dilutions of sera or antibodies and then added to monolayers of ACE-2-overexpressing 293T cells in triplicate. Three days post infection, cells are lysed, luciferase is activated with the Luciferase Assay System (Promega), and relative light units (RLU) are measured at 570 nm on a Spectramax L luminometer (Molecular Devices). After subtraction of background RLU (uninfected cells), % neutralization is calculated as $100 \times ((\text{virus only}) - (\text{virus plus antibody})) / (\text{virus only})$. Virus only is measured as the average of 8 wells. Dose-response curves are generated with a 5-parameter nonlinear function, and titers reported as the serum dilution required to achieve 50% or 80% neutralization (50% inhibitory dilution (ID₅₀) or 80% inhibitory dilution (ID₈₀)). Assays are performed twice, in separate sessions, unless laboratory capacity constraints or sample volumes preclude a second run; and data are reported as the geometric mean of the values from both runs.

The lower limit of detection is the input dilution of serum, for example 1:20, and samples that do not neutralize at the 50% level are expressed as “less than” the input dilution, in this example <20. For statistical analysis and presentation, such ID₅₀ values are plotted or reported as half of the input dilution; in this example, <20 is plotted as 10. If duplicate assays return one value above 20 and one <20, the result is reported as the geometric mean of 10 and the positive assay; therefore, some values between 10 and 20 are reported.

The ACE-2-overexpressing 293T cells, here called 293T-hACE2.MF, were the kind gift of Michael Farzan (Department of Immunology and Microbiology, the Scripps Research Institute). ACE2 is a cellular receptor for SARS-CoV-2 (Hoffmann et al. *Cell* <https://doi.org/10.1016/j.cell.2020.02.052> (2020)). Upon receipt, the HIC laboratory established a research bank of cells comprised of 40 aliquots of passage 2 cells, stored in liquid nitrogen; these were tracked in our laboratory reagent database. After 9 weeks in culture, hACE2 expression was measured by flow cytometry; as seen in Figure 1, 99.2% of cells expressed hACE2.

Figure 1. Cells Stained with Fluorescently Labelled RBD to Reveal hACE2 Expression



Left, parental 293T cells; right, 293T-hACE2.MF.

Each assay setup always includes the same six controls: three pre-pandemic sera (collected in the time period 2009-May 2019 from participants in the clinical protocol VRC500, NCT01375530), two sera from SARS-CoV-2 convalescent donors (clinical protocol VRC200, NCT00067054), and one pool of convalescent sera (pooled from five donors from clinical protocol VRC200, NCT00067054). Sufficient volumes of these sera are on hand to serve as controls. Typically, an assay setup includes 18 or 21 assay plates, with 54 or 63 samples respectively, including the six controls.

After development of our SOP, and prior to running any clinical samples, an assay fit-for-purpose test was performed. This testing included three pools of mouse sera from mice vaccinated with SARS-CoV-2 S-2P protein (Kizzmekia Corbett, unpublished data), selected to cover a range of expected ID₅₀ and ID₈₀ values; and the pool of human convalescent sera described above. A total of nine assays were performed, by 6 operators subsequent to their training. Data are deemed acceptable if ID₅₀ and ID₈₀ are within 3-fold above or below the geometric mean of all runs, similar to the reported variability for HIV-1 Env-pseudovirus assay on TZM-bl cells (Sarzotti-Kelsoe, *Immunol Methods* 409, 131-146, 2014). **Table 1** shows the data from the fit-for-purpose test. It was observed that, for all but 2 of the 36 data points, ID₅₀ and ID₈₀ values were within three-fold of the geometric mean for the sample; and in most cases, data were within 2-fold of the geometric mean.

To monitor the assay performance over time, the values for the positive and negative controls are monitored in each run. The control data from 12 assays performed with clinical samples are shown in **Table 2**. The highest and lowest values for each sample observed varied no more than 2-fold higher or lower than the geometric mean. Thus, the assay performance with the clinical samples was improved over that observed in the fit-for-purpose test.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Subsequent to the start of the assays, the SOP was modified to allow the use of the D614G variant virus. This single amino acid mutation in Spike is now present in sequences from most of the cases worldwide, and appears to be more sensitive to neutralization by convalescent serum (ref: Korber et al Cell. 2020 Aug 20;182(4):812-827.e19). To make the pseudoviruses with D614G spike, we used a modified plasmid (gift of David Montefiori): our plasmid bearing codon-optimized CMV/R-SARS-CoV-2 full-length S (parental sequence Wuhan-1, Genbank #MN908947.3) was subjected to site-directed mutagenesis to provide the D614G amino acid change. The plasmid was sequenced to ensure that this mutation was present and no others. No other aspects of the assay were changed.

Table 1. ID₅₀ and ID₈₀ Values for Assay Controls in 9 Runs of SARS-CoV-2 Pseudovirus Neutralization Assay during Fit-for-Purpose Testing

ID50										
Sample	1	2	3	4	5	6	7	8	9	geomean
nCoV mouse serum pool #1	913	1427	721	552	1070	312	531	642	586	688
nCoV mouse serum pool #2	236	383	225	142	111	68	130	145	182	162
nCoV mouse serum pool #3	168	212	190	110	83	108	148	148	115	137
Human pool SARS-COV2-POS-1	213	192	285	198	271	110	379	156	152	204
ID80										
Sample	1	2	3	4	5	6	7	8	9	geomean
nCoV mouse serum pool #1	518	491	330	196	296	118	249	287	244	278
nCoV mouse serum pool #2	85	199	95	100	20	20	64	72	77	66
nCoV mouse serum pool #3	135	121	78	65	44	20	95	112	68	72
Human pool SARS-COV2-POS-1	106	76	66	64	56	46	62	84	88	70

Table 2. ID₅₀ and ID₈₀ Values for Assay Controls in 12 Runs of SARS-CoV-2 Pseudovirus Neutralization Assay during Testing of Clinical Samples

ID ₅₀													
Run #													
Sample	1	2	3	4	5	6	7	8	9	10	11	12	geomean
Pre-pandemic Serum #1	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	n/a
Pre-pandemic Serum #2	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	n/a
Pre-pandemic Serum #3	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	n/a
Human pool SARS-COV2-POS-1	321	117	129	219	277	233	187	190	160	242	159	167	192
Convalescent Serum #1	266	98	99	125	185	140	145	127	96	155	95	106	130
Convalescent Serum #2	3554	5057	1826	6055	4839	2591	3976	4691	4298	3820	2272	3670	3689

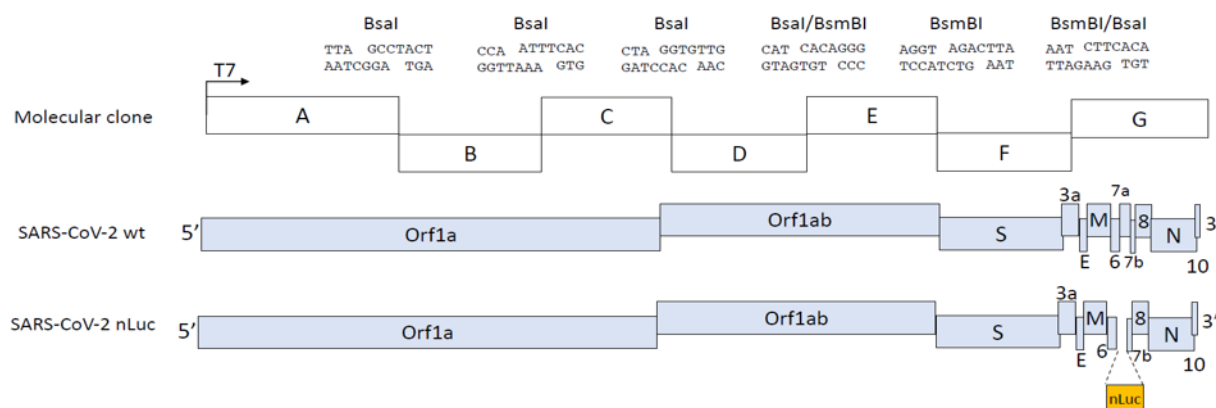
ID ₈₀													
Run #													
Sample	1	2	3	4	5	6	7	8	9	10	11	12	geomean
Pre-pandemic Serum #1	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	n/a
Pre-pandemic Serum #2	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	n/a
Pre-pandemic Serum #3	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	n/a
Human pool SARS-COV2-POS-1	84	31	59	59	54	76	61	59	41	91	46	46	56
Convalescent Serum #1	37	32	32	30	49	78	34	41	24	72	62	32	41
Convalescent Serum #2	1241	1103	1037	1797	2071	970	1417	1338	1534	716	1007	1137	1232

5. SARS-CoV-2 nLuc HIGH THROUGHPUT NEUTRALIZATION ASSAY DESCRIPTION

The SARS-CoV-2 nLuc High Throughput Neutralization Assay (HTNA) is a research assay under development to serve as a rapid, high-throughput live virus neutralization assay for evaluation of the neutralizing antibody response elicited by the mRNA-1273 investigational vaccine in the DMID 20-0003 Phase 1 clinical trial. The assay is performed in biosafety level 3 containment.

The SARS-CoV-2 recombinant virus, SARS-CoV-2-Seattle nLuc, which encodes for nLuciferase (nLuc) as an assay readout, was designed to express GFP-nLuc¹ (**Figure 2**) and recovered via reverse genetics as described previously^{2,3}, and is derived from a molecular clone based on the Seattle, Washington isolate (GenBank:MN985325)⁴. SARS-CoV-2-Seattle nLuc was titrated in Vero E6 cells (ATCC® CRL-1586; provided by USAMRIID) to obtain a relative light units (RLU) signal of at least >100X the cell only control background.

Figure 2. Genome organization of the SARS-CoV-2-Seattle nLuc recombinant virus



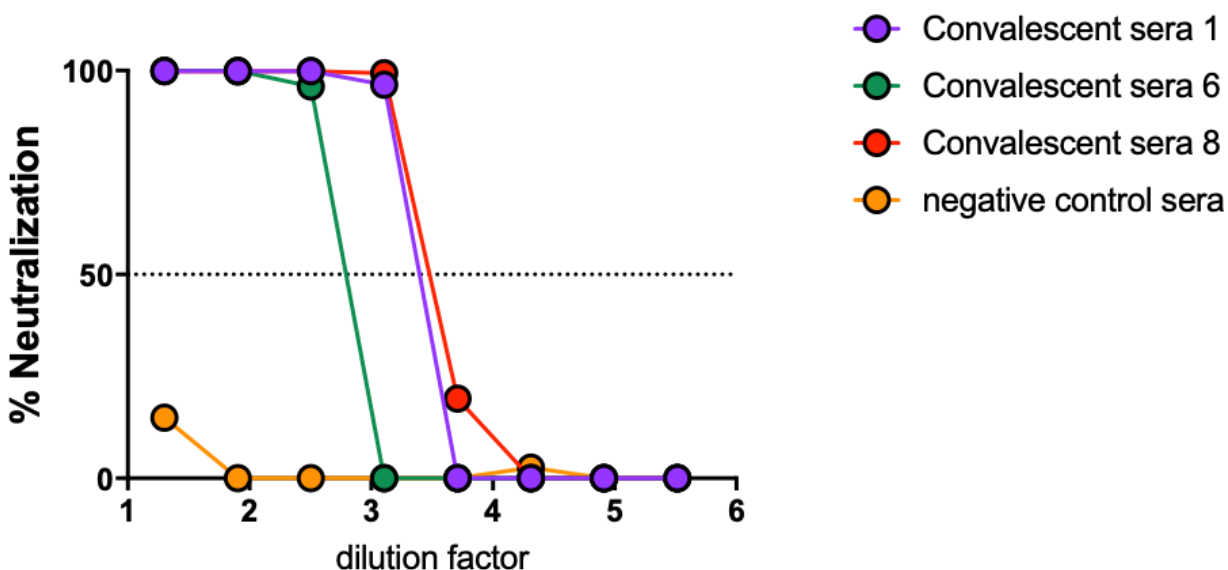
The GFP-nLuc expression cassette was inserted into the SARS-CoV-2 genome, replacing ORF7¹.

For the neutralization assay, Vero E6 cells in growth medium (DMEM, 10% FetalClone II (Hyclone), 1% penicillin streptomycin, 1% Antibiotic Antimycotic) were plated at 2×10^4 cells per well the day prior in clear bottom, black-walled 96 well plates. Clinical serum samples were heat-inactivated at 56°C for 30 minutes and were serially diluted 3-fold at a starting dilution of 1:20, with up to 8 dilution points in growth medium, on a separate 96 well plate ('serum dilution plate'). Sixty microliters of SARS-CoV-2-Seattle nLuc working stock (1500 plaque forming units (PFU) per mL, in growth medium) was plated on a separate 96 well plate ('serum/virus incubation plate'), and an equal volume of serially diluted sera was transferred from the serum dilution plate to the serum/virus incubation plate. Well contents were mixed, and antibody-virus complexes were incubated at 37°C with 5% CO₂ for 1 hour. Following incubation, growth medium was removed from the plated Vero E6 cells and 100 µL of virus-antibody dilution complexes from the serum/virus incubation plate was transferred to the cells in duplicate. Virus-only and cell-only controls were included in each neutralization assay plate. Plates were incubated at 37°C with 5% CO₂ for 48 hours. After the 48-hour incubation, plates were equilibrated to room temperature for 10 minutes, cells were lysed with the Nano-Glo Luciferase Reagent (Nano-Glo Luciferase System, Promega, Cat #N1130) for 2 min at room temperature, and luciferase activity was measured on a Spectramax M3 plate reader with the standard CellTiter Glo program. SARS-CoV-2-Seattle nLuc neutralization titers were defined as the sample dilution at which a 50% reduction in RLU was observed relative to the average of the virus control wells. GraphPad Prism was used to calculate the ID₅₀ based on a sigmoidal dose-response curve.

To demonstrate the dynamic range and specificity of the SARS-CoV-2 nLuc assay, we evaluated 9 serum samples obtained from COVID-19 survivors, who had cleared infection, under UNC IRB 20-1141 which was compliant with institutional IRB oversight. Starting at a 1:20 dilution with subsequent serial 3-fold dilutions, the SARS-CoV-2-Seattle nLuc assay afforded discrimination of a 2.5 log range of neutralization titers, with ID₅₀ values ranging from 28.2 to 3380, in this small subset of human sera samples. Importantly, negative control sera (collected under UNC IRB-approved protocol 08-0895 for collection of sera from individuals that have travelled to areas that are endemic for flaviviruses) gave values of <20 under identical conditions of treatment (**Figure 3**).

Figure 3. Human COVID-19 convalescent sera tested in the SARS-CoV-2 nLuc HTNA

Convalescent sera obtained from discharged COVID-19 patients under an IRB approved protocol were tested for neutralizing activity using the SARS-CoV-2 nLuc HTNA. The sera were serially diluted 3-fold, mixed with virus, and ID₅₀ values were calculated using a sigmoidal dose-response curve in Graph Pad Prism. % neutralization is plotted against log dilution factor. The neutralization curves for representative sera are shown as examples in the graph. ID₅₀ neutralization titers for the full set of convalescent sera are provided in the lower panel.



COVID-19 human sera										
	Convalescent sera 1	Convalescent sera 2	Convalescent sera 3	Convalescent sera 4	Convalescent sera 5	Convalescent sera 6	Convalescent sera 7	Convalescent sera 8	Convalescent sera 9	Negative control sera
Sera ID ₅₀	1689	169.5	28.21	403.7	118.2	415.2	1609	3380	1250	<20

These data demonstrate a range of neutralizing titers from natural infection, demonstrate the range and sensitivity of the assay, and provide a high-throughput assay for clinical testing.

Data for the nLuc HTNA for cohorts 1 and 2 were derived from a 3-fold dilution series at a starting dilution of 1:20. During optimization, the assay was further modified using a 2-fold-dilution series. Data for the nLuc HTNA for cohorts 5 and 8 were derived from a 2-fold dilution series at a starting dilution of 1:40. Data for the convalescent sera provided by the VRC were derived from a 2-fold dilution series at a starting dilution of 1:50. The lower limit of detection is the input dilution of serum, for example 1:20, and samples that do not neutralize at the 50% level are expressed as “less than” the input dilution, in this example <20. For statistical analysis and presentation, such ID₅₀ values are plotted or reported as half of the input dilution; in this example, <20 is plotted as 10.

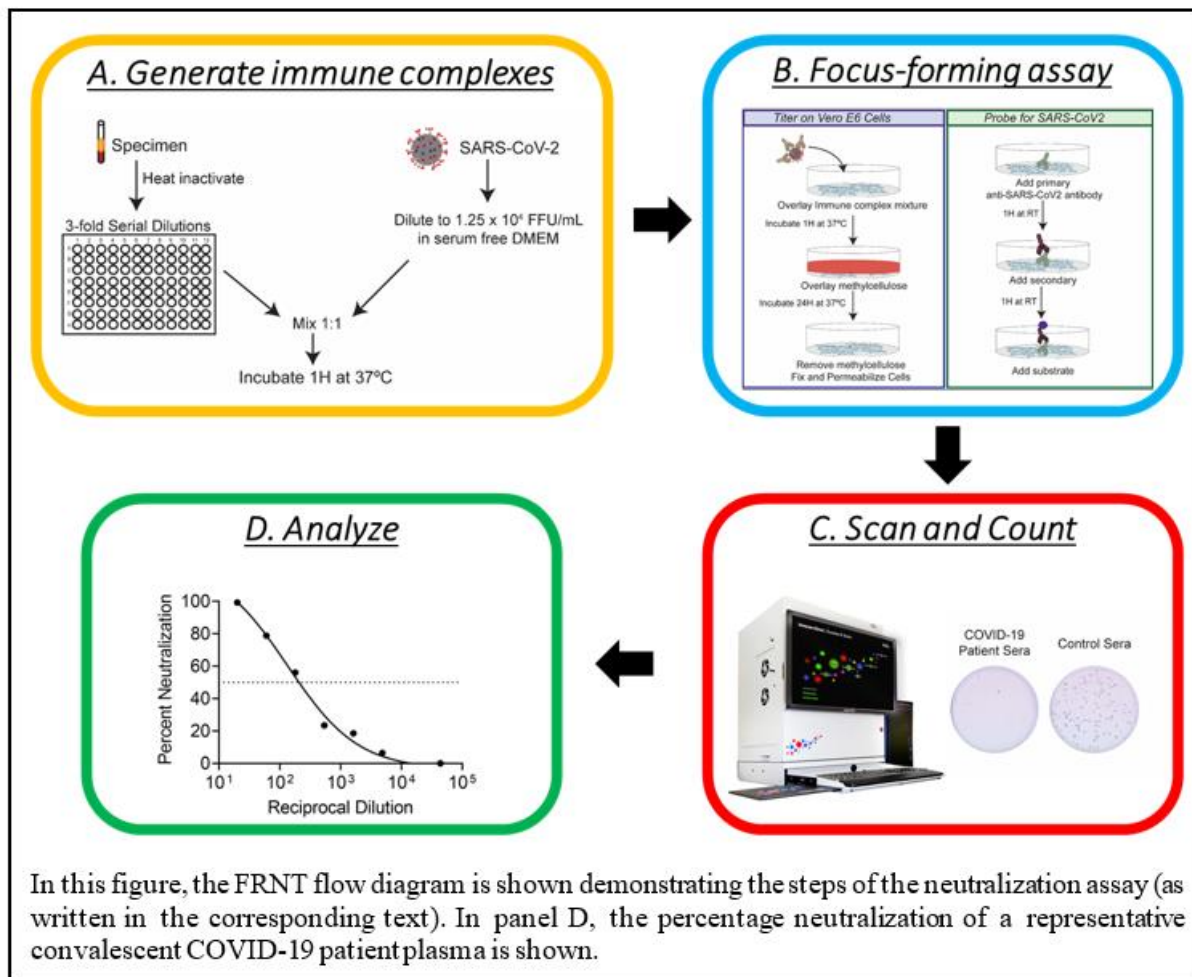
The nLuc HTNA was evaluated in concert with different virus neutralization assays (PRNT, pseudovirus neutralization assay, and nLuciferase HTNA) to identify a high-throughput SARS-CoV-2 virus neutralization assay for evaluation of the phase 1 sera from all cohorts.

1. Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH 3rd, Kato T, Lee RE, Yount BL, Mascenik TM, Chen G, Olivier KN, Ghio A, Tse LV, Leist SR, Gralinski LE, Schäfer A, Dang H, Gilmore R, Nakano S, Sun L, Fulcher ML, Livraghi-Butrico A, Nicely NI, Cameron M, Cameron C, Kelvin DJ, de Silva A, Margolis DM, Markmann A, Bartelt L, Zumwalt R, Martinez FJ, Salvatore SP, Borczuk A, Tata PR, Sontake V, Kimple A, Jaspers I, O'Neal WK, Randell SH, Boucher RC, Baric RS. (2020). SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. *Cell*. May 27:S0092-8674(2)30675-9. Online ahead of print.
2. Scobey, T., Yount, B.L., Sims, A.C., Donaldson, E.F., Agnihothram, S.S., Menachery, V.D., Graham, R.L., Swanstrom, J., Bove, P.F., Kim, J.D., et al. (2013). Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. *Proc Natl Acad Sci U S A* 110:16157-16162.
3. Yount, B., Curtis, K.M., Fritz, E.A., Hensley, L.E., Jahrling, P.B., Prentice, E., Denison, M.R., Geisbert, T.W., and Baric, R.S. (2003). Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. *Proc Natl Acad Sci U S A* 100:12995-13000.
4. Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel SK, Murray J, Queen K, Tao Y, Paden CR, Zhang J, et al. (2020). Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States. *Emerg. Infect. Dis.* 26:1266-1273

6. FOCUS-REDUCTION NEUTRALIZATION TEST (FRNT) METHOD DESCRIPTION

The ultimate functional test for evaluating immunity following SARS-CoV-2 exposure or determining the potential efficacy of a vaccine candidate is through a virus neutralization assay. A PRNT assay is a classic method for determining the neutralization capacity of a plasma sample against coronavirus infection/vaccination¹. However, this assay is labor-intensive, costly, time-consuming and low throughput. To further increase throughput, we recently developed an FRNT assay, which involves using an infectious clone (ic) derived wild type SARS-CoV-2 virus (strain USA-WA1/2020; GenBank: MN985325.1)². In this assay, a patient serum or plasma sample is incubated with icSARS-CoV-2 followed by infection of Vero E6 cells³. The neutralization potency of the serum/plasma sample is measured by the reduction in virally infected foci. The FRNT assay allows for rapid turnaround time (<24 hours), and increased throughput (~750 samples/week). The workflow is shown in **Figure 4**.

Figure 4. Schematic of the FRNT assay



Protocol:

Prior to the day of the neutralization test, Vero E6 cells (ATCC C1008 Cat# ATCC CRL-1586) were seeded at 2.5×10^4 cells/well. The Vero E6 cells were initially passaged eight times and a bank of cells were frozen down. Vero E6 cells are used until passage 25. COVID-19 patient specimen or vaccinee specimen were incubated at 56°C for 30 min to inactivate complement factors. The specimen can be either serum or plasma. Samples were manually diluted in duplicate in serum-free Dulbecco's modified Eagle's medium (DMEM) to generate a 1:10 dilution of the original specimen, which served as a starting concentration for further serial dilutions in DMEM. Three-fold serial dilutions (8-point) were performed and correspond to the following dilutions: 1/10, 1/30, 1/90, 1/270, 1/810, 1/2430, 1/7290, and 1/21,870. An equal volume of icSARS-CoV-2 virus² (strain USA-WA1/2020; GenBank: MN985325.1) was added to the diluted specimen in DMEM, producing an average final virus concentration of 1,000 focus-forming units per mL in each specimen dilution ranging from final concentrations of 1/20 to 1/43,740 of the original. Virus/specimen mixtures were incubated for 60 min at 37°C with 5% CO₂ (85-95% humidity), followed by adsorption of 0.1 mL onto a confluent Vero E6 cell monolayer in a well of a 96-well plate for

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

60 min at 37°C with 5% CO₂ (85-95% humidity). Virus only controls were included in each batch of neutralization assays, where columns within a 96-well plate were incubated with DMEM containing either no specimen or a negative control specimen (healthy control or pre-pandemic). After 60 min, the inoculum was removed and replaced with 100 µL of DMEM containing 0.85% methylcellulose and incubated for 24-30 hours at 37°C with 5% CO₂ (85-95% humidity). Methylcellulose was removed and cells washed 3 times with 150 µl Phosphate-buffered saline (PBS) followed by fixation with 100 µL of 2% paraformaldehyde in PBS for 30 min at room temperature (RT). The 2% PFA is removed and cells washed twice with 150 µL PBS. The cells were then washed once with 150 µL of PBS followed by permeabilization using 100 µl 0.1% saponin and 0.1% Bovine Serum Albumin (Perm/Wash) for 30 min at RT. The cells were then incubated with a cross-reactive biotinylated SARS-CoV anti-RBD antibody (monoclonal CR3022) at a dilution of 1:10,000 for 1 hour at room temperature or overnight at 4°C (12-18 hours; antibody is diluted in Perm/Wash). Cells were washed four times with 150 µL of PBS and incubated with Avidin-HRP (diluted in Perm/Wash) at 1:2000 for 1 hour at RT. Cells were washed four times with 150 µL of PBS and incubated with TrueBlue substrate for 30 min at RT. Cells were washed two times with 150 µL of PBS and lightly dried.

Table 3. Viridot FRNT settings

Parameter Setting	Setting
Step 1. Light Setting	Saturation
Step 2. Blur image	3
Step 3.1. Cut well edges	30
Step 4.1. Apply contrast to image based on background intensity	3
Step 4.2. Apply contrast to image based on plaque intensity	2
Step 5.1. Select difference in pixel value to distinguish plaque from background	0.09
Step 5.2. Select size (in pixels) of the window for applying the thresholding algorithm to the image	40
Step 6. Dilate your plaques to ensure they are counted as single plaques	8
Step 7. Cut overlapping plaques so they are counted separately	3
Step 8.1. Define the minimum pixel size to count as a plaque	30
Step 8.2. Define the maximum pixel size to count as a plaque	3000

Foci were visualized using an ELISPOT reader (CTL ImmunoSpot S6 Universal Analyzer) using visible light, and enumerated using Viridot⁴. The plates were visualized under the ELISPOT clear plate configuration using the visible light from the bottom of the plate (auto-exposure setting). The Lens control, Range and Precision are set to default settings and the Zoom is set to 1.6X. Once the images are acquired, the CTL extension on the filenames are changed to .TIF using Bulk Rename Utility (version 3.3.1.0). The foci in each well were enumerated using Viridot⁴. Viridot is an R-based software with a user interface in shiny that can enumerate foci. For the FRNT assay, we use the “saturation” light setting and adjusted the parameters as described in **Table 3**. The average number of foci in each virus/serum sample (duplicate) at the highest dilution were used to calculate the neutralization curves: 1 - (ratio of mean number of foci in the presence of sera and foci at the highest dilution of respective sera serum). The FRNT₅₀ titer is interpolated using a 4-parameter nonlinear regression in GraphPadPrism 8.4.3. Each specimen was tested in two independent assays performed at different times. To determine the final titer, the geometric mean titer is calculated using the titers from each of the duplicates from the two

independent runs. Specimens were retested if the geometric mean titers from the two independent assays displayed greater than a 3-fold difference in neutralization titers. In this case, the assay is run a third time. Two independent assays that fall within a 3-fold difference are used to calculate the final titer. The final titer is reported as the reciprocal dilution which neutralizes 50% of SARS-CoV-2.

Generation of CR3022 monoclonal antibody. The SARS-CoV S glycoprotein specific antibody CR3022 was generated recombinantly using previously reported heavy and light variable domain sequences deposited in GenBank under accession numbers DQ168569 and DQ168570⁵. Antibody variable domain gene sequences were synthesized by IDT and cloned into human IgG1 and human kappa expression vectors as previously described⁶. Antibodies were produced in Expi293F cells (ThermoFisher Scientific Cat# A14527) according to the manufacturer's recommendations by co-transfecting heavy and light chain plasmids at a ratio of 1:1.5. Antibodies were purified using rProtein A Sepharose Fast Flow antibody purification resin (GE Healthcare Cat# GE17-1279-03) and buffer exchanged into PBS before use. Biotinylated versions of CR3022 used in viral neutralization assays were produced by combining the antibody with a 20 molar excess of EZ-Link NHS-PEG4-Biotin (ThermoFisher Scientific Cat# 21330) for 1 hour at room temperature. Reactions were stopped by adding Tris pH 8 to a final concentration of 10 mM. The biotinylated antibody was then buffer exchanged >1000X into PBS using a 10 kDa protein spin-concentrator (Amicon).

FRNT Assay Optimization. The Emory laboratory is currently exploring conditions for assay optimization and reproducibility, including cell plating density, defining the incubation period that maximizes the accuracy of foci enumeration, viral infection titers, second operator intermediate precision, repeatability, and intermediate precision, quantitation limit/sensitivity, and specificity. We have tested over 50 combined healthy control samples that were taken either before or after the COVID-19 pandemic⁷. Some of the samples were plasma samples that were collected prior to the COVID-19 pandemic and others were samples collected by a collaborator as part of their routine analysis of evaluating Cholera or Dengue infection in humans (all of these samples that were tested are de-identified). The samples did not show any neutralization activation against SARS-CoV-2 at a titer of 1:50, which represents the lowest dilution tested in this study⁷. The SARS-CoV-2 FRNT assay is a fit-for-purpose research assay. The intent of evaluating the FRNT assay was to identify a high-throughput SARS-CoV-2 virus neutralization assay for evaluation of the phase 1 sera from all cohorts, which involved demonstration of concordance between the different virus neutralization assays (PRNT, pseudovirus neutralization assay, and nLuciferase HTNA).

References

- 1 Rockx, B. *et al.* Structural basis for potent cross-neutralizing human monoclonal antibody protection against lethal human and zoonotic severe acute respiratory syndrome coronavirus challenge. *J Virol* **82**, 3220-3235, doi:10.1128/JVI.02377-07 (2008).
- 2 Xie, X. *et al.* An Infectious cDNA Clone of SARS-CoV-2. *Cell host & microbe* **27**, 841-848 e843, doi:10.1016/j.chom.2020.04.004 (2020).
- 3 Harcourt, J. *et al.* Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States. *Emerg Infect Dis* **26**, 1266-1273, doi:10.3201/eid2606.200516 (2020).
- 4 Katzelnick, L. C. *et al.* Viridot: An automated virus plaque (immunofocus) counter for the measurement of serological neutralizing responses with application to dengue virus. *PLoS neglected tropical diseases* **12**, e0006862, doi:10.1371/journal.pntd.0006862 (2018).

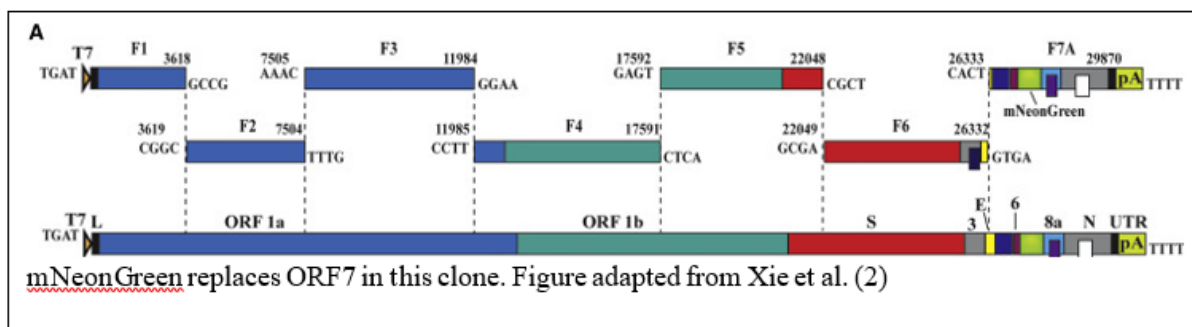
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

- 5 Meulen, J. *et al.* Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. *PLoS Med* **3**, e237, doi:10.1371/journal.pmed.0030237 (2006).
- 6 Smith, K. *et al.* Rapid generation of fully human monoclonal antibodies specific to a vaccinating antigen. *Nature protocols* **4**, 372-384, doi:10.1038/nprot.2009.3 (2009).
- 7 Suthar, M. S. *et al.* Rapid Generation of Neutralizing Antibody Responses in COVID-19 Patients. *Cell Rep Med* **1**, 100040, doi:10.1016/j.xcrm.2020.100040 (2020).

7. FOCUS-REDUCTION NEUTRALIZATION TEST mNEONGREEN (FRNT-MNG) METHOD DESCRIPTION

The FRNT-mNG assay involves using a recombinant infectious clone (ic) SARS-CoV-2-mNeonGreen (mNG) live virus (**Figure 5**)¹. In this assay, a patient serum or plasma sample is incubated with icSARS-CoV-2-mNG followed by infection of Vero E6 cells. The neutralization potency of the serum/plasma sample is measured by the reduction in fluorescent foci. The FRNT-mNG assay allows for fewer post-processing steps (no antibody cell staining), rapid turnaround time (<24 hours), and increased throughput (~750 samples/week). The workflow is described in **Figure 6**.

Figure 5. Schematic of icSARS-CoV-2-mNG



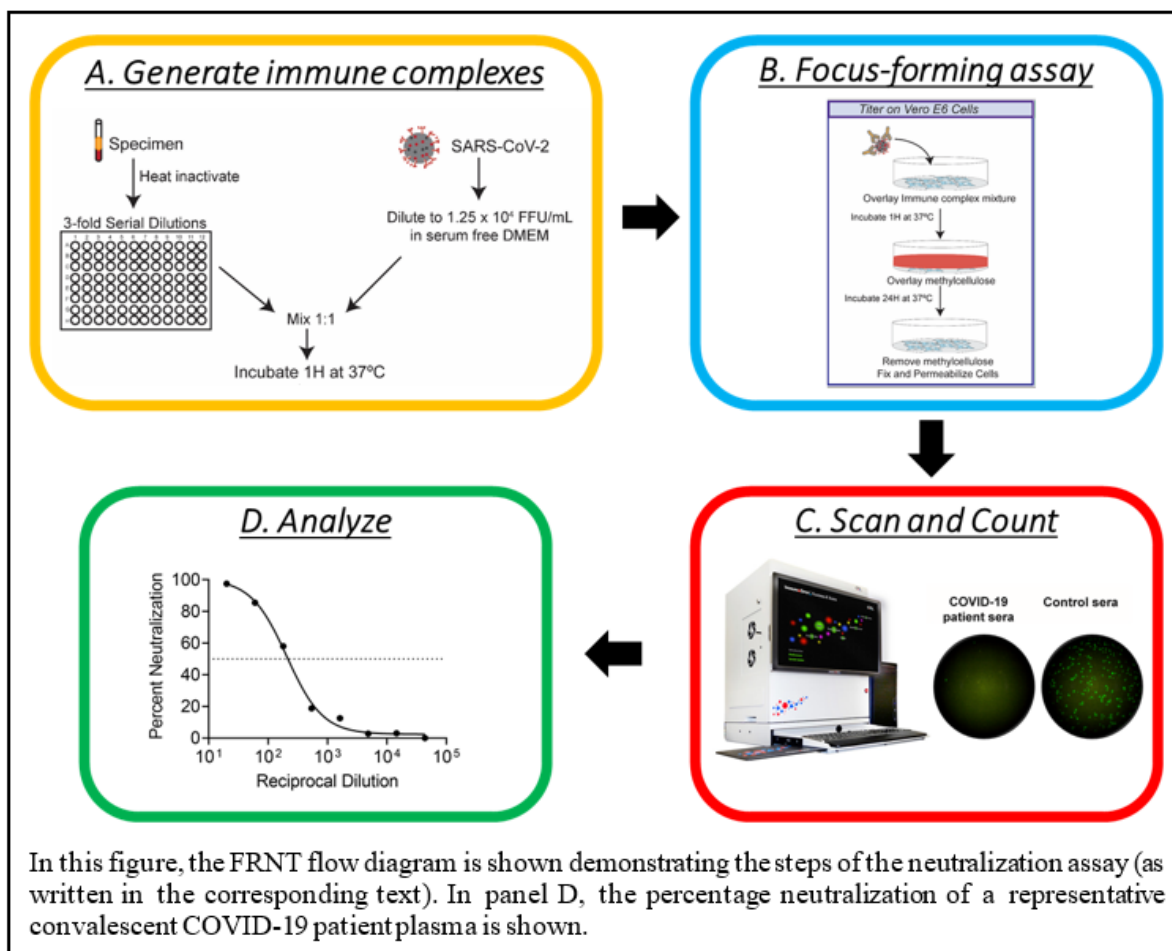
Protocol:

Prior to the day of the neutralization test, Vero E6 cells (ATCC C1008 Cat# ATCC CRL-1586) were seeded at 2.5×10^4 cells/well. The Vero E6 cells were initially passaged eight times, and a bank of cells was frozen down. Vero E6 cells are used until passage 25. COVID-19 vaccinee or patient specimens were incubated at 56°C for 30 min to inactivate complement factors. The specimen can be either serum or plasma. Samples were manually diluted in duplicate in serum-free Dulbecco's modified Eagle's medium (DMEM) to generate a 1:10 dilution of the original specimen, which served as a starting concentration for further serial dilutions in DMEM. Three-fold serial dilutions (8-point) were performed and correspond to the following dilutions: 1/10, 1/30, 1/90, 1/270, 1/810, 1/2430, 1/7290, and 1/21,870. An equal volume of icSARS-CoV-2-mNG virus¹ (strain USA-WA1/2020; GenBank: MN985325.1; **Figure 5**) was added to the diluted specimen in DMEM, producing an average final virus concentration of 1,667 GFP focus forming units per mL in each serum dilution ranging from final concentrations of 1/20 to 1/43,740 of the original. Virus/specimen mixtures were incubated for 60 min at 37°C with 5% CO₂ (85-95% humidity), followed by adsorption of 0.1 ml onto a confluent Vero E6 cell monolayer in a well of a 96-well plate for 60 min at 37°C with 5% CO₂ (85-95% humidity). Virus only controls were included in each batch of neutralization assays, wherein a column within a 96-well plate was incubated with DMEM containing no

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

sera or a negative control specimen (healthy control or pre-pandemic). After 60 min, the inoculum was removed and replaced with 100 μ l DMEM containing 0.85% methylcellulose and incubated for 24-30 hours at 37°C with 5% CO₂ (85-95% humidity). Methylcellulose was removed, and cells washed 3 times with 150 μ l Phosphate-buffered saline (PBS) followed by fixation with 100 μ l of 2% paraformaldehyde in PBS for 30 min at room temperature (RT). The 2% PFA is removed and cells washed twice with 150 μ l PBS. A final volume of 35 μ l PBS is added to each well for visualization (**Figure 6**, blue panel). Foci were visualized using an ELISPOT reader (CTL ImmunoSpot S6 Universal Analyzer) under a FITC channel (**Figure 6**, red panel).

Figure 6. Schematic of the FRNT-mNG assay

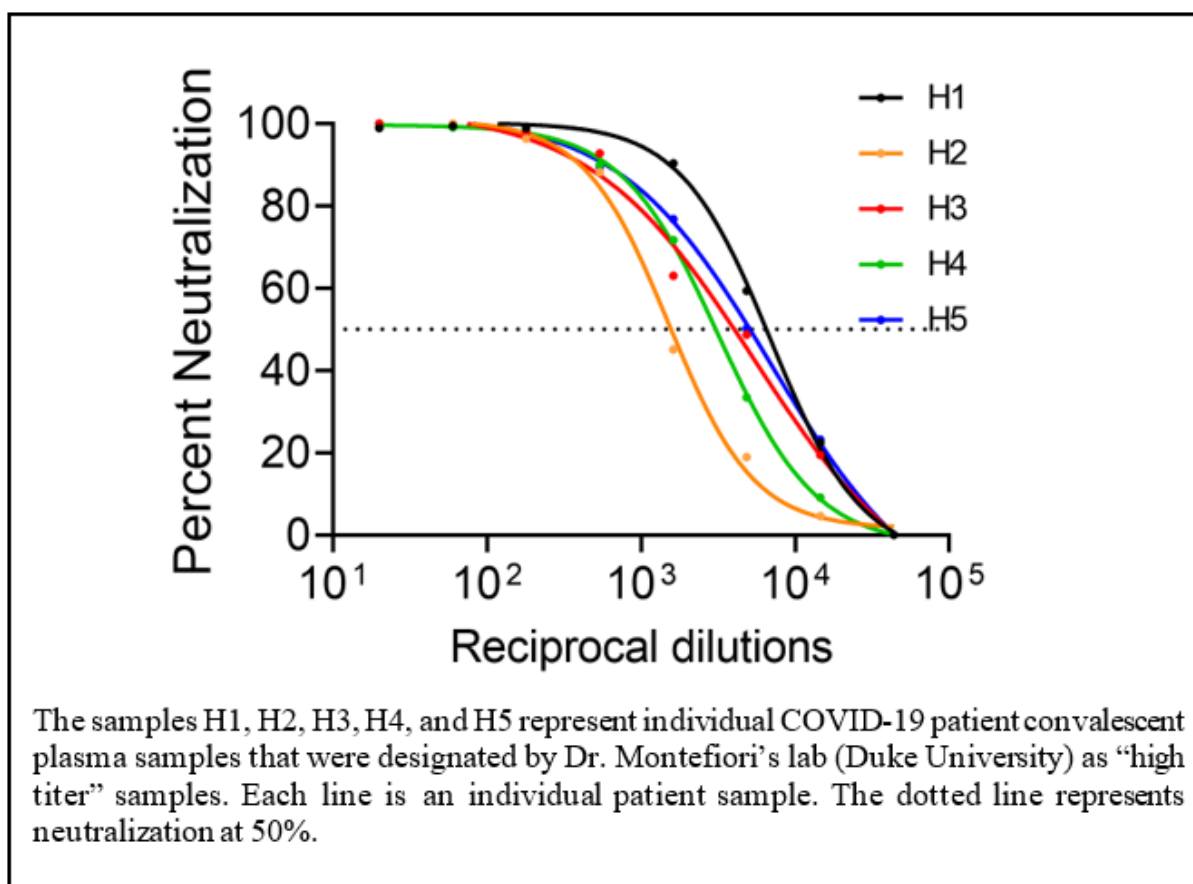


Plates can be visualized immediately following fixation, or stored at 4°C for up to 3 days. The plates were visualized under the Fluorospot plate configuration using an excitation band of 480 nm, gain setting of 28.0 and time setting of 2800. The Lens control is set to the position at 6196, Range at 600, Precision at 10, and Zoom at 1.8X. Once the images are acquired, the CTL extension on the filenames is changed to .TIF using Bulk Rename Utility (version 3.3.1.0). The foci in each well were enumerated using Viridot (version 1.0)². Viridot is an R-based software with a user interface in shiny that can enumerate foci. For

accurate counting, a two-step process was involved for counting foci with the FRNT-mNG assay. First, foci were counted, which included automated counting of both green foci, which represent virally infected cells, and background signal. Thus, a correction is needed to remove the background signal. To do this, we set the Viridot parameters to the FRNT-mNG settings. (Table 4). Next, each well is counted again under the Background correction settings (Table 5). The foci counted under the Background correction settings are subtracted from the foci counted under the FRNT-mNG parameter settings for each individual well. This represents the foci counts per well. Then, the average number of foci in each virus/serum sample (duplicate) at the highest dilution was used to calculate the neutralization curves: $1 - (\text{ratio of the mean number of foci in the presence of sera and foci at the highest dilution of respective sera serum})$.

Figure 7 shows representative neutralization curves. The FRNT₅₀ titer is interpolated using a 4-parameter nonlinear regression in GraphPad Prism 8.4.3. Each specimen was tested in two independent assays performed at different times. To determine the final titer, the geometric mean titer is calculated using the titers from each of the duplicates from the two independent runs. Specimens were retested if the geometric mean titers from the two independent assays displayed greater than a 3-fold difference in neutralization. In this case, the assay is run a third time. Two independent assay that fall within a 3-fold difference are used to calculate the final titer. The final titer is reported as the reciprocal dilution which neutralizes 50% of SARS-CoV-2.

Figure 7. Sample Curves



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

FRNT-mNG Assay Optimization. The Emory laboratory has explored conditions for assay optimization and reproducibility, including cell plating density, defining the incubation period that maximizes the accuracy of foci enumeration, viral infection titers, second operator intermediate precision, repeatability, and intermediate precision, quantitation limit/sensitivity and specificity. In terms of sensitivity, we tested COVID-19 convalescent patient samples provided by Duke University (David Montefiori), which were grouped between pre-determined low, medium, and high titer ranges, as part of a Pilot Concordance Assay. We also tested serial dilutions of COVID-19 patient samples to determine the endpoint titer. The average coefficient of variation (CV) for the low titer samples (GM= 212.2) was 38.0%, with a range from 17.9% to 58.6%. 3. The average CV for the medium titer samples (GM= 1577.2) was 47.7%, with a range from 28.0% to 67.5%. The average CV for the high titer samples (GM= 3647.5) was 31.9%, with a range from 12.4% to 43.6%. We have also tested over 50 healthy control samples that were taken either before or after the COVID-19 pandemic. Some of these samples were plasma samples that were collected prior to the COVID-19 pandemic and others were samples collected by a collaborator as part of their routine analysis of evaluating Cholera or Dengue infection in humans (all of these samples are de-identified). 0/50 samples displayed any neutralization activity against SARS-CoV-2 at a titer of 1:50, which represents the lowest dilution tested in this study. The SARS-CoV-2 FRNT-mNG neutralization assay is a high-throughput fit-for-purpose research assay. The intent of evaluating the FRNT-mNG assay was to identify a high-throughput SARS-CoV-2 virus neutralization assay for evaluation of the phase 1 sera from all cohorts, which involved demonstration of concordance between the different virus neutralization assays (PRNT, pseudovirus neutralization assay and, nLuciferase HTNA). The FRNT-mNG assay will be employed to quantify neutralizing antibody responses in sera from the mRNA-1273 phase 1 clinical trial going forward, in addition to the pseudovirion neutralization assay. It was important to demonstrate that the data from the FRNT and the reporter virus-based FRNT-mNG assays were highly correlative.

Table 4. Viridot FRNT-mNG settings

Parameter Setting	Setting
Step 1. Light Setting	Red
Step 2. Blur image	3
Step 3.1. Cut well edges	30
Step 4.1. Apply contrast to image based on background intensity	1.3
Step 4.2. Apply contrast to image based on plaque intensity	1
Step 5.1. Select difference in pixel value to distinguish plaque from background	0.13
Step 5.2. Select size (in pixels) of the window for applying the thresholding algorithm to the image	40
Step 6. Dilate your plaques to ensure they are counted as single plaques	13
Step 7. Cut overlapping plaques so they are counted separately	3
Step 8.1. Define the minimum pixel size to count as a plaque	30
Step 8.2. Define the maximum pixel size to count as a plaque	3000

Table 5. Viridot background correction settings

Parameter Setting	Setting
Step 1. Light Setting	Green
Step 2. Blur image	3
Step 3.1. Cut well edges	30
Step 4.1. Apply contrast to image based on background intensity	1.4
Step 4.2. Apply contrast to image based on plaque intensity	1
Step 5.1. Select difference in pixel value to distinguish plaque from background	0.14
Step 5.2. Select size (in pixels) of the window for applying the thresholding algorithm to the image	40
Step 6. Dilate your plaques to ensure they are counted as single plaques	8
Step 7. Cut overlapping plaques so they are counted separately	2
Step 8.1. Define the minimum pixel size to count as a plaque	30
Step 8.2. Define the maximum pixel size to count as a plaque	3000

References

- 1 Xie, X., Muruato, A., Lokugamage, K. G., Narayanan, K., Zhang, X., Zou, J., Liu, J., Schindewolf, C., Bopp, N. E., Aguilar, P. V., Plante, K. S., Weaver, S. C., Makino, S., LeDuc, J. W., Menachery, V. D. & Shi, P. Y. An Infectious cDNA Clone of SARS-CoV-2. *Cell host & microbe* 27, 841-848 e843, doi:10.1016/j.chom.2020.04.004 (2020). PMID: PMC7153529.
- 2 Katzelnick, L. C., Coello Escoto, A., McElvany, B. D., Chavez, C., Salje, H., Luo, W., Rodriguez-Barraquer, I., Jarman, R., Durbin, A. P., Diehl, S. A., Smith, D. J., Whitehead, S. S. & Cummings, D. A. T. Viridot: An automated virus plaque (immunofocus) counter for the measurement of serological neutralizing responses with application to dengue virus. *PLoS neglected tropical diseases* 12, e0006862, doi:10.1371/journal.pntd.0006862 (2018). PMID: PMC6226209.

8. CONVALESCENT SERA DESCRIPTION

Convalescent sera were collected from a total of 41 individuals with confirmed Covid-19 diagnosis. These samples were included in convalescent sera panels and tested along with the vaccine trial participant samples as comparators for the ELISA and PsVNA vaccine-induced responses and to establish correlations across the ELISA (S-2P and RBD) and the PsVNA. Binding and PsVNA data are shown for one serum sample from each of the 41 individuals. The samples were collected under IRB approved protocols at the National Institutes of Health, Bethesda MD (NCT00067054); Aaron Diamond AIDS Research Center, Columbia University, New York NY (NCT04342195); the University of Washington, Seattle WA (HAARVI study and STUDY00000959); and Vanderbilt University Medical Center, Nashville, TN (NCT04362176 and IRB VUMC protocol # 070258). Time since diagnosis (onset of symptoms or positive PCR test) ranged from 23-54 days (median 34 days). Ages ranged from 20-77 years (median 49); 19 were female, 22 were male. Race and ethnicity were reported as follows: 4 were Asian, 2 were Black, 4 were White/Hispanic or Latino, 5 were White/ethnicity unreported, and 26 were White/Not Hispanic or Latino. The Covid-19 illness severity was known for 38 of these individuals and was classified as mild in 63%, moderate in 22%, and severe (hospitalization requiring intensive care and/or ventilation) in 15%. Data for these convalescent sera are shown as comparators for the various serological assays (see **Table 6** below).

Table 6. Convalescent Sera Tested in Serological Assays

SN	ELISA	PSVN	PRNT	FRNT	nLuc
0044-0002-01	Y	Y	-	Y	-
0044-0007-01	Y	Y	-	Y	-
0044-0008-01	Y	Y	-	Y	-
0044-0010-01	Y	Y	-	Y	-
0044-0011-01	Y	Y	-	Y	-
0044-0012-01	Y	Y	-	Y	-
0047-0008-01	Y	Y	-	Y	-
0047-0009-01	Y	Y	-	Y	-
0047-0014-02	Y	Y	-	Y	-
0047-0015-01	Y	Y	-	Y	-
0047-0016-01	Y	Y	-	Y	-
0047-0018-01	Y	Y	-	Y	-
0047-0019-01	Y	Y	-	Y	-
0047-0020-01	Y	Y	-	Y	-
0047-0021-01	Y	Y	-	Y	-
0047-0022-01	Y	Y	-	Y	-
20A-768-30	-	-	-	-	Y
20A-769-30	Y	Y	-	Y	Y
20A-770-30	Y	Y	-	Y	Y
20A-771-30	Y	Y	-	Y	Y
20A-772-30	Y	Y	-	Y	Y
20A-773-30	Y	Y	-	Y	Y
20A-774-30	Y	Y	-	Y	Y
20A-776-30	Y	Y	-	Y	Y
20A-777-30	Y	Y	-	Y	Y
20A-778-30	Y	Y	-	Y	Y
20A-779-30	Y	Y	-	Y	Y
20A-780-30	Y	Y	-	Y	Y
20A-781-30	Y	Y	-	Y	Y
20A-782-30	Y	Y	-	Y	Y
20A-783-30	Y	Y	-	Y	-
20A-785-30	Y	Y	-	Y	Y
20A-786-30	Y	Y	-	Y	Y
20A-787-30	Y	Y	-	Y	Y
20A-788-30	Y	Y	-	Y	Y
20A-789-30	Y	Y	-	Y	Y
20A-790-30	Y	Y	-	Y	Y
20A-791-30	Y	Y	-	Y	Y
20A-792-30	Y	Y	-	Y	Y
20A-793-30	-	-	-	-	Y

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

SN	ELISA	PSVN	PRNT	FRNT	nLuc
20A-794-30	-	-	-	-	Y
20A-795-30	-	-	-	-	Y
VUMC1	Y	Y	Y	Y	Y
VUMC2	Y	Y	Y	Y	Y
VUMC3	Y	Y	Y	Y	Y

9. T CELL MEDIATED IMMUNE RESPONSE ANALYSIS DESCRIPTION

Intracellular cytokine stimulation (ICS) assay was used to evaluate T cell responses elicited by the mRNA-1273 vaccine in clinical samples collected on day 1, day 29, and day 43 post-vaccination. The protocol for this assay is detailed in VRC-VIP SOP 5521 *Intracellular Cytokine Staining Assay*. Briefly, frozen peripheral blood mononuclear cells (PBMC) were thawed, counted and rested in R10 culture media (90% RPMI 1640 with 10% Fetal Bovine Serum (FBS) and 1% Penicillin Streptomycin and L-Glutamine) overnight at 37°C with 5% CO₂. Following the rest period, cells were counted on day 2 and resuspended in R10 cell culture media. 0.5-1.5 x 10⁶ cells were transferred to individual wells of a 96-well V-bottom plate(s) and incubated with pools of 15-mer peptides overlapping by 10 amino acids covering the N-terminus of SARS-CoV-2 Spike protein up to the furin cleavage site (S1 pool), the C-terminus of the SARS-CoV-2 Spike protein up to the furin cleavage site (S2 pool) for 6 hours at 37°C with 5% CO₂. Peptide pools were custom ordered from JPT Peptide Technologies and were >85% pure. Cells were also stimulated with *Staphylococcus* enterotoxin B (SEB; positive control), or Dimethylsulfoxide (DMSO; no stimulation control) for 6 hours at 37°C with 5% CO₂. Following stimulation, cells were washed and stained with viability dye for 20 minutes at room temperature, followed by surface stain for 20 minutes at room temperature, cell fixation and permeabilization with BD cytofix/cytoperm kit (catalog # 554714) for 20 minutes at room temperature, and then intracellular stain for 20 minutes at room temperature (see **Table 7** for complete list of antibodies). Upon completion of staining, cells were collected on a BD FACSymphony Flow Cytometer. A single SARS-CoV-2 naïve, CMV-reactive control PBMC sample (VRC 200-002-37) was included with each batch. This sample was stimulated with all of the stimuli listed above (negative control for SARS-CoV-2 stimulation) as well as a Cytomegalovirus (CMV) peptide pool (quantitative control for peptide stimulated cytokine response).

Samples were analyzed using FlowJo 10.6.2. Anomalous “bad” events were separated from “good” events using FlowAI. “Good events” were used to determine cytokine responses. Cytokine positive cells were determined by gating on singlets, lymphocytes, viability dye⁻CD3⁺, followed by CD4⁺ or CD8⁺ (**Figure 8**). Individual cytokines were plotted on the Y-axis vs. CD69 on the X-axis and only the CD69⁺cytokine⁺ events were used to determine positive responses. Positive cytokine gates were determined using unstimulated samples during qualification testing. A template of gating was created during assay qualification and was applied to all vaccine samples without manipulation. “Any responses” are any combination of the indicated individual cytokines by a population of CD4 or CD8 T cells and were calculated using Boolean combination gates. All antigen-specific cytokine frequencies are reported after background subtraction of identical gates from the same sample incubated with the control stimulation (DMSO).

Assay Development and Optimization

This 13-color flow cytometry panel was developed at the VRC (**Table 7**). Seven-point titrations were performed on each new lot of antibody-fluorochrome to determine the concentration that yielded optimal marker resolution while minimizing fluorochrome spreading and spillover. The assay was qualified for evaluation of the phase 1 mRNA-1273 clinical trial samples. Briefly, Th1 specificity was qualified using PBMCs from patients with CMV-reactive T cells stimulated with CMV peptide pools, which generate robust Th1-type responses. Th2 specificity was qualified using PBMCs from patients with *Filaria* parasite-reactive T cells stimulated with *Filaria* crude antigen, which generate strong Th2-type cytokines. For the VRC 200-002-37 quantitative control used in each experimental batch, cytokine tolerance ranges were determined following 20 different ICS assays performed on this sample.

Table 7. 13-color ICS Antibody Panel

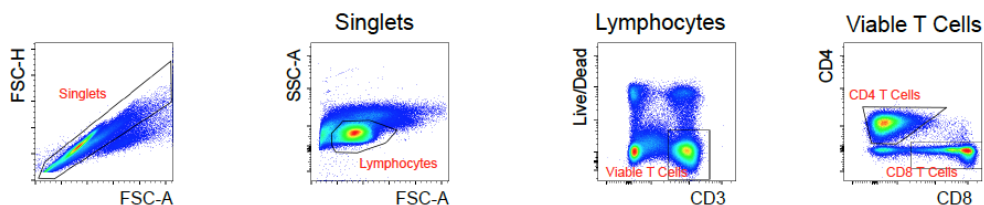
Detector	Fluorophore	Specificity	Clone	Dilution	Stain
B710	BB700	IL-4	MP4-25D2	1:160	intracellular
G560	PE	CD28	CD28.2	1:20	surface
G610	ECD	CD69	TP1.55.3	1:20	intracellular
G660	PE/Cy5	CD8a	RPA-T8	1:40	surface
G780	PE/Cy7	IFN γ	B27	1:160	intracellular
R660	APC	IL-2	MQ1-17H12	1:20	intracellular
R780	APC/H7	CD3	SK7	1:80	intracellular
U395	BUV395	CCR7	150503	1:40	surface
U450	UV-Blue	Viability	-	1:500	surface
U570	BUV563	TNF	Mab11	1:10	intracellular
U785	BUV805	CD4	SK3	1:10	surface
V450	BV421	IL-13	JES10-SA2	1:10	intracellular
V785	BV785	CD45RO	UCHL1	1:20	surface

The specificity of the ICS assay for SARS-CoV-2 S-specific T cell responses was demonstrated using a small panel of COVID-19 convalescent patient samples and naïve SARS-CoV-2 PBMC samples (**Figure 9**). Importantly, Th1 predominant responses (CD4⁺ T cells expressing IL-2, IFN- γ and/or TNF- α) with minimal to no detectable Th2 responses (IL-4, IL-13 expression in CD4⁺ T cells) were observed in a small panel of convalescent PBMC samples stimulated with either the S1 or S2 S-specific peptide pools; detection of S-specific CD4⁺ responses were negligible in SARS-CoV-2 naïve samples. S-specific CD8⁺ T cell responses were only marginally observed in either convalescent and naïve PBMCs following stimulation with S1 and S2 peptides.

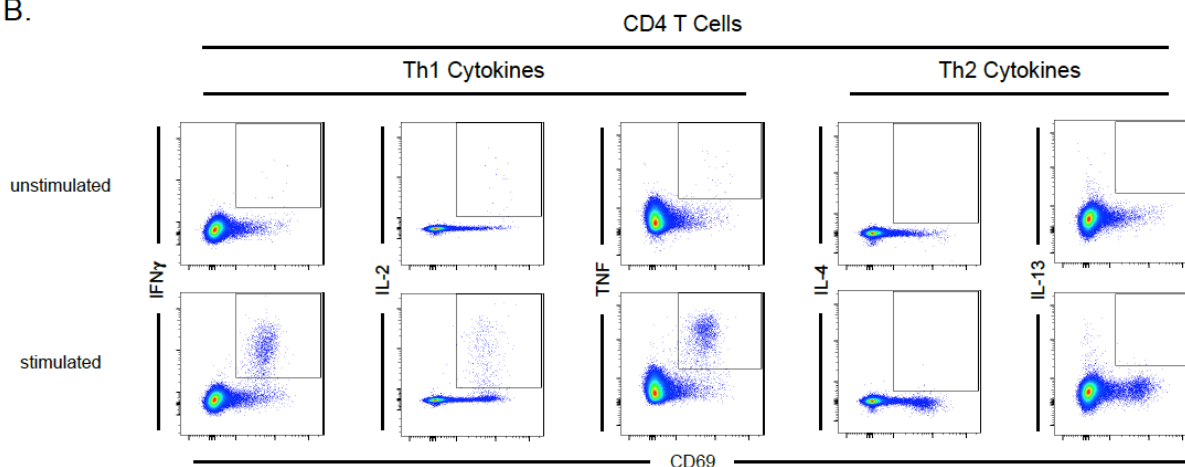
Figure 8. Flow Cytometric Gating Strategy

PBMCs stained with the 13-color panel. (A) Lineage gating of T cells. After gating on single cells, lymphocytes, and viable CD3⁺ T cells, T cells were further subdivided into CD4⁺ and CD8⁺ T cells. (B,C) Gating to measure T cell function following no stimulation or stimulation with CMV peptide pool. Cytokine staining is shown following gating on CD4⁺ T cells (B) or CD8⁺ T cells (C).

A.



B.



C.

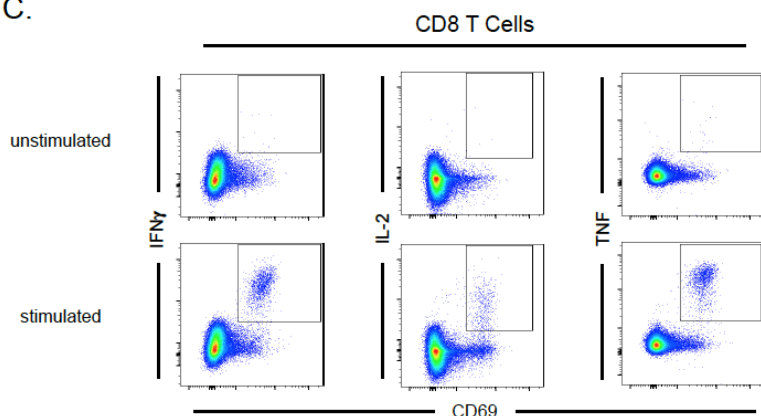
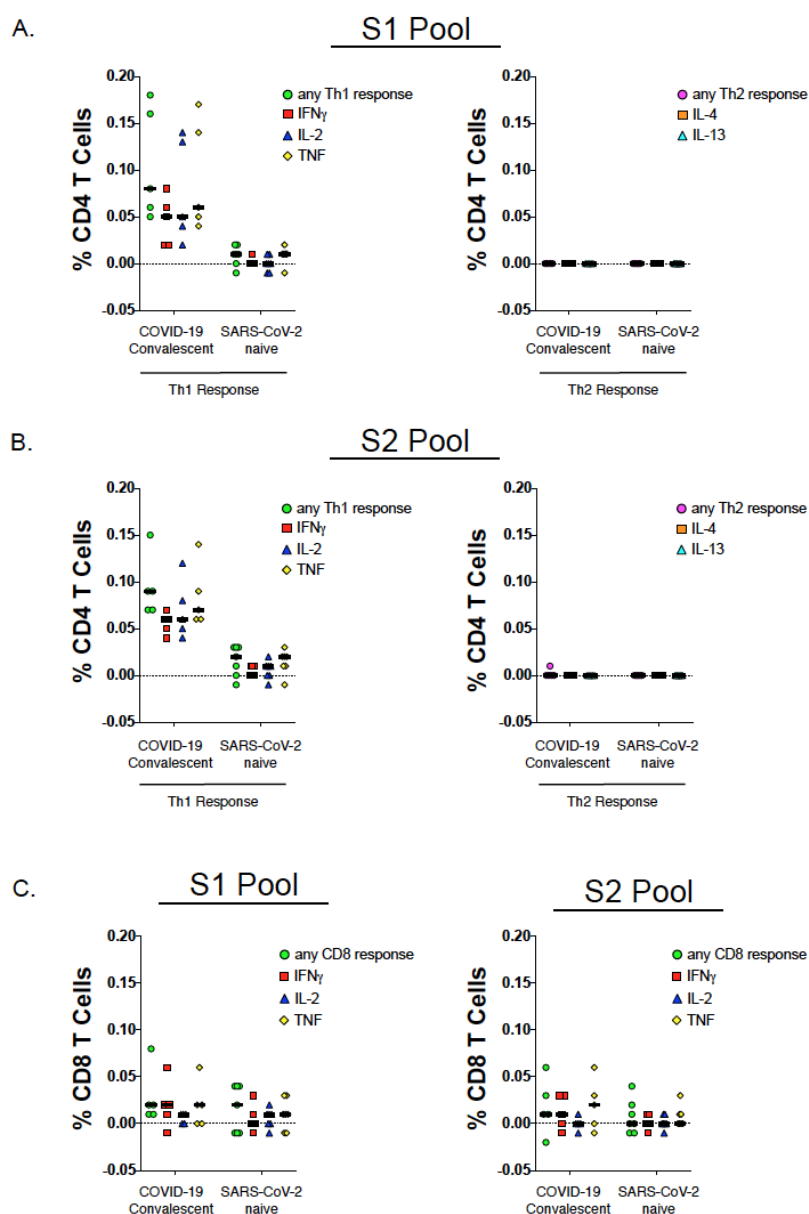


Figure 9. SARS-CoV-2-Specific T Cell Responses in COVID-19 Convalescent Patient Samples.

PBMCs from COVID-19 convalescent patients at 1 month post onset of symptoms or SARS-CoV-2 naïve patients were stimulated with indicated SARS-CoV-2 peptide pools. (A) Frequencies of CD4⁺ T cell Th1 (left) or Th2 (right) cytokines following stimulation with SARS-CoV-2 S1 peptide pool. (B) Frequencies of CD4⁺ T cell Th1 (left) or Th2 (right) cytokines following stimulation with SARS-CoV-2 S2 peptide pool. (C) Frequencies of CD8⁺ T cell cytokines following stimulation with SARS-CoV-2 S1 peptide pool (left) or SARS-CoV-2 S2 peptide pool (right). “Any response” indicates the frequencies of cells producing any combination of the individual cytokines.



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

10. TABLES AND FIGURES

Implementation Note: There are four subjects (2 in cohort 1, 1 in cohort 3, and 1 in cohort 5) who discontinued vaccination prior to receiving the second dose yet continued to provide samples for immunogenicity assessments; each display omits the data from visits post second dose for these four subjects. Further, one subject in cohort 2 and one subject in cohort 10 had a phone visits on Day 43 and therefore had no samples drawn. One subject in cohort 2 had a phone visit on Day 57 and therefore had no samples drawn.

Table 8. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age 18 -55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	15	15	15	15	60	41
	GM	1	7	1	1	2	14157
	95% CI	0, 1	2, 22	0, 4	0, 5	1, 3	7616, 26312
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60	
	GM	5674	11200	19068	30642	13881	
	95% CI	3224, 9983	6140, 20431	12424, 29265	18029, 52078	10356, 18607	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59	
	GM	8304	15767	20525	38448	17699	
	95% CI	5221, 13209	8969, 27716	14234, 29595	22899, 64555	13623, 22993	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57	
	GM	94988	141838	213076	315723	175366	
	95% CI	64997, 138817	99921, 201339	165185, 274852	255688, 389853	146363, 210117	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55	
	GM	91081	146045	221956	254374	167352	
	95% CI	61317, 135293	102400, 208290	182108, 270524	200737, 322342	140601, 199193	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56	
	GM	77904	105398	147332	217813	128097	
	95% CI	56717, 107006	73793, 150539	113898, 190580	170240, 278681	108110, 151778	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42	
	GM	42487		81372	88852	68526	
	95% CI	29443, 61310		61678, 107354	66277, 119115	56531, 83066	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point.							

Table 9. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GM	2	12	9	6	14157
	95% CI	0, 5	4, 30	1, 37	3, 11	7616, 26312
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GM	702	6183	9822	3494	
	95% CI	103, 4751	2881, 13271	6539, 14753	1649, 7400	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GM	2037	6847	20493	6588	
	95% CI	496, 8355	3454, 13570	14413, 29137	3656, 11869	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29	
	GM	45869	33012	1070250	108844	
	95% CI	23165, 90823	17514, 62224	224760, 5096239	50021, 236839	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29	
	GM	64533	78050	277751	108388	
	95% CI	34146, 121959	55858, 109059	115183, 669765	72488, 162068	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29	
	GM	49990	70851	266183	94736	
	95% CI	27060, 92349	54739, 91706	94155, 752518	61197, 146656	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19	
	GM	16624		62227	31065	
	95% CI	9084, 30422		39421, 98228	19315, 49963	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 10. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age ≥ 71

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GM	1	6	15	5	14157
	95% CI	0, 3	1, 25	6, 39	2, 10	7616, 26312
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GM	1664	661	16174	2612	
	95% CI	679, 4076	138, 3144	3350, 78072	1103, 6185	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GM	5949	2073	23933	6658	
	95% CI	2934, 12061	608, 7067	10375, 55204	3614, 12265	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30	
	GM	95566	16091	557510	94999	
	95% CI	57468, 158922	6431, 40257	191748, 1620960	47126, 191502	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30	
	GM	70620	48716	1273343	163625	
	95% CI	41797, 119320	22221, 106801	403930, 4014062	81035, 330389	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30	
	GM	142653	40430	523632	144545	
	95% CI	66931, 304041	20221, 80832	167243, 1639475	79535, 262692	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20	
	GM	18687		48171	30003	
	95% CI	12303, 28385		29102, 79735	20677, 43537	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 11. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - S-2P – Age 18-55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60
	GMFR ^a	13517.3	2876.2	28300.6	39579.8	14445.8
	95% CI	4705.476, 38830.697	783.075, 10563.891	8319.721, 96267.799	11551.751, 135612.504	7864.232, 26535.639
	4-Fold Rise ^b	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	60/60 (100%)
	95% CI	78.2%, 100%	78.2%, 100%	78.2%, 100%	78.2%, 100%	94%, 100%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59
	GMFR ^a	19786.2	4048.9	30463	53290.8	18661.1
	95% CI	7164.525, 54643.201	957.412, 17122.958	9185.964, 101023.082	14486.651, 196036.104	10042.396, 34676.463
	4-Fold Rise ^b	15/15 (100%)	15/15 (100%)	15/15 (100%)	14/14 (100%)	59/59 (100%)
	95% CI	78.2%, 100%	78.2%, 100%	78.2%, 100%	76.8%, 100%	93.9%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57
	GMFR ^a	197551.8	36425.6	316250.4	437609.9	174206.2
	95% CI	76518.696, 510028.44	7798.346, 170141.319	95307.907, 1049380.846	116316.547, 1646389.964	91812.04, 330542.815
	4-Fold Rise ^b	13/13 (100%)	15/15 (100%)	15/15 (100%)	14/14 (100%)	57/57 (100%)
	95% CI	75.3%, 100%	78.2%, 100%	78.2%, 100%	76.8%, 100%	93.7%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55
	GMFR ^a	189426.3	49759.1	348444.2	352576.7	184380.7
	95% CI	73319.816, 489394.379	12225.925, 202517.648	104253.422, 1164598.06	89054.744, 1395886.778	100441.111, 338469.387
	4-Fold Rise ^b	13/13 (100%)	14/14 (100%)	14/14 (100%)	14/14 (100%)	55/55 (100%)
	95% CI	75.3%, 100%	76.8%, 100%	76.8%, 100%	76.8%, 100%	93.5%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56
	GMFR ^a	162021.8	27067.3	231292.3	301901.8	128125
	95% CI	65611.312, 400099.793	6418.715, 114140.817	71428.076, 748950.815	72359.981, 1259601.233	68057.78, 241207.102

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)
	4-Fold Rise ^b	13/13 (100%)	15/15 (100%)	14/14 (100%)	14/14 (100%)	56/56 (100%)
	95% CI	75.3%, 100%	78.2%, 100%	76.8%, 100%	76.8%, 100%	93.6%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42
	GMFR ^a	88363.1		120772.9	123153.4	110355.3
	95% CI	37510.935, 208153.648		38894.992, 375012.131	30463.478, 497866.665	59654.813, 204145.977
	4-Fold Rise ^b	13/13 (100%)		15/15 (100%)	14/14 (100%)	42/42 (100%)
	95% CI	75.3%, 100%		78.2%, 100%	76.8%, 100%	91.6%, 100%

Note: N=Number of Subjects.

Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Table 12. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - S-2P – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30
	GMFR ^a	646.1	577.2	1554.2	833.7
	95% CI	60.428, 6908.027	208.511, 1597.533	294.228, 8209.33	336.185, 2067.639
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30
	GMFR ^a	1901.4	639.1	3242.6	1579.5
	95% CI	254.995, 14177.838	229.702, 1778.131	510.697, 20588.28	651.345, 3830.068
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	42875.4	3081.7	201812	27970.8
	95% CI	9113.733, 201706.45	1034.776, 9177.489	11404.31, 3571288.573	8765.993, 89250.256
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	60321.7	7286	52374.3	27853.9
	95% CI	13316.359, 273250.697	2379.815, 22306.921	4541.146, 604048.204	10890.6, 71239.625
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	46727.4	6614	50193	24345.5
	95% CI	9097.671, 240000.904	2415.043, 18113.736	3790.03, 664726.375	9249.316, 64081.009

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19
	GMFR ^a	15538.9		11733.9	13603.2
	95% CI	3376.496, 71511.032		1533.426, 89789.288	4432.543, 41747.468
	4-Fold Rise ^b	10/10 (100%)		9/9 (100%)	19/19 (100%)
	95% CI	69.2%, 100%		66.4%, 100%	82.4%, 100%

Note: N=Number of Subjects.

Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Table 13. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - S-2P – Age ≥ 71

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30
	GMFR ^a	2675.4	195.8	1099.6	832
	95% CI	703.62, 10172.812	36.077, 1062.215	276.216, 4377.487	355.27, 1948.376
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30
	GMFR ^a	9566.4	619.7	1627.2	2128.8
	95% CI	1889.759, 48427.363	106.352, 3611.298	674.532, 3925.353	902.747, 5019.917
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	153704.3	4816.8	37906.4	30389.1
	95% CI	32850.917, 719158.116	827.715, 28030.652	8871.888, 161960.176	11562.54, 79869.971
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	113582.3	14583.4	86577.5	52343
	95% CI	22921.287, 562836.24	1979.471, 107441.31	17699.503, 423496.07	20415.194, 134203.499
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	229435.8	12102.9	35602.9	46239.4
	95% CI	44400.689, 1185585.328	1786.003, 82016.051	9408.664, 134723.639	17901.765, 119434.343

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20
	GMFR ^a	30055.9		3275.3	9921.7
	95% CI	6099.06, 148113.781		1284.014, 8354.491	3692.908, 26656.653
	4-Fold Rise ^b	10/10 (100%)		10/10 (100%)	20/20 (100%)
	95% CI	69.2%, 100%		69.2%, 100%	83.2%, 100%

Note: N=Number of Subjects.

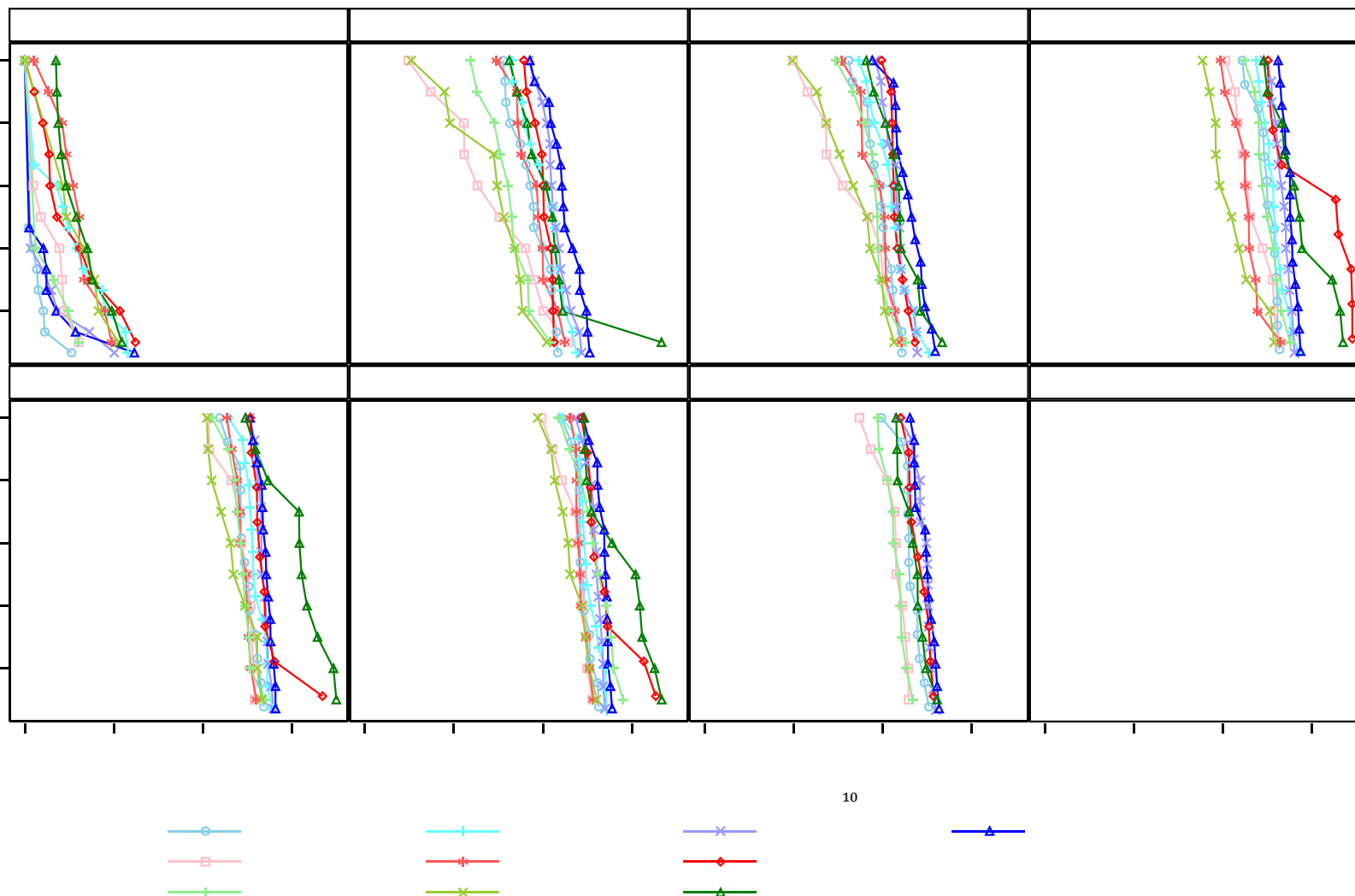
Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

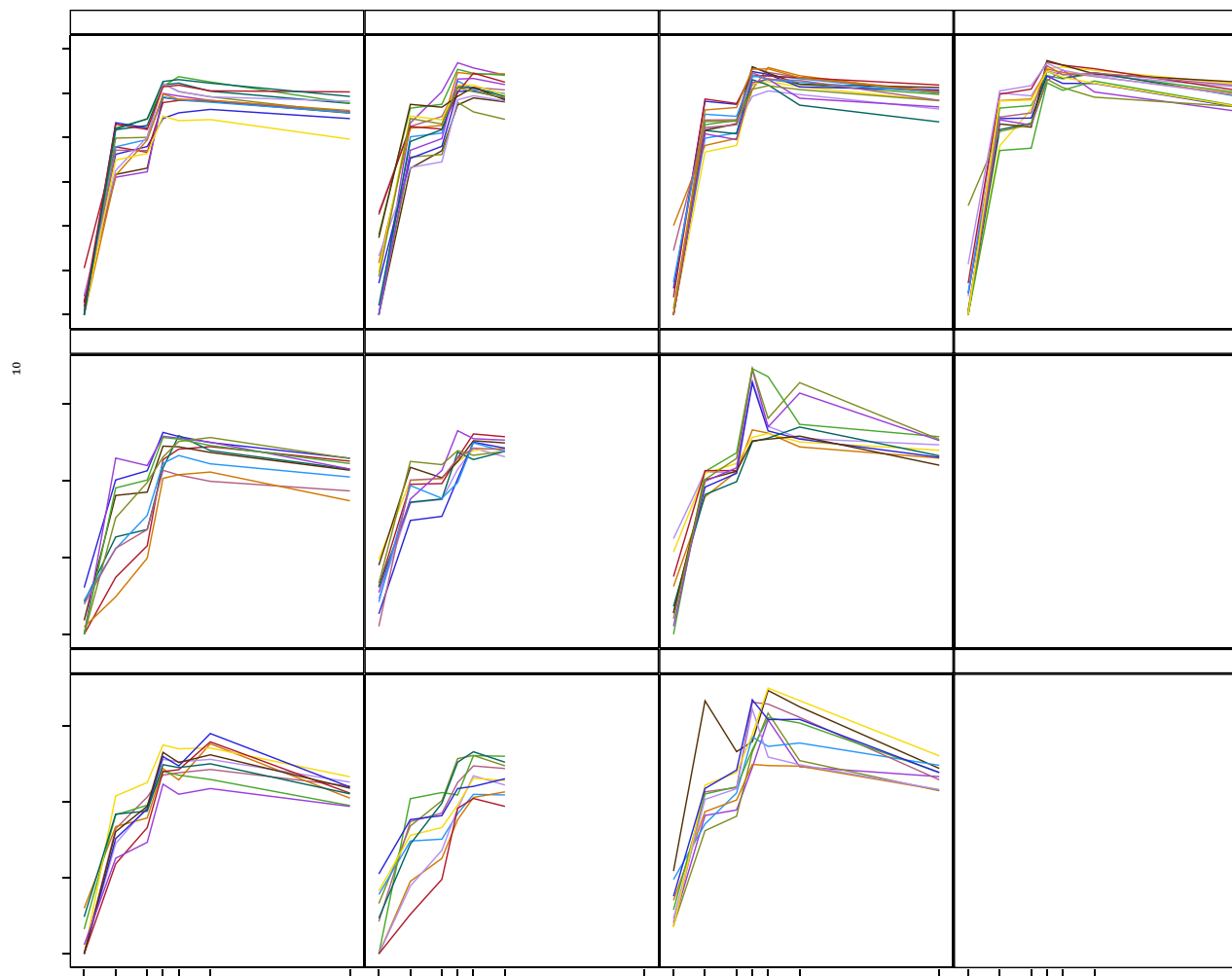
AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Figure 10. Reverse Cumulative Distribution of Serum IgG ELISA Area Under the Curve (AUC) Values by Time Point and Vaccination Group - S-2P



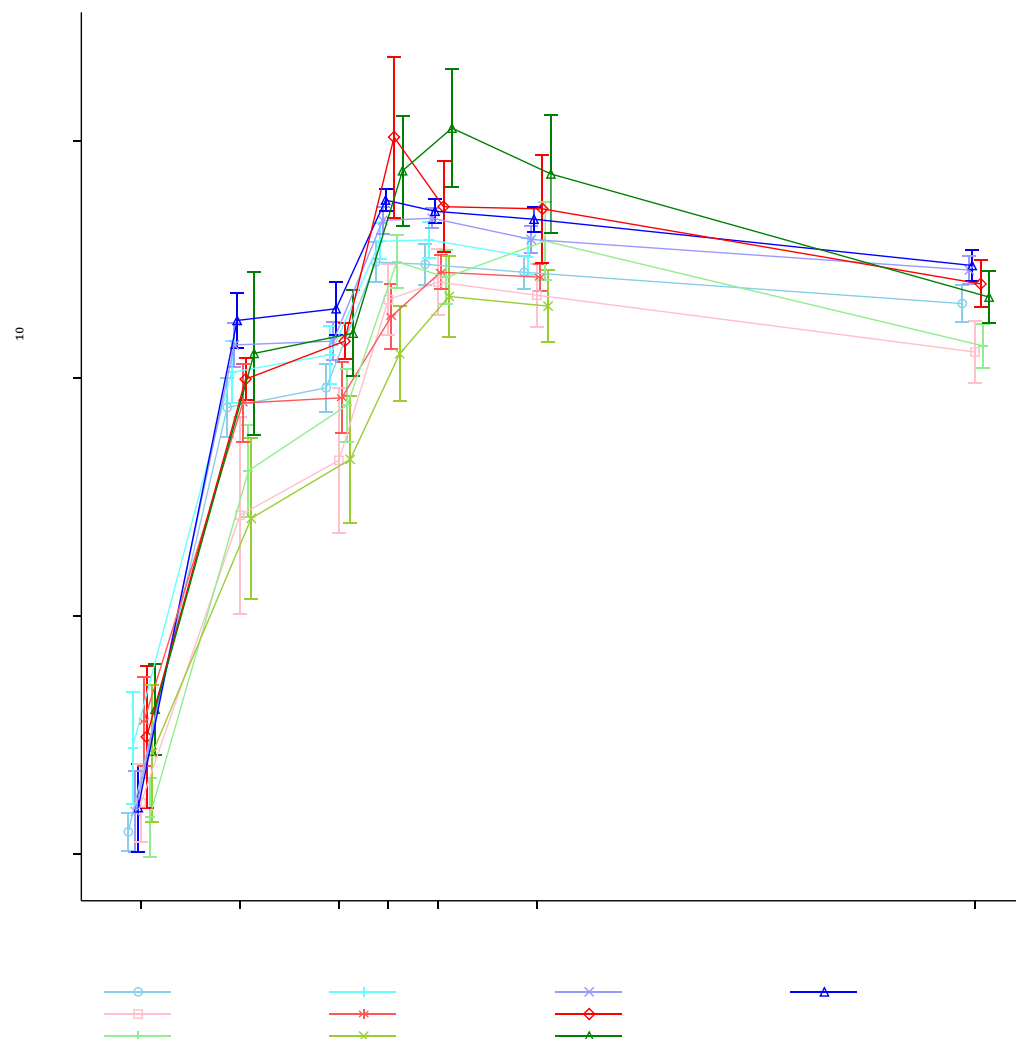
10

Figure 11. Serum IgG ELISA Area Under the Curve (AUC) Values by Time Point and Vaccination Group - S-2P



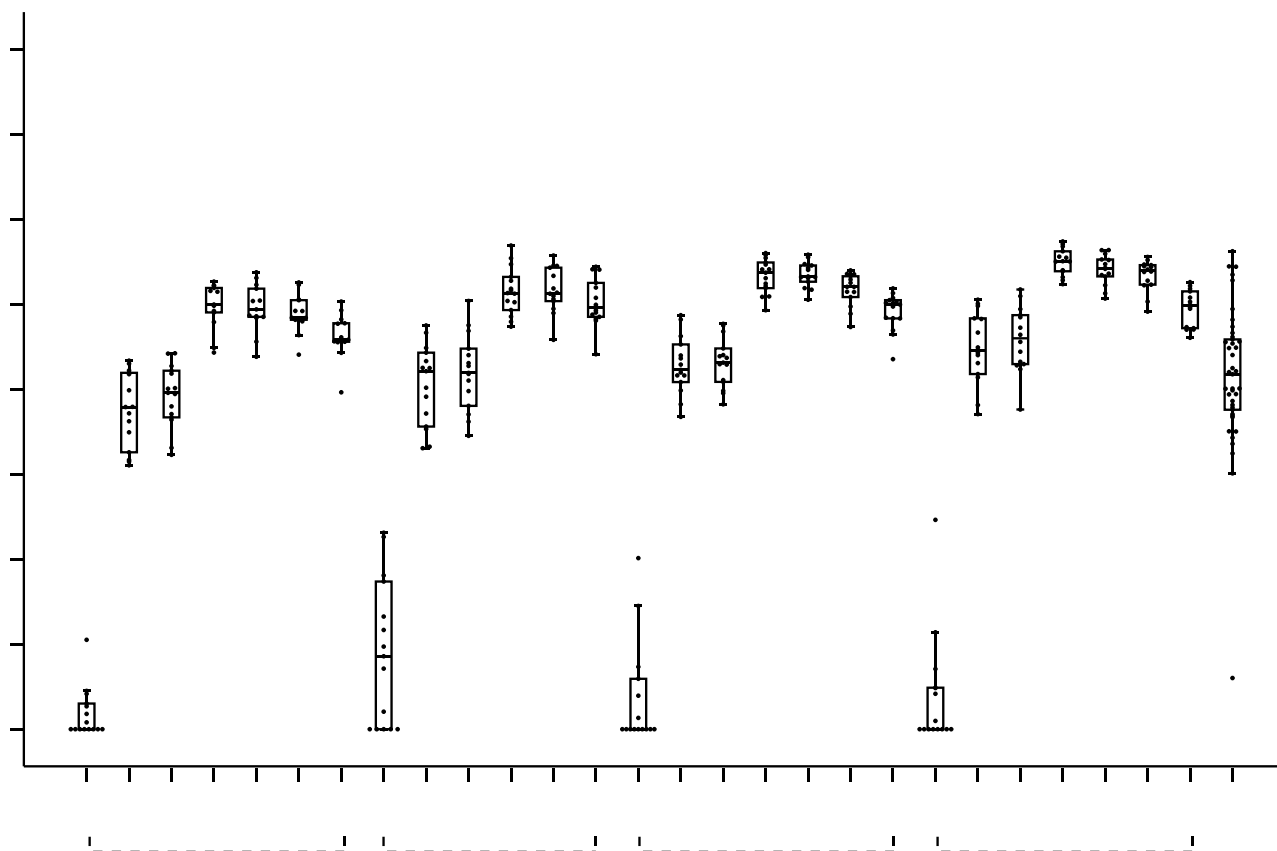
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 12. Geometric Mean Area Under the Curve (AUC) Values by Time Point and Vaccination Group - S-2P



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

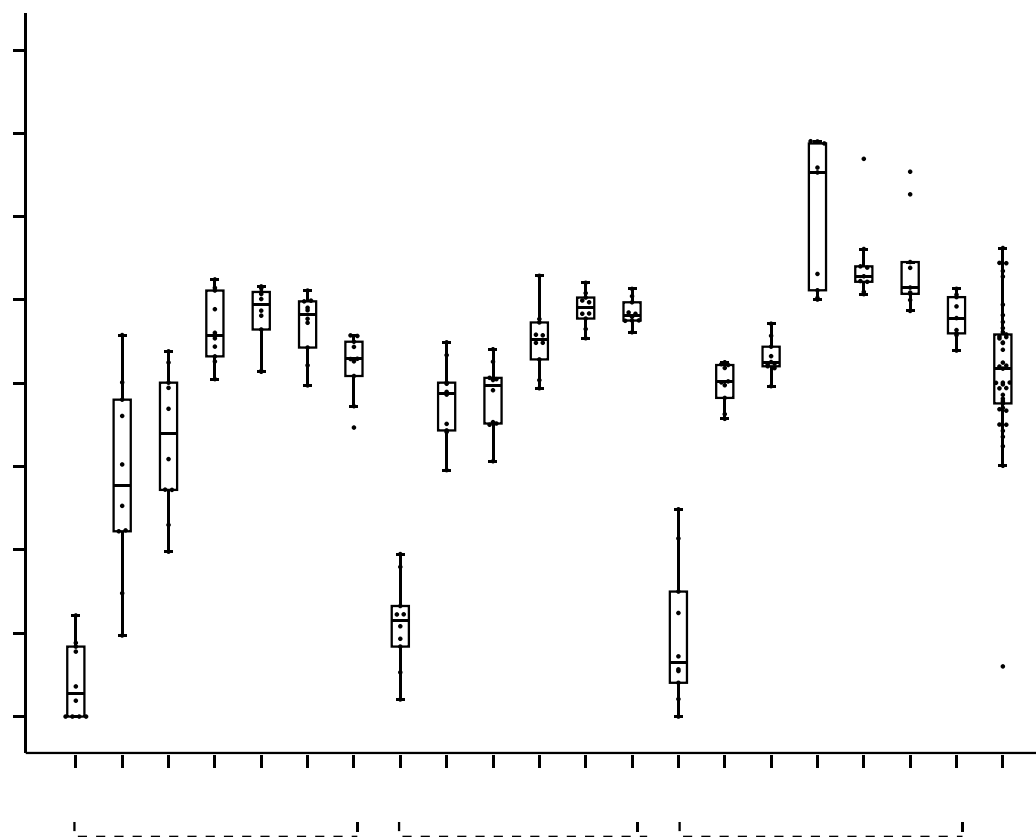
Figure 13. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - S-2P – Age 18-55



Note: Boxes and horizontal bars denote interquartile range (IQR) and median AUC, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

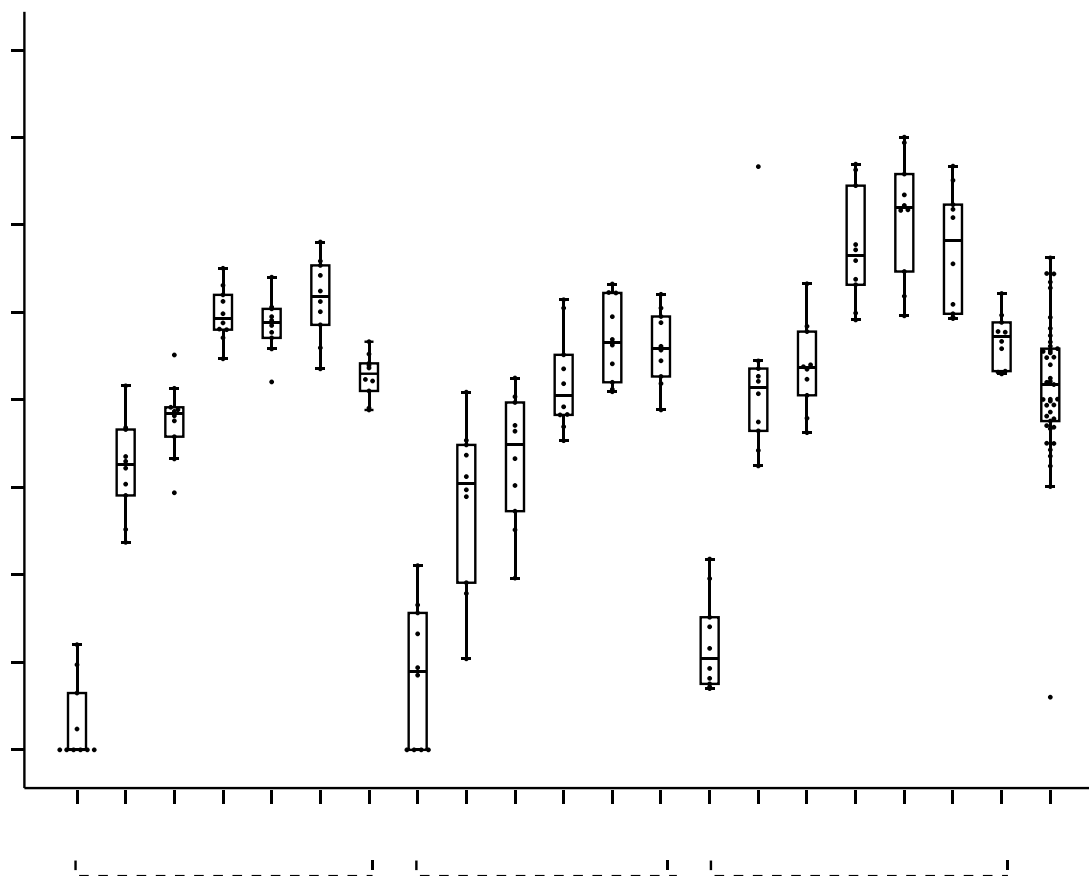
Figure 14. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - S-2P – Age 56-70



Note: Boxes and horizontal bars denote interquartile range (IQR) and median AUC, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 15. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - S-2P – Age ≥ 71



Note: Boxes and horizontal bars denote interquartile range (IQR) and median AUC, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Table 14. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age 18-55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	15	15	15	15	60	56
	GM	0	9	2	14	3	4365
	95% CI	0, 0	5, 16	0, 4	6, 33	2, 6	2446, 7788
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60	
	GM	596	4523	5642	15337	3910	
	95% CI	258, 1376	2479, 8252	3130, 10170	9094, 25866	2555, 5983	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59	
	GM	2110	9202	12130	17556	7913	
	95% CI	1130, 3939	4941, 17137	8447, 17418	10869, 28358	5734, 10920	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57	
	GM	43973	104431	126250	200639	105802	
	95% CI	30848, 62681	71079, 153431	91696, 173824	160276, 251167	86107, 130001	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55	
	GM	45792	110554	141713	170112	106706	
	95% CI	30246, 69327	74467, 164130	110096, 182410	133715, 216415	87238, 130517	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56	
	GM	55071	81161	106248	113627	86303	
	95% CI	36135, 83930	54190, 121557	76429, 147701	82246, 156983	71807, 103727	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42	
	GM	25750		45905	43401	37672	
	95% CI	14395, 46060		32315, 65210	30790, 61179	29669, 47835	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable							

Table 15. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GM	2	8	3	4	4222
	95% CI	1, 5	2, 28	0, 10	2, 7	2021, 8819
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GM	161	1109	3817	880	
	95% CI	18, 1341	718, 1712	2303, 6327	390, 1986	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GM	522	2187	10045	2256	
	95% CI	91, 2970	1147, 4171	5718, 17645	1091, 4664	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29	
	GM	31501	23593	301411	57469	
	95% CI	14520, 68343	12720, 43758	101394, 895996	31550, 104681	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29	
	GM	41848	56750	248702	80817	
	95% CI	21034, 83258	37471, 85949	109350, 565638	52054, 125472	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29	
	GM	24347	44732	109975	47948	
	95% CI	13051, 45416	31318, 63891	66446, 182018	33877, 67863	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19	
	GM	9828		36516	18302	
	95% CI	4667, 20697		22002, 60604	10822, 30950	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 16. Serum IgG ELISA Area Under the Curve (AUC) Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age ≥ 71

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GM	1	3	6	3	4222
	95% CI	0, 5	1, 10	1, 24	1, 6	2021, 8819
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GM	317	108	3007	469	
	95% CI	122, 825	14, 792	1219, 7417	196, 1121	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GM	2354	728	8229	2417	
	95% CI	935, 5927	145, 3644	4332, 15630	1220, 4788	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30	
	GM	44554	14239	153562	46014	
	95% CI	24426, 81270	4940, 41038	72220, 326519	26382, 80256	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30	
	GM	48343	31589	111806	55477	
	95% CI	26849, 87043	11858, 84151	69029, 181092	36739, 83773	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30	
	GM	41781	25439	95909	46714	
	95% CI	23064, 75689	9476, 68291	57611, 159664	30652, 71191	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20	
	GM	11062		29736	18137	
	95% CI	5901, 20739		17627, 50162	11704, 28106	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 17. Serum IgG ELISA Area Under the Curve (AUC) Geometric Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age 18-55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60
	GMFR ^a	3303	527.8	6446.6	1227.7	1927.3
	95% CI	1407.253, 7752.747	214.752, 1297.039	2392.284, 17371.725	428.962, 3513.952	1170.438, 3173.575
	4-Fold Rise ^b	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	60/60 (100%)
	95% CI	78.2%, 100%	78.2%, 100%	78.2%, 100%	78.2%, 100%	94%, 100%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59
	GMFR ^a	11712.5	1073.8	13861.2	1530.8	4108.8
	95% CI	6299.847, 21775.67	457.196, 2521.868	5106.887, 37622.531	521.232, 4495.926	2470.808, 6832.809
	4-Fold Rise ^b	15/15 (100%)	15/15 (100%)	15/15 (100%)	14/14 (100%)	59/59 (100%)
	95% CI	78.2%, 100%	78.2%, 100%	78.2%, 100%	76.8%, 100%	93.9%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57
	GMFR ^a	242797.8	12186.6	144272.7	17495.1	50494.7
	95% CI	167055.334, 352881.711	6675.661, 22246.954	57782.398, 360224.231	5949.763, 51443.577	30607.609, 83303.211
	4-Fold Rise ^b	13/13 (100%)	15/15 (100%)	15/15 (100%)	14/14 (100%)	57/57 (100%)
	95% CI	75.3%, 100%	78.2%, 100%	78.2%, 100%	76.8%, 100%	93.7%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55
	GMFR ^a	252840.4	14976	182400.6	14833.2	55055.9
	95% CI	164562.484, 388474.008	8439.084, 26576.497	63232.542, 526152.966	5002.234, 43985.075	32435.923, 93450.436
	4-Fold Rise ^b	13/13 (100%)	14/14 (100%)	14/14 (100%)	14/14 (100%)	55/55 (100%)
	95% CI	75.3%, 100%	76.8%, 100%	76.8%, 100%	76.8%, 100%	93.5%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56
	GMFR ^a	304079.9	9471.2	136753.2	9907.9	41775.8
	95% CI	196663.913, 470165.358	5167.895, 17357.688	38437.242, 486544.503	3214.987, 30534.211	23127.207, 75461.527

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)
	4-Fold Rise ^b	13/13 (100%)	15/15 (100%)	14/14 (100%)	14/14 (100%)	56/56 (100%)
	95% CI	75.3%, 100%	78.2%, 100%	76.8%, 100%	76.8%, 100%	93.6%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42
	GMFR ^a	142175.1		52458	3784.5	29732.8
	95% CI	79548.615, 254105.676		17308.628, 158986.983	1219.519, 11744.035	14604.921, 60530.402
	4-Fold Rise ^b	13/13 (100%)		15/15 (100%)	14/14 (100%)	42/42 (100%)
	95% CI	75.3%, 100%		78.2%, 100%	76.8%, 100%	91.6%, 100%

Note: N=Number of Subjects.

Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Table 18. Serum IgG ELISA Area Under the Curve (AUC) Geometric Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30
	GMFR ^a	89.5	195.1	2849.1	367.8
	95% CI	6.846, 1170.081	42.257, 901.162	533.109, 15226.08	117.043, 1155.856
	4-Fold Rise ^b	8/10 (80%)	10/10 (100%)	10/10 (100%)	28/30 (93.3%)
	95% CI	44.4%, 97.5%	69.2%, 100%	69.2%, 100%	77.9%, 99.2%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30
	GMFR ^a	347.8	385	7497.3	1001.2
	95% CI	37.101, 3259.692	93.031, 1592.975	1412.603, 39791.469	347.112, 2888.01
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	21294.4	4153	179249.6	23474.7
	95% CI	4211.05, 107681.108	995.916, 17318.269	46618.695, 689217.73	9097.09, 60575.656
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	28288.8	9989.7	147903.1	33012
	95% CI	6621.785, 120852.198	1677.624, 59485.631	45858.866, 477014.403	13833.348, 78780.012
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	16457.8	7874.1	65402.2	19585.5
	95% CI	4037.599, 67083.981	1433.14, 43263.202	14720.933, 290569.194	8428.564, 45510.743

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19
	GMFR ^a	6643.5		21716.1	11642.6
	95% CI	1526.9, 28905.311		4197.282, 112355.335	4181.509, 32416.721
	4-Fold Rise ^b	10/10 (100%)		9/9 (100%)	19/19 (100%)
	95% CI	69.2%, 100%		66.4%, 100%	82.4%, 100%

Note: N=Number of Subjects.

Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Table 19. Serum IgG ELISA Area Under the Curve (AUC) Geometric Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age ≥ 71

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30
	GMFR ^a	626.5	44.3	688.3	267.3
	95% CI	94.036, 4174.46	4.798, 408.419	138.787, 3413.586	90.536, 788.984
	4-Fold Rise ^b	9/10 (90%)	8/10 (80%)	10/10 (100%)	27/30 (90%)
	95% CI	55.5%, 99.7%	44.4%, 97.5%	69.2%, 100%	73.5%, 97.9%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30
	GMFR ^a	4659.7	379.7	1884	1493.8
	95% CI	554.772, 39137.681	49.209, 2929.205	359.621, 9870.316	517.686, 4310.176
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	88208.5	7489.9	35161.6	28533.3
	95% CI	13191.21, 589843.201	1266.959, 44277.866	7357.378, 168040.734	10885.684, 74790.586
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	95709.6	16616.4	25600.5	34401.7
	95% CI	14085.922, 650318.174	2736.074, 100912.603	5177.369, 126586.998	13452.348, 87975.711
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	82718.3	13381.2	21960.5	28967.6
	95% CI	11765.849, 581540.787	2229.93, 80296.828	4231.851, 113960.787	11172.458, 75106.502

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20
	GMFR ^a	21900.8		6808.6	12211.2
	95% CI	3161.376, 151719.789		1190.524, 38938.675	3655.465, 40792.118
	4-Fold Rise ^b	10/10 (100%)		10/10 (100%)	20/20 (100%)
	95% CI	69.2%, 100%		69.2%, 100%	83.2%, 100%

Note: N=Number of Subjects.

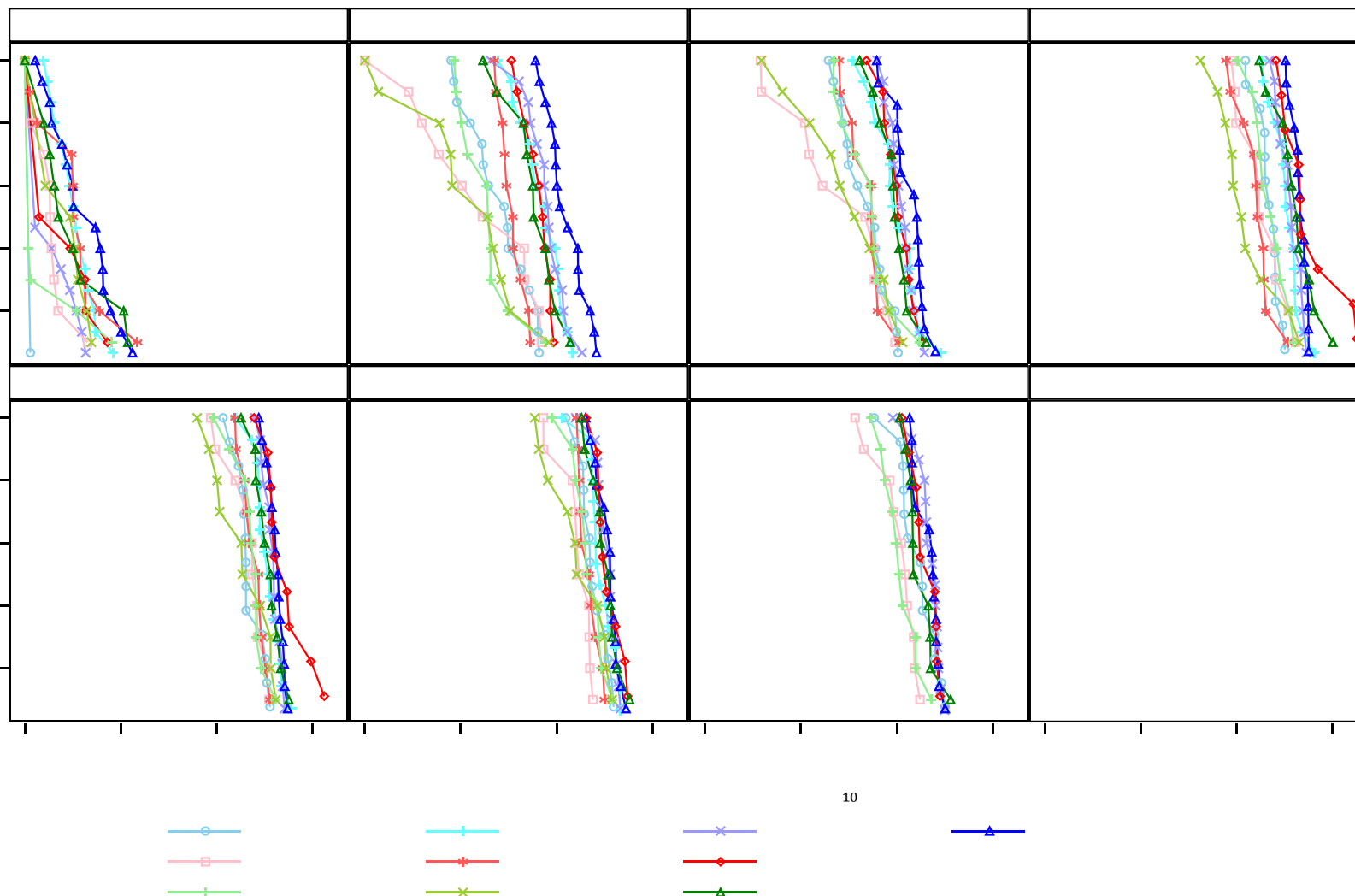
Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

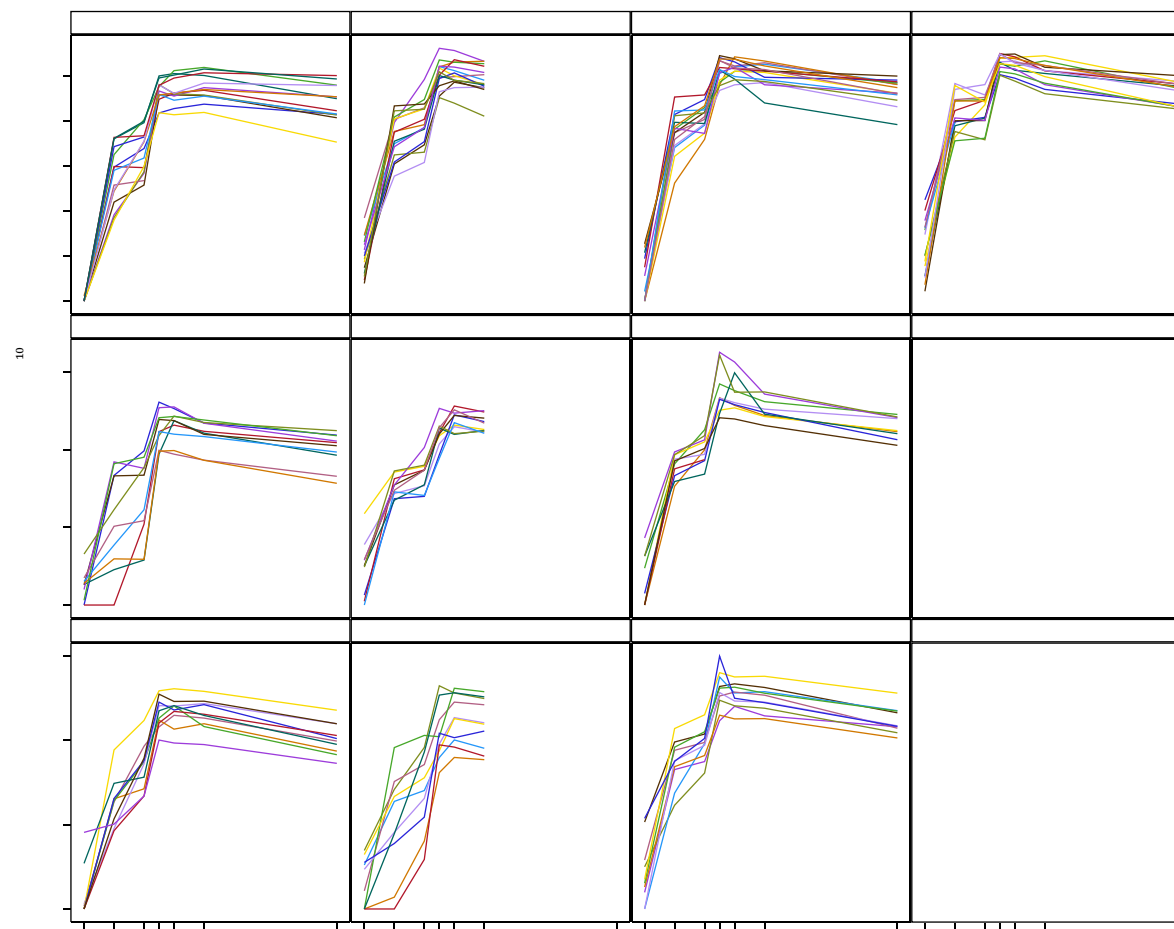
Figure 16. Reverse Cumulative Distribution of Serum IgG ELISA Area Under the Curve (AUC) Values by Time Point and Vaccination Group – RBD



10

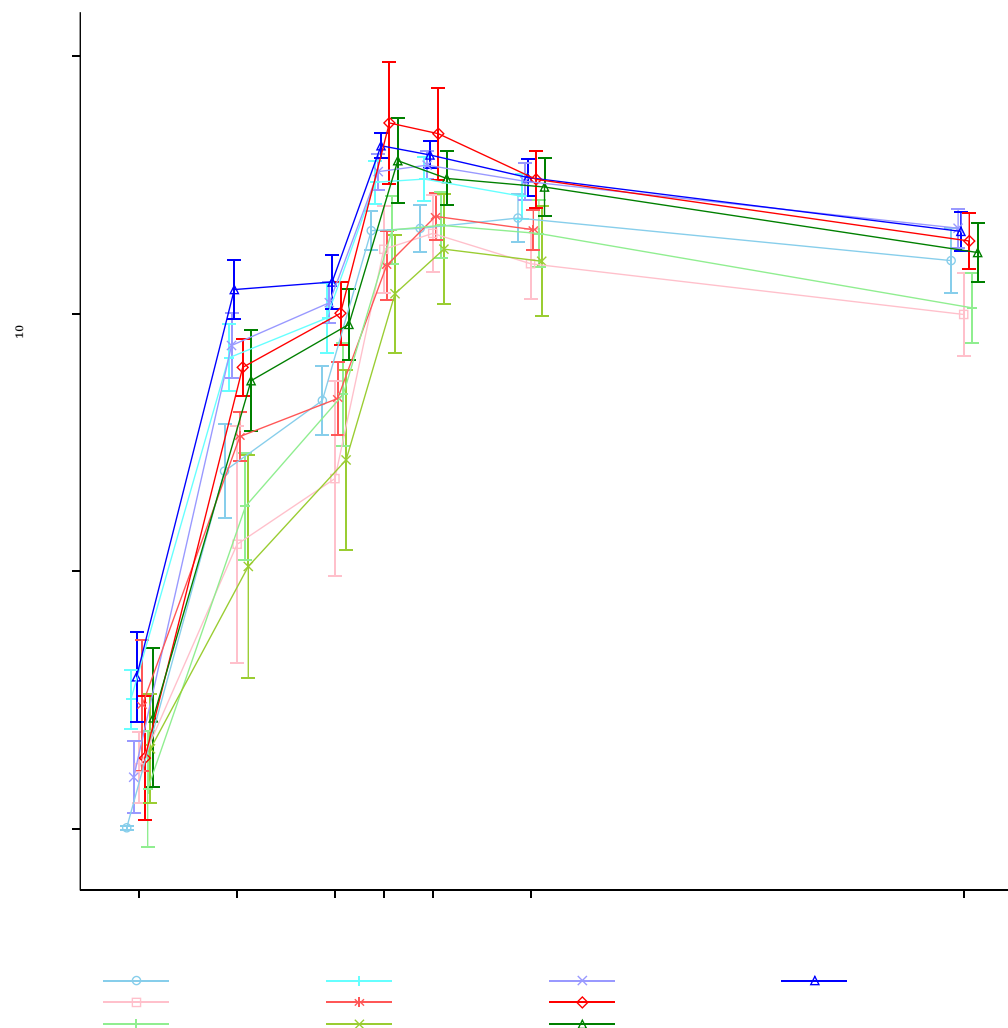
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 17. Serum IgG ELISA Area Under the Curve (AUC) Values by Time Point and Vaccination Group – RBD



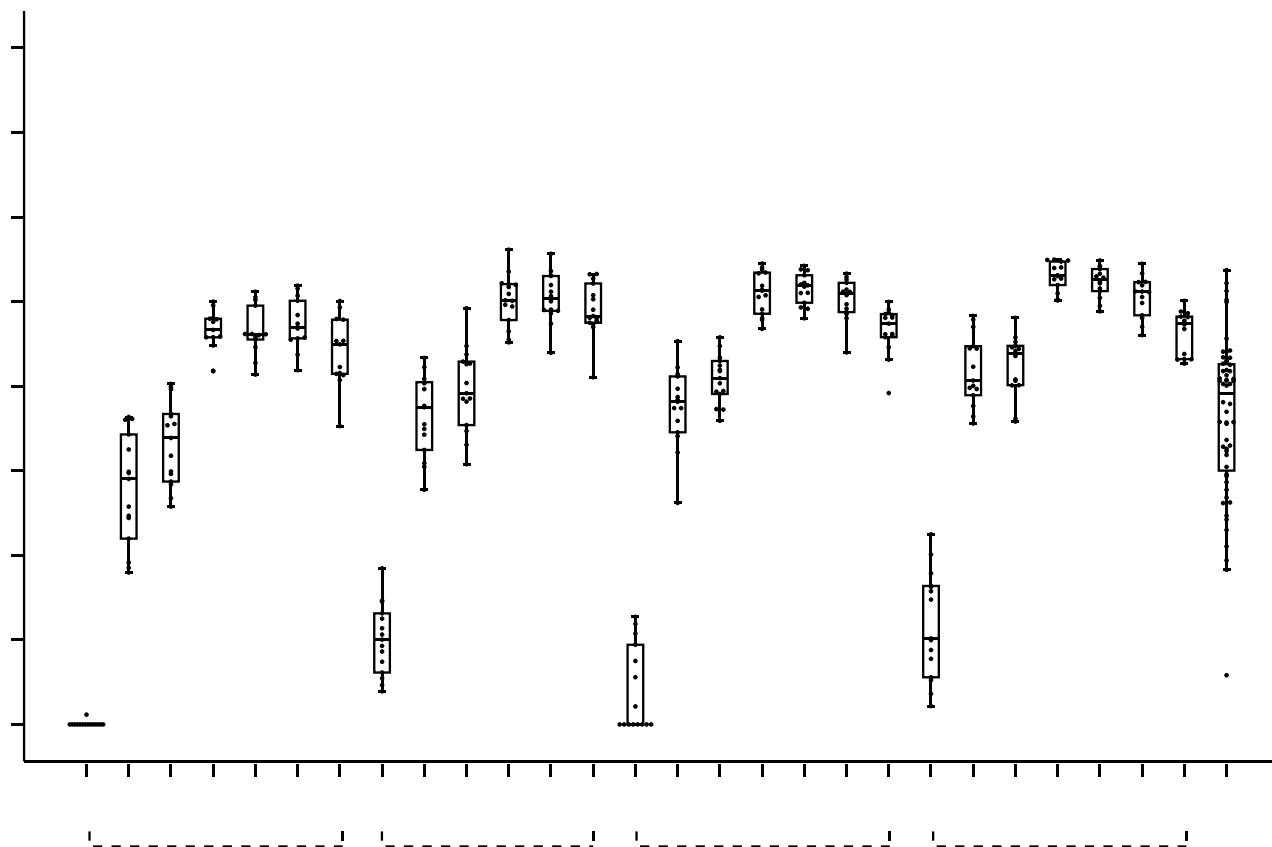
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 18. Geometric Mean Area Under the Curve (AUC) Values by Time Point and Vaccination Group – RBD



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

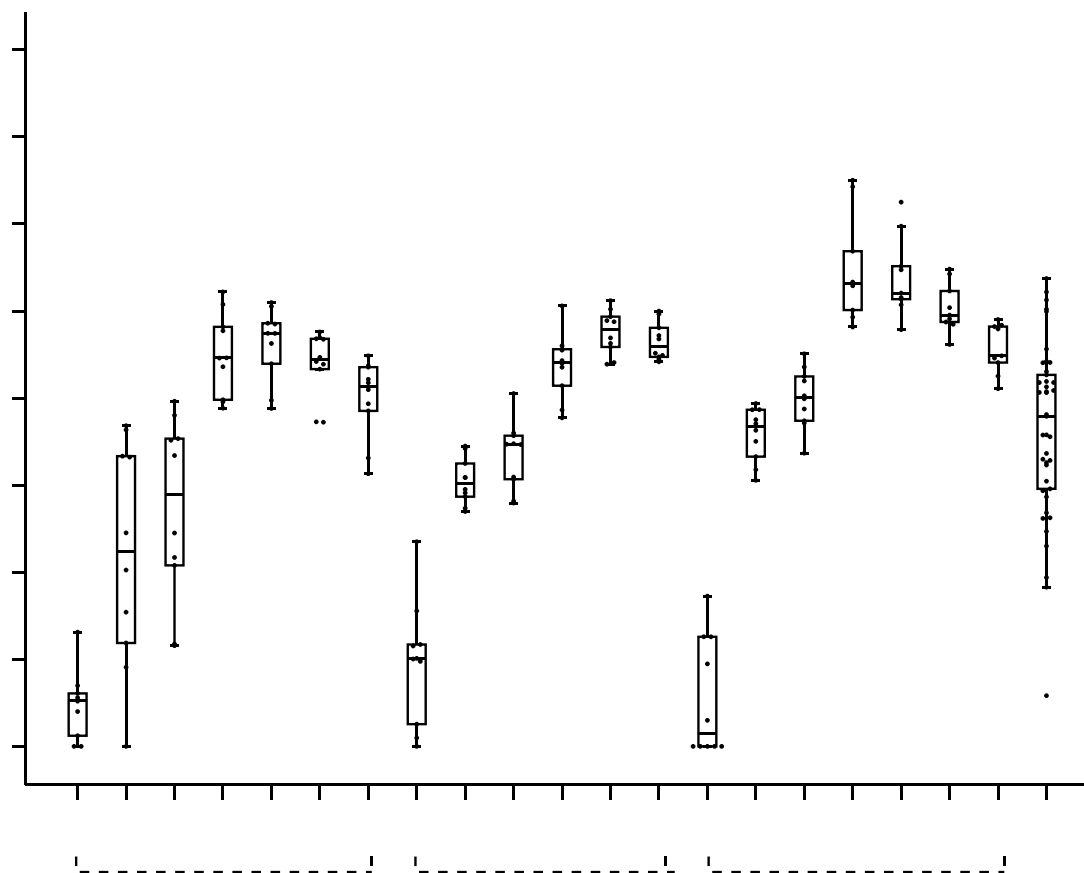
Figure 19. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - RBD – Age 18-55



Note: Boxes and horizontal bars denote interquartile range (IQR) and median AUC, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

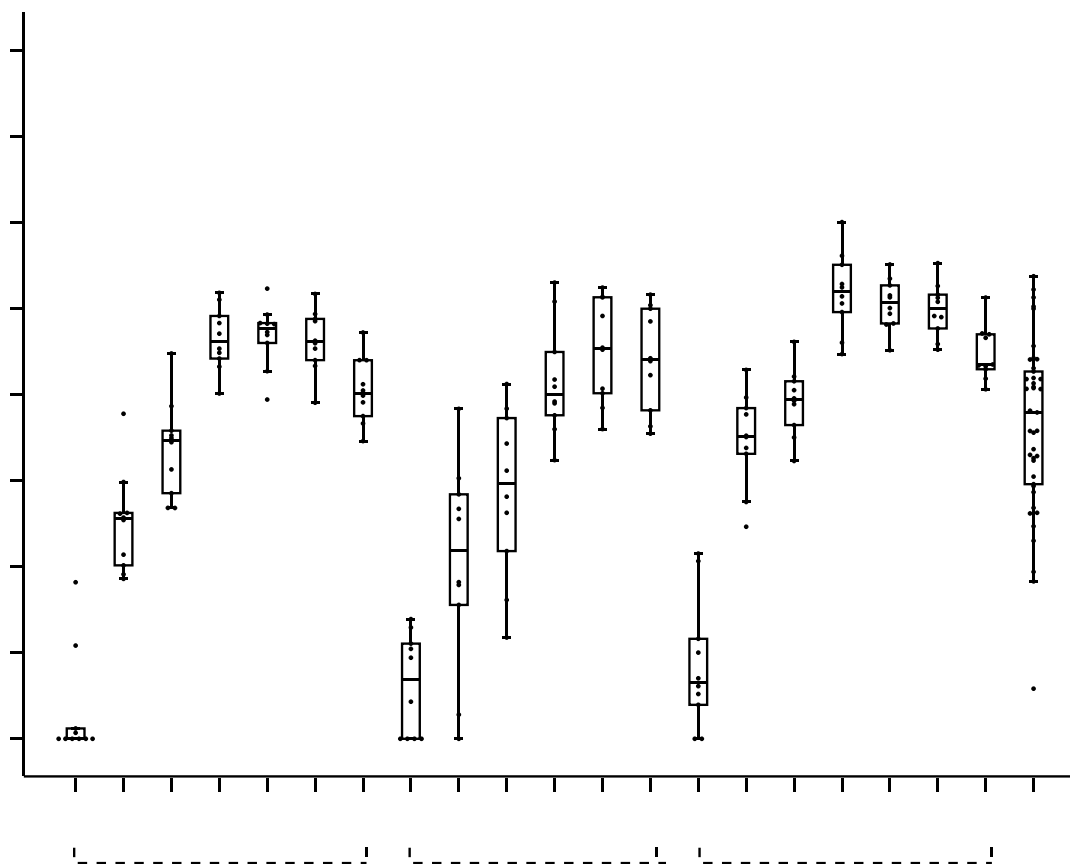
Figure 20. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - RBD – Age 56-70



Note: Boxes and horizontal bars denote interquartile range (IQR) and median AUC, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 21. Serum IgG Area Under the Curve (AUC) Distribution by Time Point and Treatment Group - RBD – Age ≥ 71



Note: Boxes and horizontal bars denote interquartile range (IQR) and median AUC, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals..

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Table 20. Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age 18 -55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	15	15	15	15	60	41
	GMT	116	341	131	178	174	138901
	95% CI	72, 187	127, 914	65, 266	81, 392	121, 251	82876, 232799
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60	
	GMT	32261	67403	86291	163449	74418	
	95% CI	18723, 55587	32438, 140056	56403, 132016	102155, 261520	55452, 99870	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59	
	GMT	40227	118294	109209	213526	101369	
	95% CI	29094, 55621	71948, 194495	79051, 150874	128832, 353896	79236, 129685	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57	
	GMT	391018	866617	781399	1261975	771364	
	95% CI	267402, 571780	641450, 1170823	606247, 1007156	973972, 1635140	648287, 917807	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55	
	GMT	379764	734025	811119	994629	696133	
	95% CI	281597, 512152	588266, 915900	656336, 1002404	806189, 1227115	602999, 803652	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56	
	GMT	299751	562064	782719	1255376	645070	
	95% CI	206070, 436020	407368, 775505	619310, 989244	969516, 1625521	531541, 782848	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42	
	GMT	301540		413971	604507	425777	
	95% CI	217148, 418729		322891, 530744	451387, 809568	357332, 507332	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable							

Table 21. Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GMT	189	563	655	411	138901
	95% CI	76, 466	316, 1006	270, 1591	261, 649	82876, 232799
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GMT	10509	43985	55532	29498	
	95% CI	2841, 38868	20837, 92849	40611, 75935	17406, 49992	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GMT	17684	44878	115831	45131	
	95% CI	5300, 59001	23417, 86006	73288, 183069	27073, 75236	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29	
	GMT	313720	201990	5033017	637766	
	95% CI	160451, 613395	99732, 409099	1113760, 22743909	306259, 1328109	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29	
	GMT	476136	419777	1305996	623535	
	95% CI	263956, 858874	303166, 581241	581138, 2934971	438214, 887230	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29	
	GMT	323945	315031	1183066	479594	
	95% CI	182202, 575958	217897, 455465	379698, 3686201	310929, 739751	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19	
	GMT	86391		366252	171244	
	95% CI	51215, 145728		213031, 629675	104822, 279757	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 22. Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - S-2P - ≥71 Years

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GMT	111	325	953	325	138901
	95% CI	55, 222	104, 1015	493, 1842	188, 561	82876, 232799
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GMT	14837	6720	104909	21869	
	95% CI	6925, 31787	1734, 26038	22445, 490343	10094, 47380	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GMT	57986	16197	203365	57590	
	95% CI	31452, 106905	5220, 50257	97384, 424686	32150, 103160	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30	
	GMT	460094	96574	2636979	489333	
	95% CI	272951, 775548	39656, 235186	1072782, 6481893	255624, 936714	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30	
	GMT	303630	251461	8091439	851688	
	95% CI	167743, 549597	119950, 527158	2546249, 25712881	404186, 1794648	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30	
	GMT	1128391	157016	3638522	863858	
	95% CI	636087, 2001717	82004, 300641	1316233, 10058130	461325, 1617623	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20	
	GMT	75677		195272	121563	
	95% CI	53020, 108016		117647, 324112	84822, 174220	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 23. Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - Vaccination Group - S-2P – Age 18-55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60
	GMFR ^a	278.9	197.4	656.6	915.8	426.5
	95% CI	120.712, 644.309	94.925, 410.543	327.061, 1318.056	418.821, 2002.329	290.797, 625.661
	4-Fold Rise ^b	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	60/60 (100%)
	95% CI	78.2%, 100%	78.2%, 100%	78.2%, 100%	78.2%, 100%	94%, 100%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59
	GMFR ^a	347.7	346.5	831	1259.5	588.4
	95% CI	170.898, 707.61	134.007, 895.728	379.435, 1819.776	533.04, 2975.799	392.847, 881.246
	4-Fold Rise ^b	15/15 (100%)	15/15 (100%)	15/15 (100%)	14/14 (100%)	59/59 (100%)
	95% CI	78.2%, 100%	78.2%, 100%	78.2%, 100%	76.8%, 100%	93.9%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57
	GMFR ^a	2971	2538.1	5945.5	7443.6	4287.1
	95% CI	1440.586, 6127.2	860.349, 7487.837	2824.739, 12514.226	3258.379, 17004.433	2842.637, 6465.518
	4-Fold Rise ^b	13/13 (100%)	15/15 (100%)	15/15 (100%)	14/14 (100%)	57/57 (100%)
	95% CI	75.3%, 100%	78.2%, 100%	78.2%, 100%	76.8%, 100%	93.7%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55
	GMFR ^a	2885.5	2609.5	6381.4	5866.7	4123.7
	95% CI	1489.891, 5588.303	1052.283, 6471.24	2845.746, 14310.027	2489.211, 13826.839	2805.55, 6061.279
	4-Fold Rise ^b	13/13 (100%)	14/14 (100%)	14/14 (100%)	14/14 (100%)	55/55 (100%)
	95% CI	75.3%, 100%	76.8%, 100%	76.8%, 100%	76.8%, 100%	93.5%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56
	GMFR ^a	2277.5	1646.2	6158	7404.7	3595
	95% CI	1061.856, 4884.982	691.442, 3919.159	3200.137, 11849.779	2850.706, 19233.461	2385.725, 5417.337

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)
	4-Fold Rise ^b	13/13 (100%)	15/15 (100%)	14/14 (100%)	14/14 (100%)	56/56 (100%)
	95% CI	75.3%, 100%	78.2%, 100%	76.8%, 100%	76.8%, 100%	93.6%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42
	GMFR ^a	2291.1		3149.8	3565.6	2974.7
	95% CI	1210.086, 4337.908		1628.983, 6090.592	1347.745, 9433.154	1970.928, 4489.774
	4-Fold Rise ^b	13/13 (100%)		15/15 (100%)	14/14 (100%)	42/42 (100%)
	95% CI	75.3%, 100%		78.2%, 100%	76.8%, 100%	91.6%, 100%

Note: N=Number of Subjects.

Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Table 24. Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - Vaccination Group - S-2P – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30
	GMFR ^a	55.7	78.1	84.7	71.7
	95% CI	9.591, 323.362	39.516, 154.316	33.769, 212.561	38.792, 132.487
	4-Fold Rise ^b	9/10 (90%)	10/10 (100%)	10/10 (100%)	29/30 (96.7%)
	95% CI	55.5%, 99.7%	69.2%, 100%	69.2%, 100%	82.8%, 99.9%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30
	GMFR ^a	93.7	79.7	176.7	109.7
	95% CI	16.087, 545.965	35.91, 176.774	61.08, 511.281	56.984, 211.117
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	1662.5	358.6	7919.9	1590.2
	95% CI	445.872, 6199.194	157.078, 818.701	960.785, 65284.929	663.046, 3813.998
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	2523.3	745.3	2055.1	1554.8
	95% CI	856.026, 7437.66	390.824, 1421.134	391.227, 10795.348	839.174, 2880.524
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	1716.7	559.3	1861.7	1195.8
	95% CI	428.352, 6880.259	319.822, 978.087	306.421, 11310.5	599.411, 2385.743

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19
	GMFR ^a	457.8		576.3	510.6
	95% CI	131.067, 1599.219		173.445, 1915.046	233.761, 1115.163
	4-Fold Rise ^b	10/10 (100%)		9/9 (100%)	19/19 (100%)
	95% CI	69.2%, 100%		66.4%, 100%	82.4%, 100%

Note: N=Number of Subjects.

Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Table 25. Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - Vaccination Group - S-2P - ≥71 Years

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30
	GMFR ^a	133.7	20.7	110	67.3
	95% CI	54.367, 328.996	6.406, 66.923	31.725, 381.607	35.284, 128.329
	4-Fold Rise ^b	10/10 (100%)	9/10 (90%)	10/10 (100%)	29/30 (96.7%)
	95% CI	69.2%, 100%	55.5%, 99.7%	69.2%, 100%	82.8%, 99.9%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30
	GMFR ^a	522.7	49.9	213.3	177.2
	95% CI	177.45, 1539.595	15.523, 160.463	102.234, 444.994	94.529, 332.171
	4-Fold Rise ^b	10/10 (100%)	9/10 (90%)	10/10 (100%)	29/30 (96.7%)
	95% CI	69.2%, 100%	55.5%, 99.7%	69.2%, 100%	82.8%, 99.9%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	4147.3	297.6	2765.7	1505.6
	95% CI	1610.973, 10676.865	97.451, 908.669	796.806, 9599.661	739.531, 3065.403
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	2736.9	774.8	8486.4	2620.6
	95% CI	913.801, 8197.41	213.685, 2809.551	2252.854, 31967.873	1266.553, 5422.157
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	10171.4	483.8	3816.1	2658
	95% CI	3722.344, 27793.388	164.278, 1424.865	1263.313, 11527.479	1292.484, 5466.303

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20
	GMFR ^a	682.2		204.8	373.8
	95% CI	271.77, 1712.245		103.019, 407.152	206.704, 675.884
	4-Fold Rise ^b	10/10 (100%)		10/10 (100%)	20/20 (100%)
	95% CI	69.2%, 100%		69.2%, 100%	83.2%, 100%

Note: N=Number of Subjects.

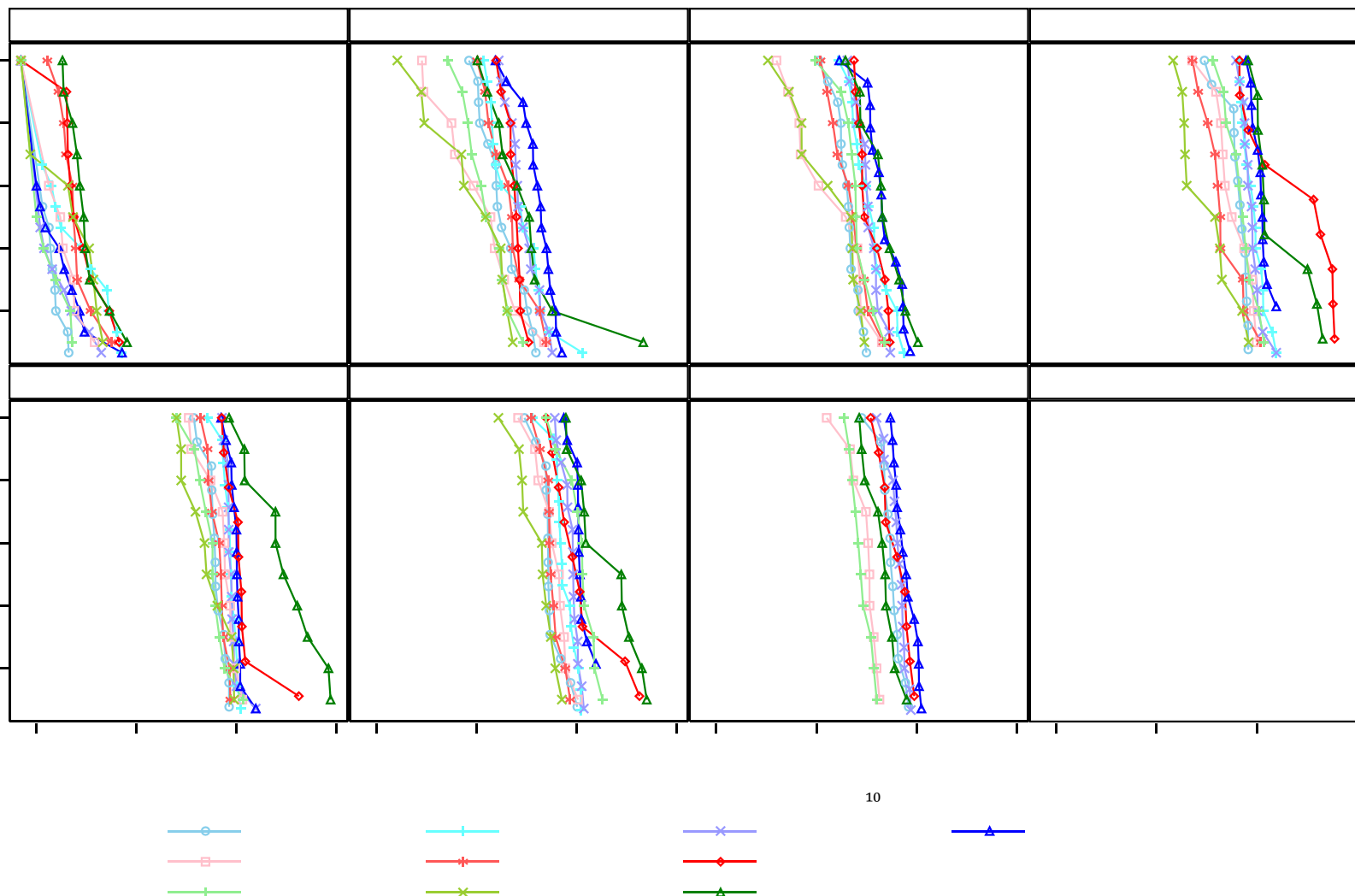
Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

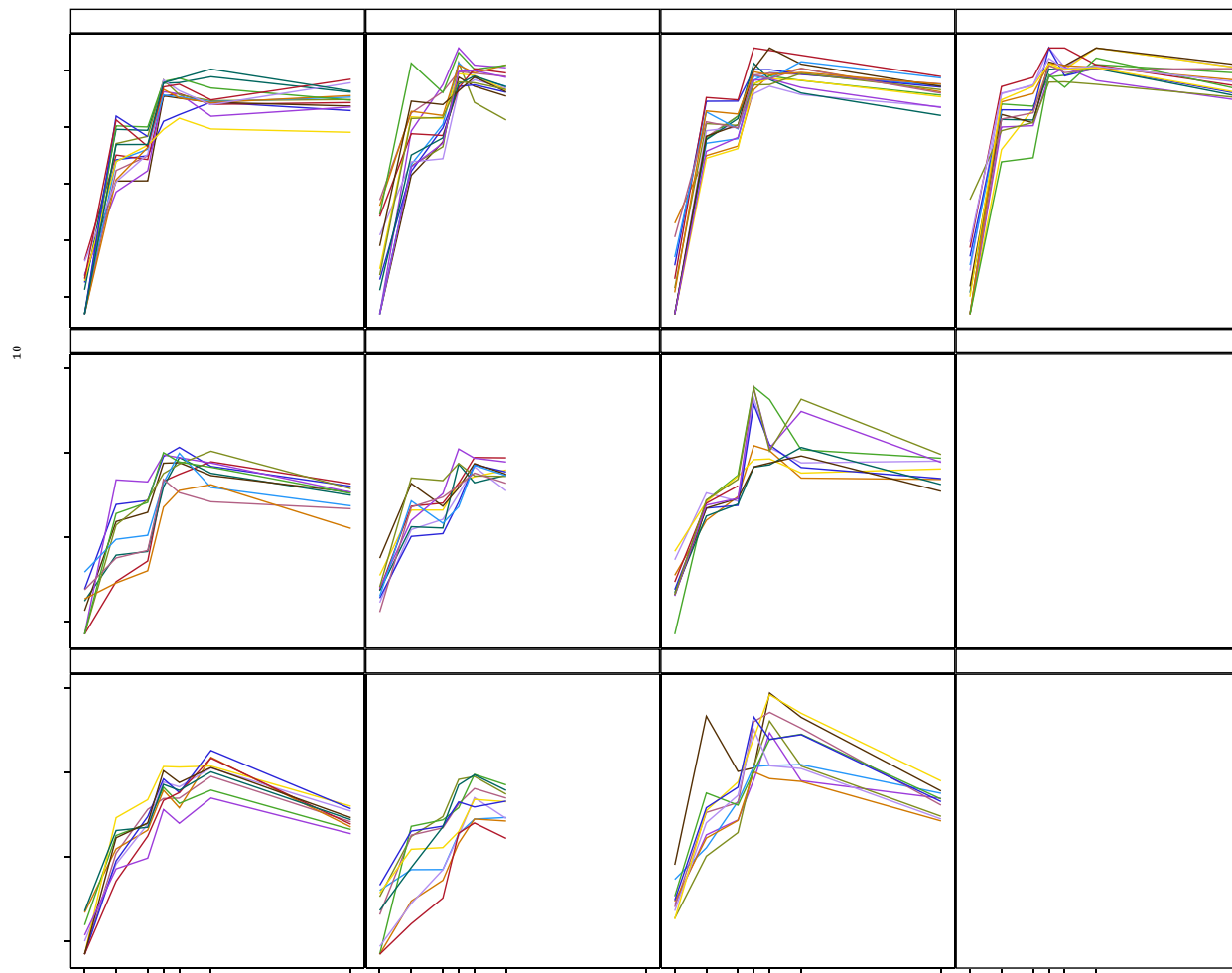
AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Figure 22. Reverse Cumulative Distribution of Serum IgG ELISA Endpoint Titer Values by Time Point and Vaccination Group - S-2P



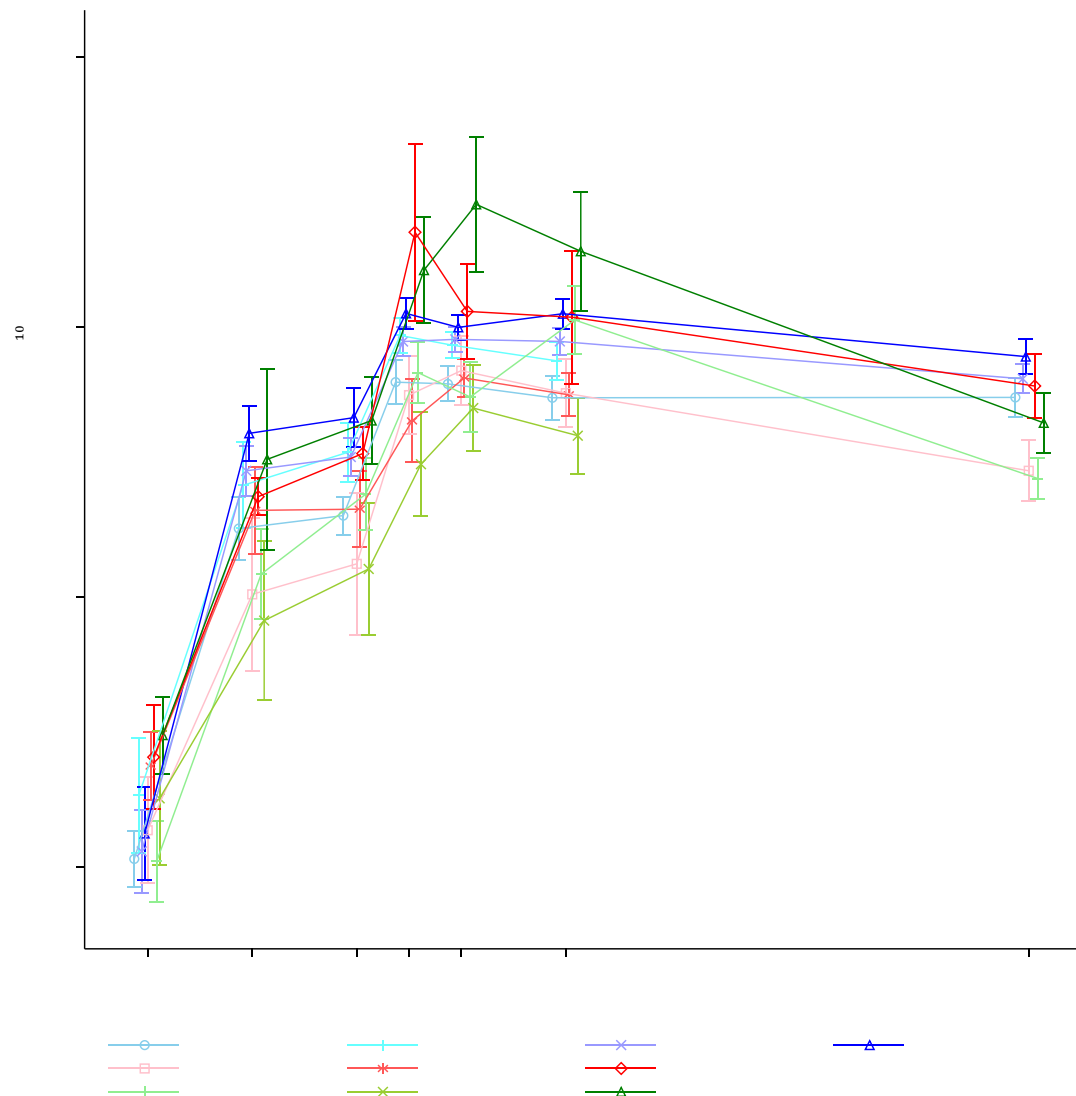
10

Figure 23. Serum IgG ELISA Endpoint Titer Values by Time Point and Vaccination Group - S-2P



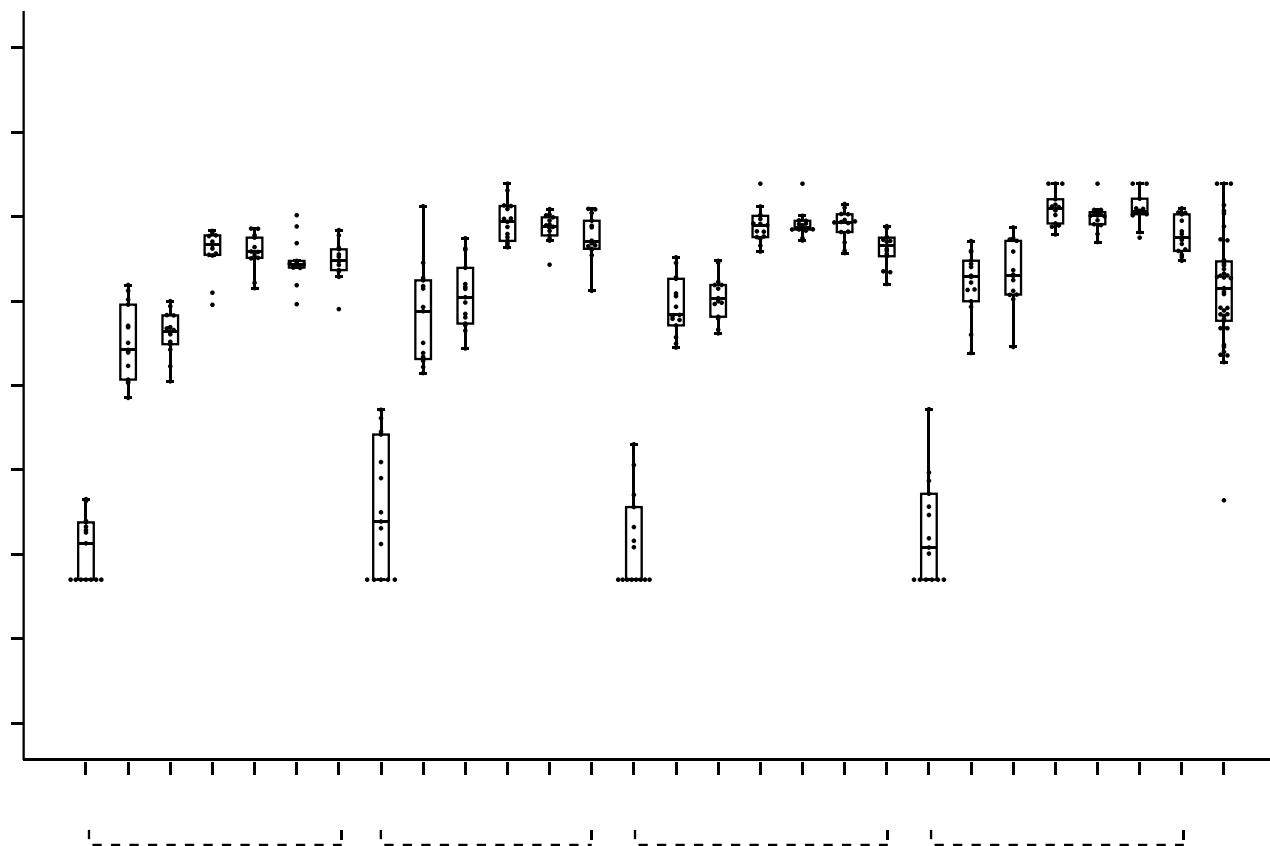
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 24. Geometric Mean Endpoint Titer Values by Time Point and Vaccination Group - S-2P



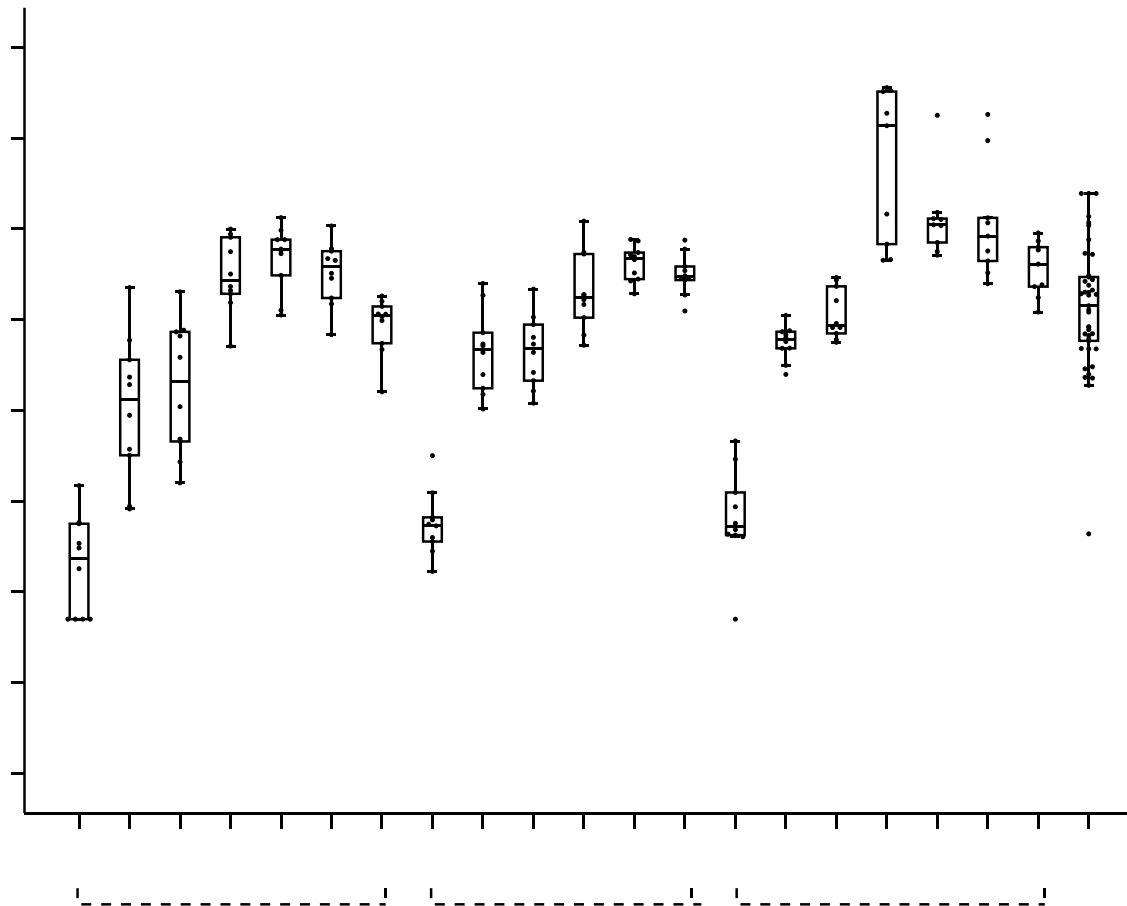
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 25. Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - S-2P – Age 18-55



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 26. Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - S-2P – Age 56-70



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 27. Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - S-2P - ≥ 71 Years

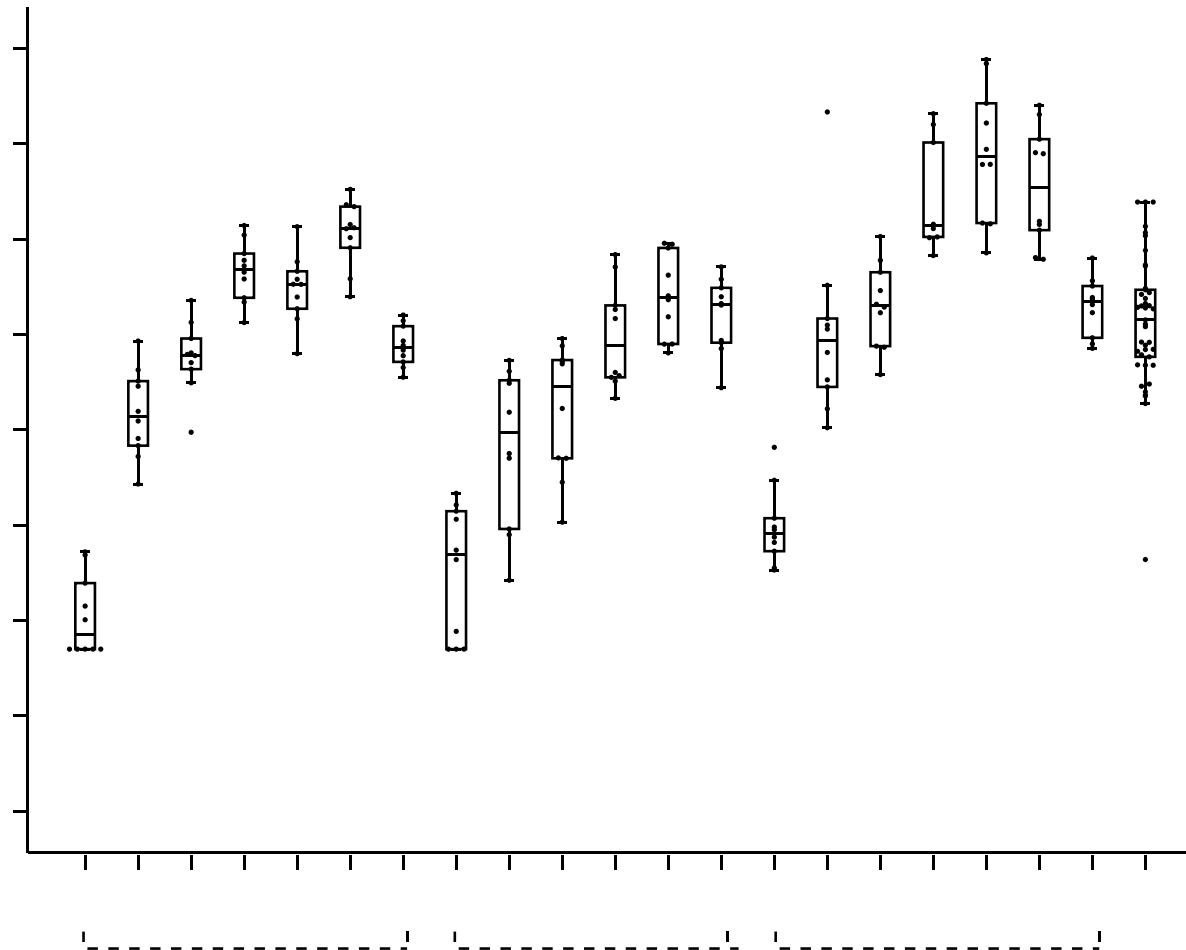


Table 26. Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age 18-55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	15	15	15	15	60	56
	GMT	56	588	166	576	236	34648
	95% CI	44, 70	411, 840	82, 337	349, 949	169, 331	21443, 55985
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60	
	GMT	6567	27297	34073	87480	27037	
	95% CI	3651, 11813	15907, 46843	21688, 53531	51868, 147544	19239, 37995	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59	
	GMT	18149	66630	93231	120088	59961	
	95% CI	11091, 29700	35968, 123430	59895, 145123	71013, 203077	44203, 81336	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57	
	GMT	208652	659829	499539	720907	481983	
	95% CI	142803, 304864	466377, 933523	400950, 622370	591860, 878090	401121, 579145	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55	
	GMT	233264	572950	558905	644395	474371	
	95% CI	164756, 330259	398765, 823221	462908, 674810	495808, 837510	399349, 563487	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56	
	GMT	183652	515720	371271	564241	382295	
	95% CI	122763, 274741	328463, 809732	266721, 516804	396948, 802039	309541, 472150	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42	
	GMT	140985		235228	224653	197705	
	95% CI	79938, 248653		177236, 312195	151320, 333524	156955, 249036	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable							

Table 27. Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GMT	204	349	223	251	37244
	95% CI	114, 365	165, 737	64, 775	158, 399	20170, 68771
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GMT	2924	8405	30981	9132	
	95% CI	576, 14833	5860, 12056	15901, 60362	4828, 17269	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GMT	4841	13600	45690	14436	
	95% CI	1531, 15304	7948, 23271	26314, 79330	8476, 24586	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29	
	GMT	198643	105925	1471882	297747	
	95% CI	98719, 399707	50968, 220142	560108, 3867893	166078, 533806	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29	
	GMT	201496	244967	1005639	354975	
	95% CI	115918, 350251	154504, 388398	445521, 2269948	235731, 534538	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29	
	GMT	78045	201801	506364	193486	
	95% CI	42847, 142159	135928, 299598	235654, 1088051	127566, 293473	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19	
	GMT	45421		151761	80431	
	95% CI	23045, 89526		88571, 260033	49072, 131830	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 28. Serum IgG ELISA Endpoint Titer Geometric Mean Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD – ≥71 Years

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GMT	111	194	503	221	37244
	95% CI	46, 270	92, 408	174, 1455	132, 370	20170, 68771
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GMT	4676	2135	25670	6352	
	95% CI	2236, 9777	514, 8871	12394, 53168	3328, 12123	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GMT	15338	8245	56343	19243	
	95% CI	7085, 33203	2487, 27335	35052, 90567	11299, 32771	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30	
	GMT	160591	78965	711752	208206	
	95% CI	82611, 312177	25685, 242760	368657, 1374153	120213, 360610	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30	
	GMT	295194	127341	694471	296649	
	95% CI	167293, 520878	44080, 367871	465032, 1037111	188585, 466637	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30	
	GMT	218268	121389	453506	229043	
	95% CI	106743, 446314	43515, 338622	255624, 804573	146129, 359004	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20	
	GMT	65987		157946	102090	
	95% CI	33240, 130995		94345, 264420	65789, 158421	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 29. Serum IgG ELISA Endpoint Titer Geometric Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age 18-55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60
	GMFR ^a	118.2	46.4	205.2	152	114.4
	95% CI	60.898, 229.597	24.51, 87.967	105.889, 397.681	74.295, 310.933	81.634, 160.303
	4-Fold Rise ^b	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	60/60 (100%)
	95% CI	78.2%, 100%	78.2%, 100%	78.2%, 100%	78.2%, 100%	94%, 100%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59
	GMFR ^a	326.8	113.3	561.5	222.1	261.4
	95% CI	194.534, 549.007	51.602, 248.935	264.941, 1189.944	107.021, 461.001	183.349, 372.712
	4-Fold Rise ^b	15/15 (100%)	15/15 (100%)	15/15 (100%)	14/14 (100%)	59/59 (100%)
	95% CI	78.2%, 100%	78.2%, 100%	78.2%, 100%	76.8%, 100%	93.9%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57
	GMFR ^a	3696.8	1122.4	3008.5	1333.4	1991.9
	95% CI	2273.806, 6010.434	669.718, 1881.009	1610.699, 5619.25	768.976, 2312.173	1499.044, 2646.88
	4-Fold Rise ^b	13/13 (100%)	15/15 (100%)	15/15 (100%)	14/14 (100%)	57/57 (100%)
	95% CI	75.3%, 100%	78.2%, 100%	78.2%, 100%	76.8%, 100%	93.7%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55
	GMFR ^a	4132.9	1073.8	3527.7	1191.9	2052.6
	95% CI	2714.982, 6291.356	726.77, 1586.535	1637.818, 7598.401	640.566, 2217.76	1508.041, 2793.749
	4-Fold Rise ^b	13/13 (100%)	14/14 (100%)	14/14 (100%)	14/14 (100%)	55/55 (100%)
	95% CI	75.3%, 100%	76.8%, 100%	76.8%, 100%	76.8%, 100%	93.5%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56
	GMFR ^a	3253.9	877.3	2343.4	1043.6	1587.9
	95% CI	1989.818, 5320.999	557.835, 1379.568	963.92, 5697.1	494.474, 2202.725	1135.132, 2221.189

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)
	4-Fold Rise ^b	13/13 (100%)	15/15 (100%)	14/14 (100%)	14/14 (100%)	56/56 (100%)
	95% CI	75.3%, 100%	78.2%, 100%	76.8%, 100%	76.8%, 100%	93.6%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42
	GMFR ^a	2497.9		1416.7	415.5	1121.9
	95% CI	1363.387, 4576.607		685.114, 2929.333	209.466, 824.301	731.156, 1721.439
	4-Fold Rise ^b	13/13 (100%)		15/15 (100%)	14/14 (100%)	42/42 (100%)
	95% CI	75.3%, 100%		78.2%, 100%	76.8%, 100%	91.6%, 100%

Note: N=Number of Subjects.

Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Table 30. Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30
	GMFR ^a	14.4	24.1	138.9	36.4
	95% CI	2.731, 75.518	12.171, 47.635	47.299, 407.876	17.842, 74.059
	4-Fold Rise ^b	6/10 (60%)	10/10 (100%)	10/10 (100%)	26/30 (86.7%)
	95% CI	26.2%, 87.8%	69.2%, 100%	69.2%, 100%	69.3%, 96.2%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30
	GMFR ^a	23.8	39	204.8	57.5
	95% CI	5.815, 97.222	19.878, 76.362	54.657, 767.682	28.825, 114.556
	4-Fold Rise ^b	8/10 (80%)	10/10 (100%)	9/10 (90%)	27/30 (90%)
	95% CI	44.4%, 97.5%	69.2%, 100%	55.5%, 99.7%	73.5%, 97.9%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	975.7	303.4	5588.7	1121.1
	95% CI	330.172, 2883.052	136.067, 676.699	2486.983, 12558.802	586.253, 2143.832
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	989.7	701.8	3818.4	1336.6
	95% CI	411.217, 2381.821	272.086, 1809.923	2065.08, 7060.283	806.259, 2215.649
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29
	GMFR ^a	383.3	578.1	1922.6	728.5
	95% CI	156.909, 936.467	249.122, 1341.482	379.042, 9752.418	390.374, 1359.569

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	9/9 (100%)	29/29 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	66.4%, 100%	88.1%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19
	GMFR ^a	223.1		576.2	349.7
	95% CI	82.895, 600.403		168.76, 1967.559	167.59, 729.704
	4-Fold Rise ^b	10/10 (100%)		9/9 (100%)	19/19 (100%)
	95% CI	69.2%, 100%		66.4%, 100%	82.4%, 100%

Note: N=Number of Subjects.

Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

Table 31. Serum IgG ELISA Geometric Mean Fold Rise (GMFR) and 4-Fold Rise Results by Time Point and Vaccination Group - RBD - ≥71 Years

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30
	GMFR ^a	42	11	51	28.7
	95% CI	14.344, 123.269	2.664, 45.538	17.028, 152.836	14.816, 55.577
	4-Fold Rise ^b	9/10 (90%)	6/10 (60%)	9/10 (90%)	24/30 (80%)
	95% CI	55.5%, 99.7%	26.2%, 87.8%	55.5%, 99.7%	61.4%, 92.3%
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30
	GMFR ^a	137.9	42.5	112	86.9
	95% CI	37.219, 511.138	11.334, 159.667	39.4, 318.233	45.361, 166.61
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	1444.1	407.4	1414.5	940.6
	95% CI	386.702, 5392.916	109.903, 1510.464	487.842, 4101.419	485.178, 1823.658
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	2654.5	657	1380.2	1340.2
	95% CI	760.064, 9270.938	173.077, 2494.327	475.685, 4004.465	697.978, 2573.364
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30
	GMFR ^a	1962.8	626.3	901.3	1034.8
	95% CI	480.831, 8012.1	180.418, 2174.381	270.671, 3001.108	527.738, 2028.964

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)
	4-Fold Rise ^b	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)
	95% CI	69.2%, 100%	69.2%, 100%	69.2%, 100%	88.4%, 100%
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20
	GMFR ^a	593.4		313.9	431.6
	95% CI	150.964, 2332.394		88.358, 1115.129	183.754, 1013.644
	4-Fold Rise ^b	10/10 (100%)		10/10 (100%)	20/20 (100%)
	95% CI	69.2%, 100%		69.2%, 100%	83.2%, 100%

Note: N=Number of Subjects.

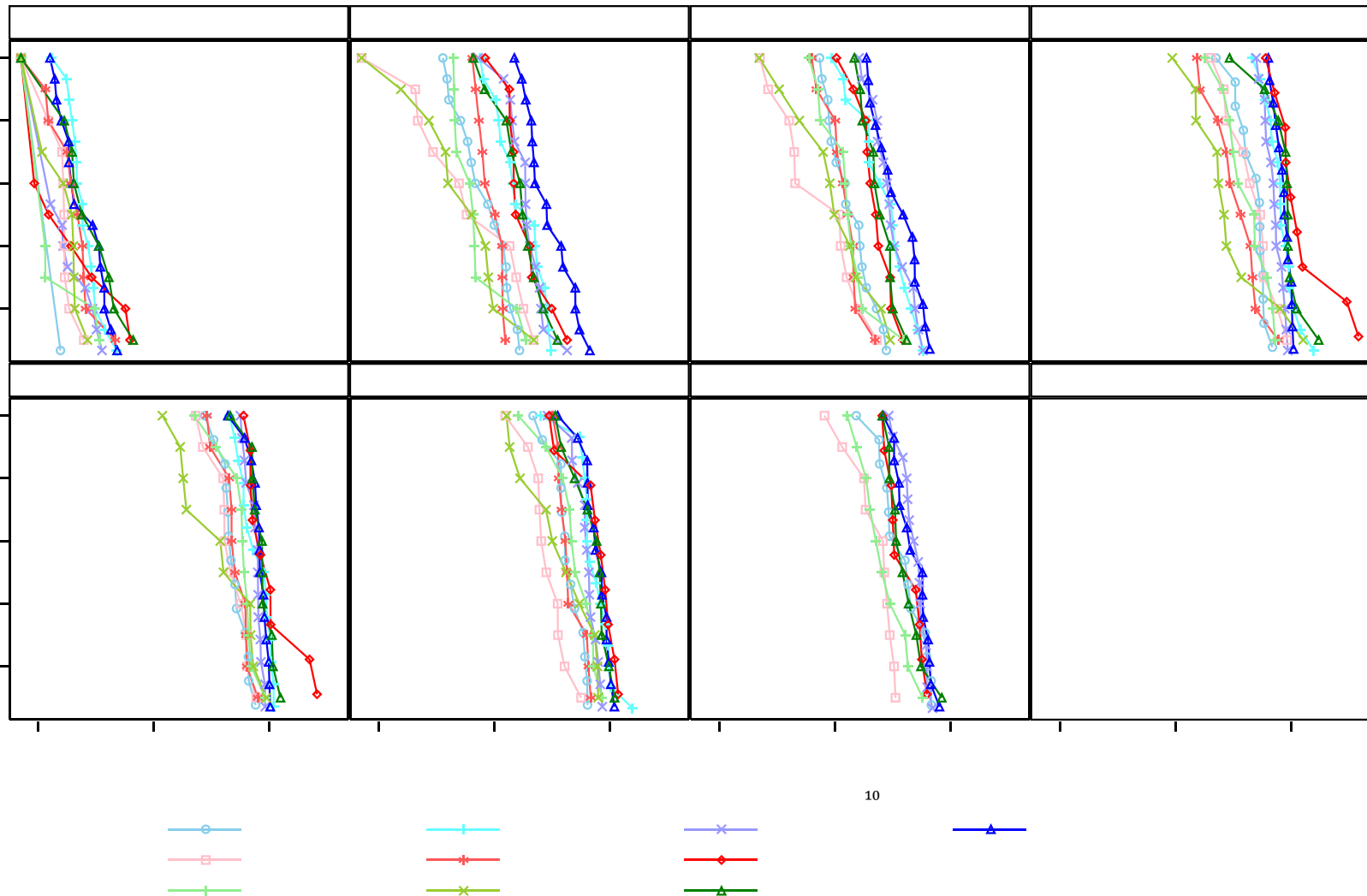
Note: n=number of subjects with baseline and data at corresponding visit.

^aGMFR represents the geometric mean fold rise in AUC compared to pre-dose 1

^b4-Fold Rise represents the percentage of subjects with at least a 4-Fold Rise in AUC compared to pre-dose 1

AUC results reported as 0 were imputed to the lowest non-zero reported value for the purposes of fold-rise calculations.

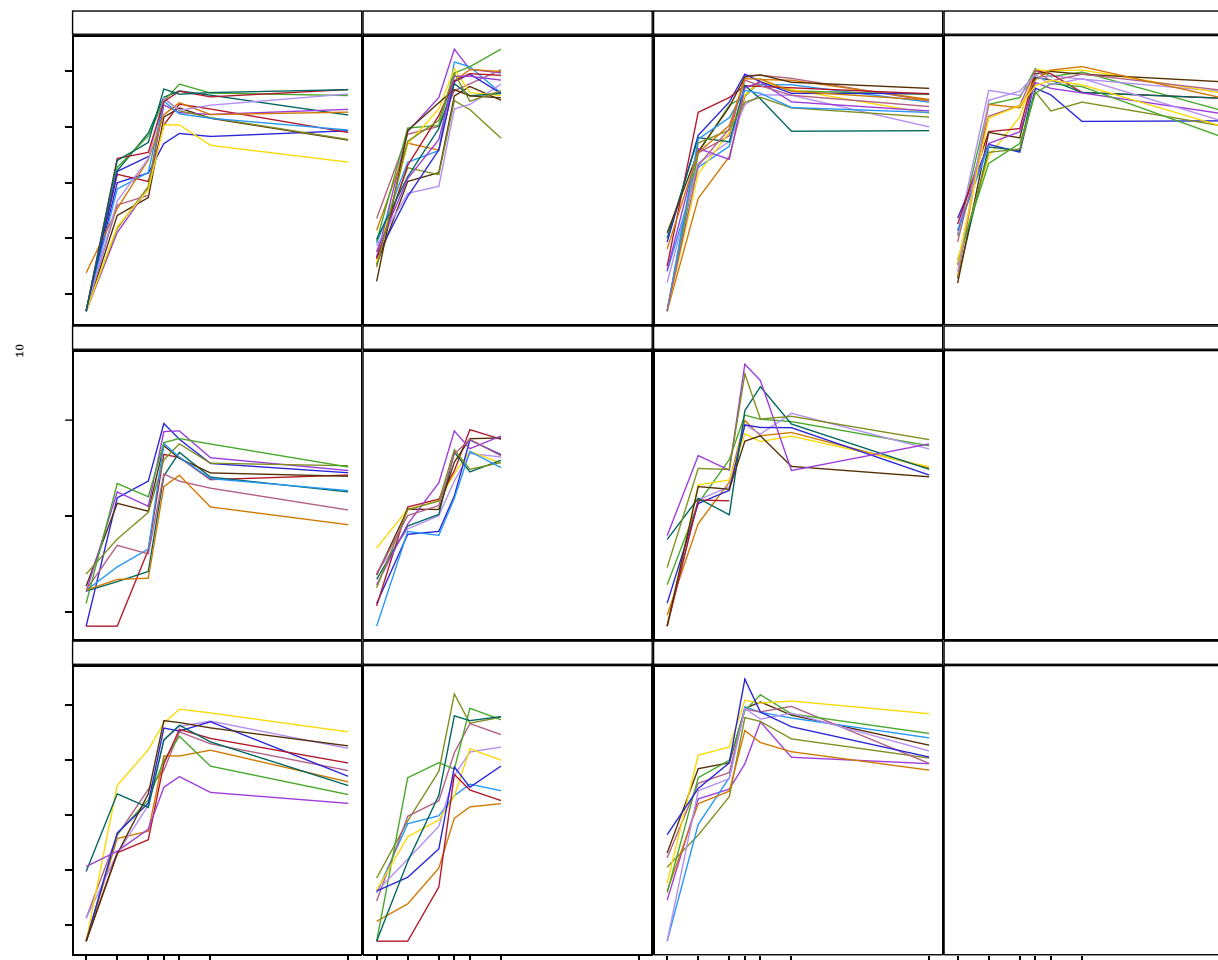
Figure 28. Reverse Cumulative Distribution of Serum IgG ELISA Endpoint Titer Values by Time Point and Vaccination Group - RBD



10

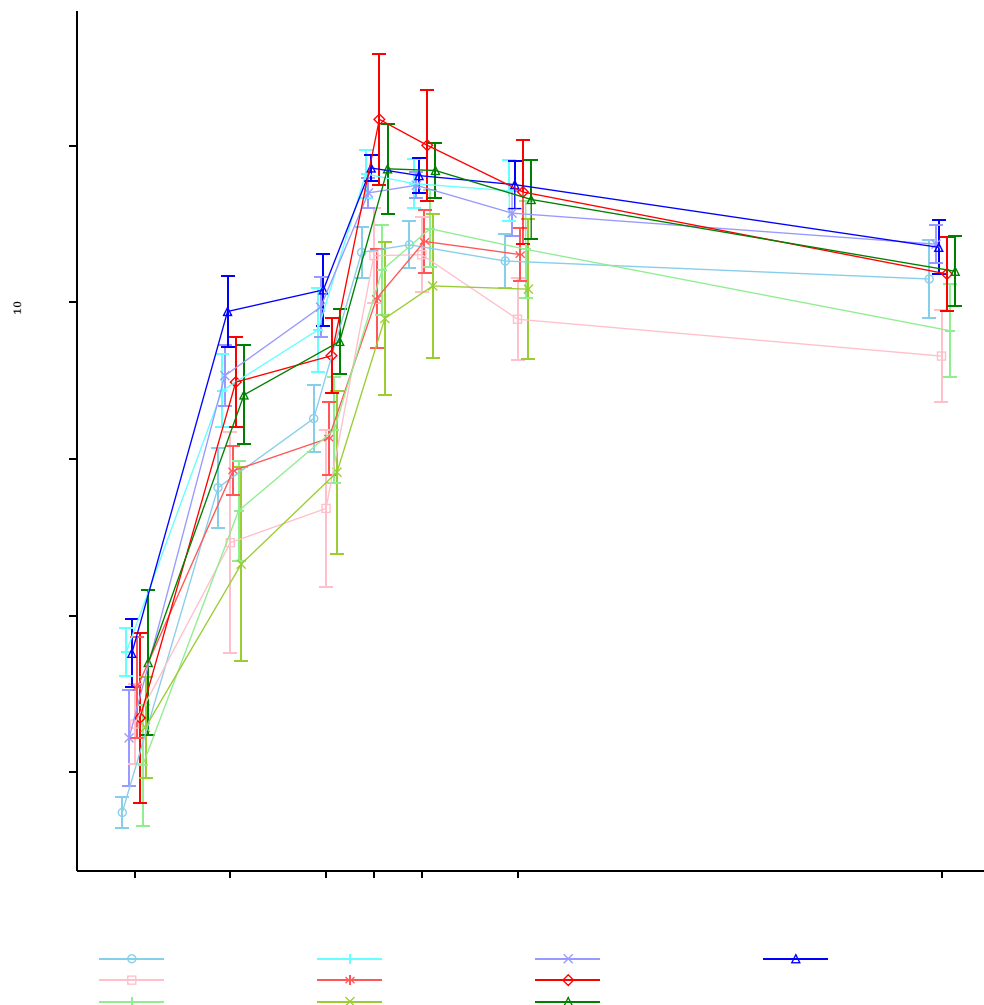
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 29. Serum IgG ELISA Endpoint Titer Values by Time Point and Vaccination Group – RBD



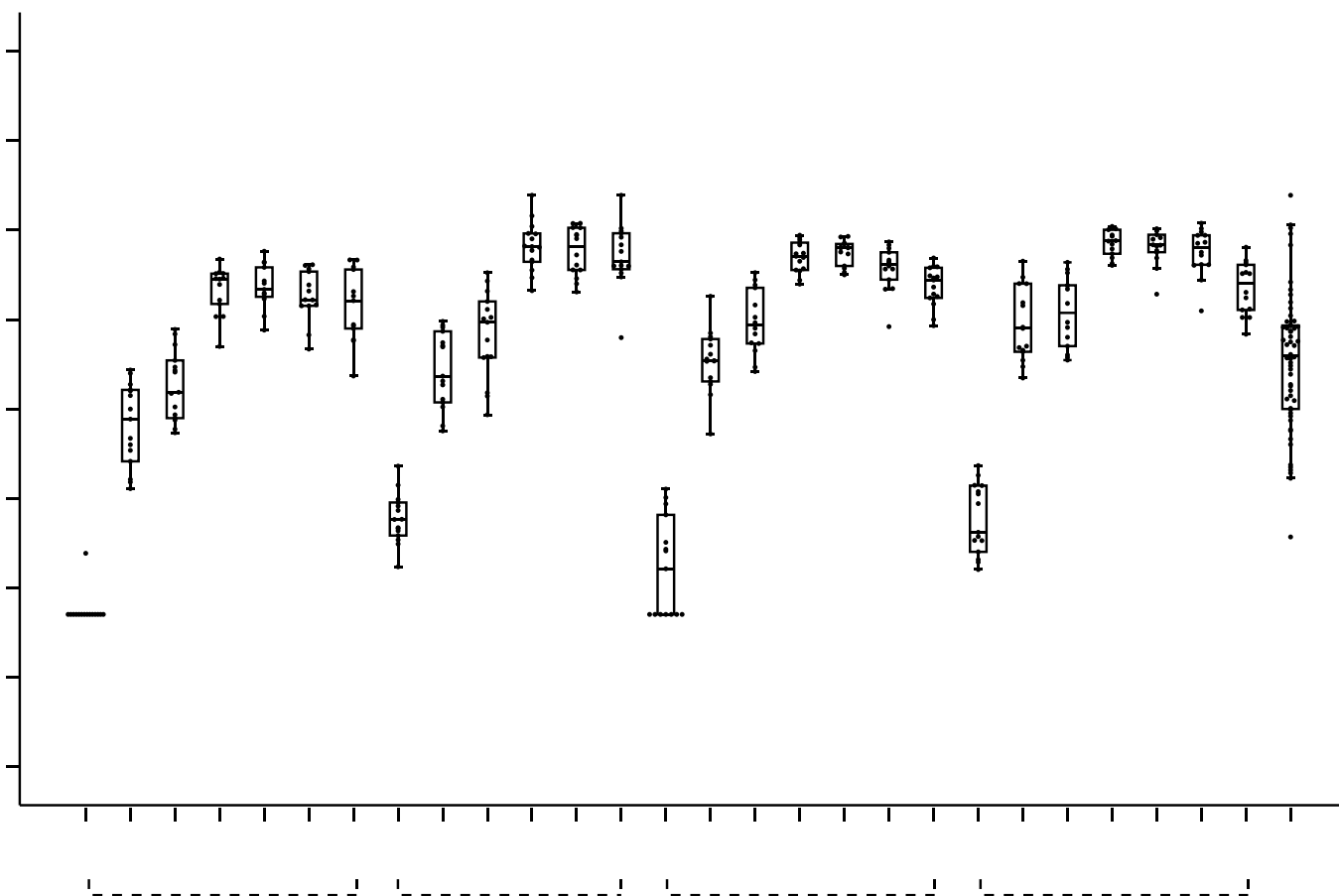
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 30. Serum IgG ELISA Geometric Mean Endpoint Titer Values by Time Point and Vaccination Group – RBD



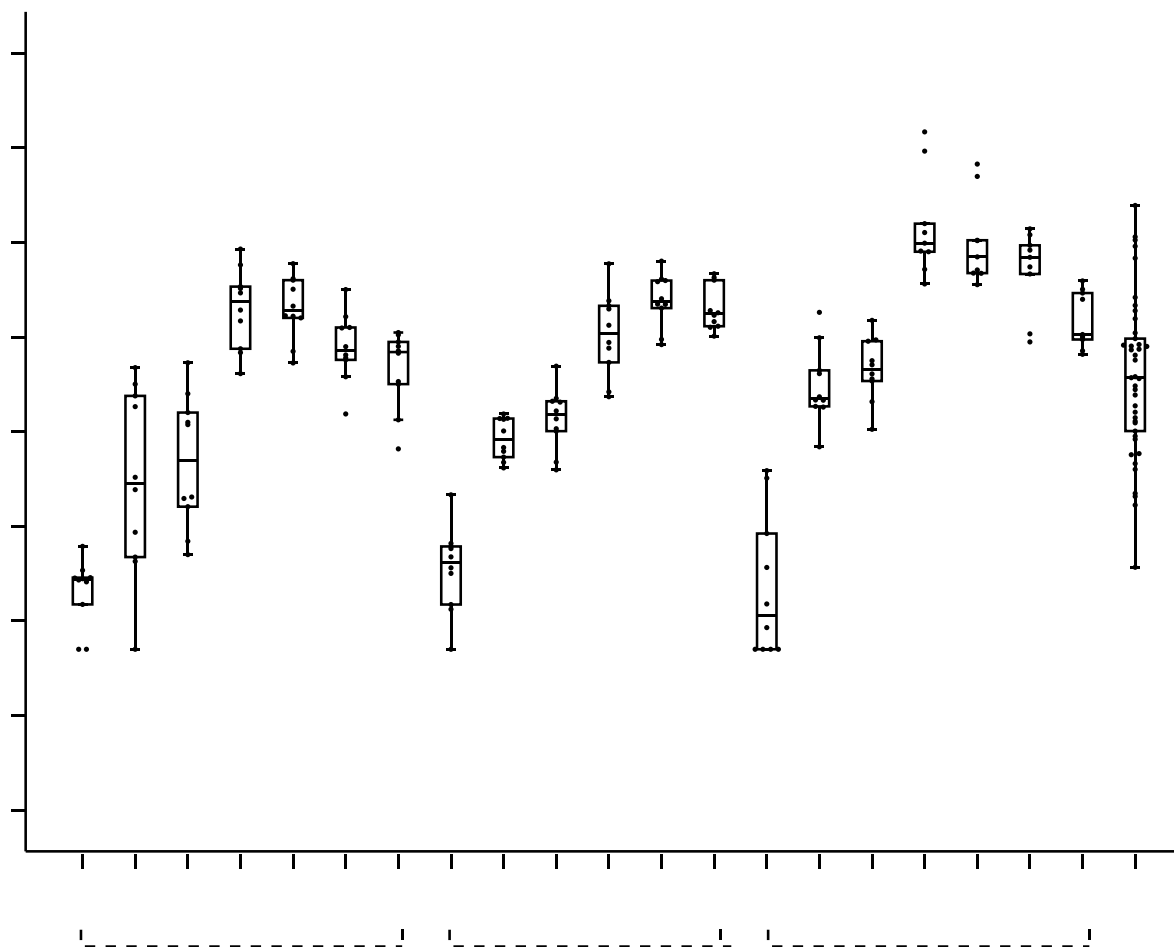
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 31. Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - RBD – Age 18-55



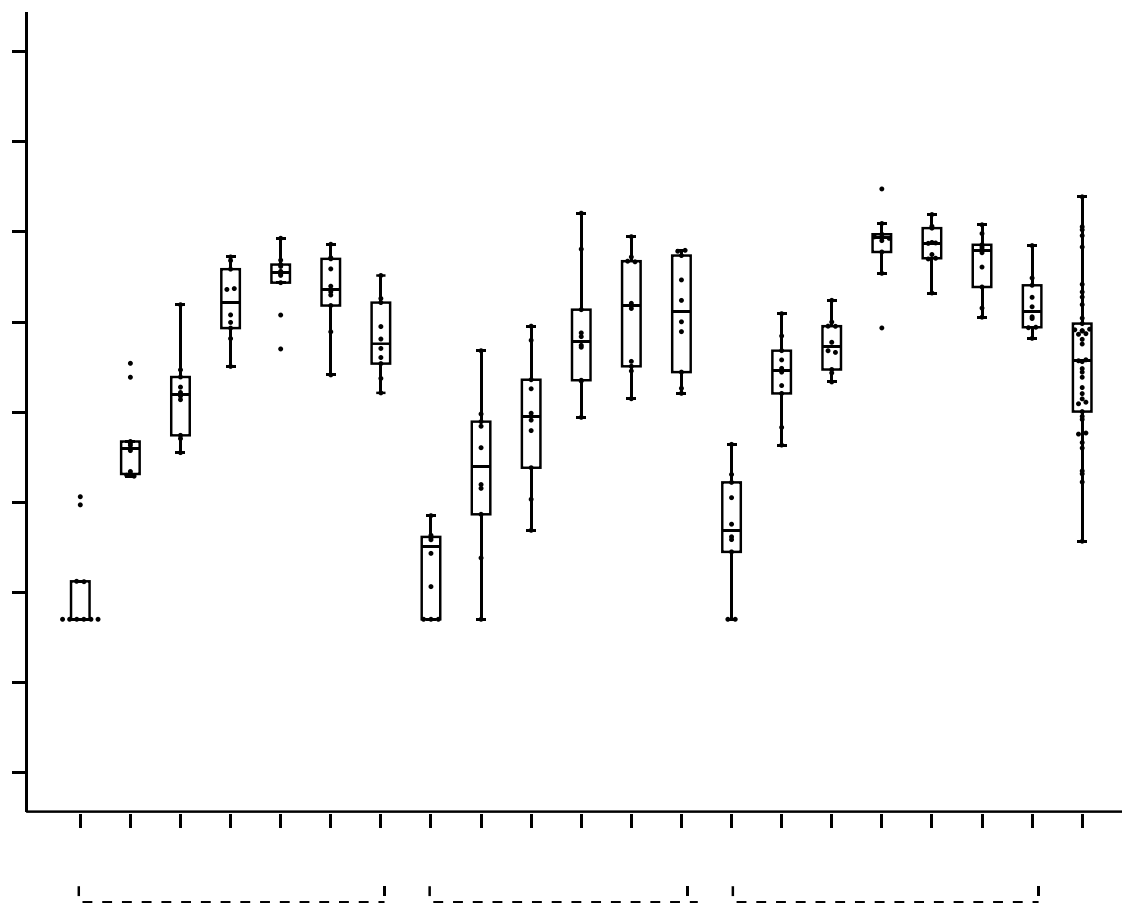
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 32. Serum IgG ELISA Endpoint Titer Distribution by Time Point and Treatment Group - RBD – Age 56-70



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 33. Serum IgG Endpoint Titer Distribution by Time Point and Treatment Group - RBD - ≥ 71 Years



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Table 32. Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ – Age 18-55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	15	15	15	15	60	41
	GM	10	10	10	10	10	106
	95% CI	NE	NE	NE	NE	NE	60, 189
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60	
	GM	14	23	24	26	21	
	95% CI	10, 21	13, 40	13, 42	14, 48	16, 27	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59	
	GM	12	14	18	21	16	
	95% CI	10, 14	9, 21	12, 27	13, 32	13, 19	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57	
	GM	106	294	263	378	241	
	95% CI	70, 160	178, 487	188, 368	306, 468	194, 298	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55	
	GM	112	351	360	342	268	
	95% CI	71, 177	214, 575	273, 476	267, 438	216, 333	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56	
	GM	90	234	276	277	204	
	95% CI	57, 143	153, 358	193, 393	231, 332	166, 251	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42	
	GM	54		182	185	126	
	95% CI	29, 100		112, 296	128, 269	92, 173	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable							

Table 33. Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GM	10	10	10	10	106
	95% CI	NE	NE	NE	NE	60, 189
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GM	10	12	12	11	
	95% CI	NE	10, 14	10, 15	10, 12	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GM	10	12	11	11	
	95% CI	NE	9, 16	10, 12	10, 12	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29	
	GM	85	108	340	142	
	95% CI	51, 142	56, 211	219, 527	99, 204	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29	
	GM	119	220	404	215	
	95% CI	68, 209	162, 299	292, 561	162, 286	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29	
	GM	100	163	424	185	
	95% CI	49, 204	115, 230	267, 673	130, 264	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19	
	GM	44		167	83	
	95% CI	20, 98		88, 318	47, 146	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

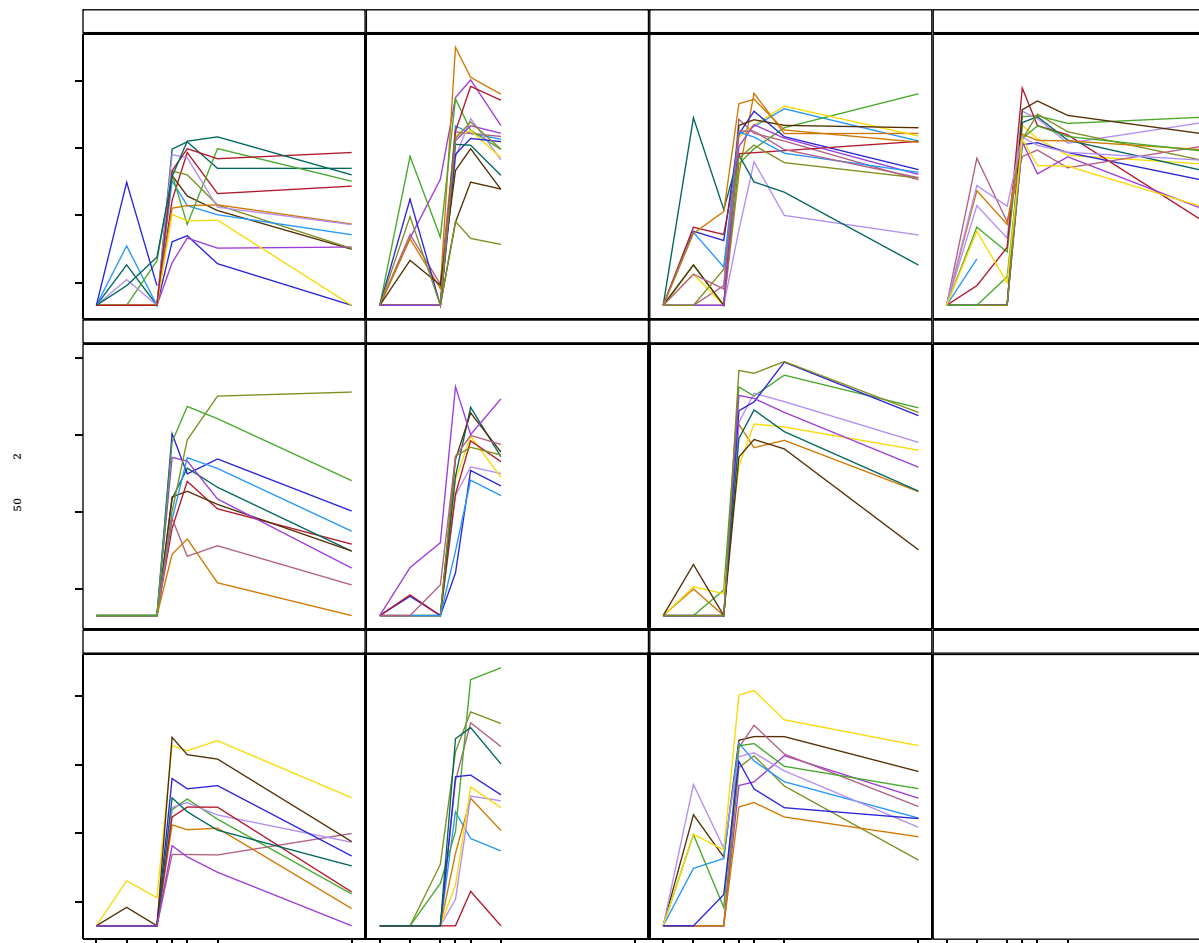
Table 34. Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ – Age ≥ 71

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GM	10	10	10	10	106
	95% CI	NE	NE	NE	NE	60, 189
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GM	11	10	27	15	
	95% CI	9, 14	NE	12, 60	11, 19	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GM	11	12	20	14	
	95% CI	9, 12	9, 17	12, 33	11, 17	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30	
	GM	121	75	310	141	
	95% CI	69, 211	30, 190	202, 475	94, 212	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30	
	GM	112	217	317	197	
	95% CI	67, 188	86, 542	198, 508	136, 287	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30	
	GM	100	150	231	151	
	95% CI	56, 179	53, 419	150, 356	103, 223	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20	
	GM	33		109	60	
	95% CI	19, 59		68, 175	39, 93	

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

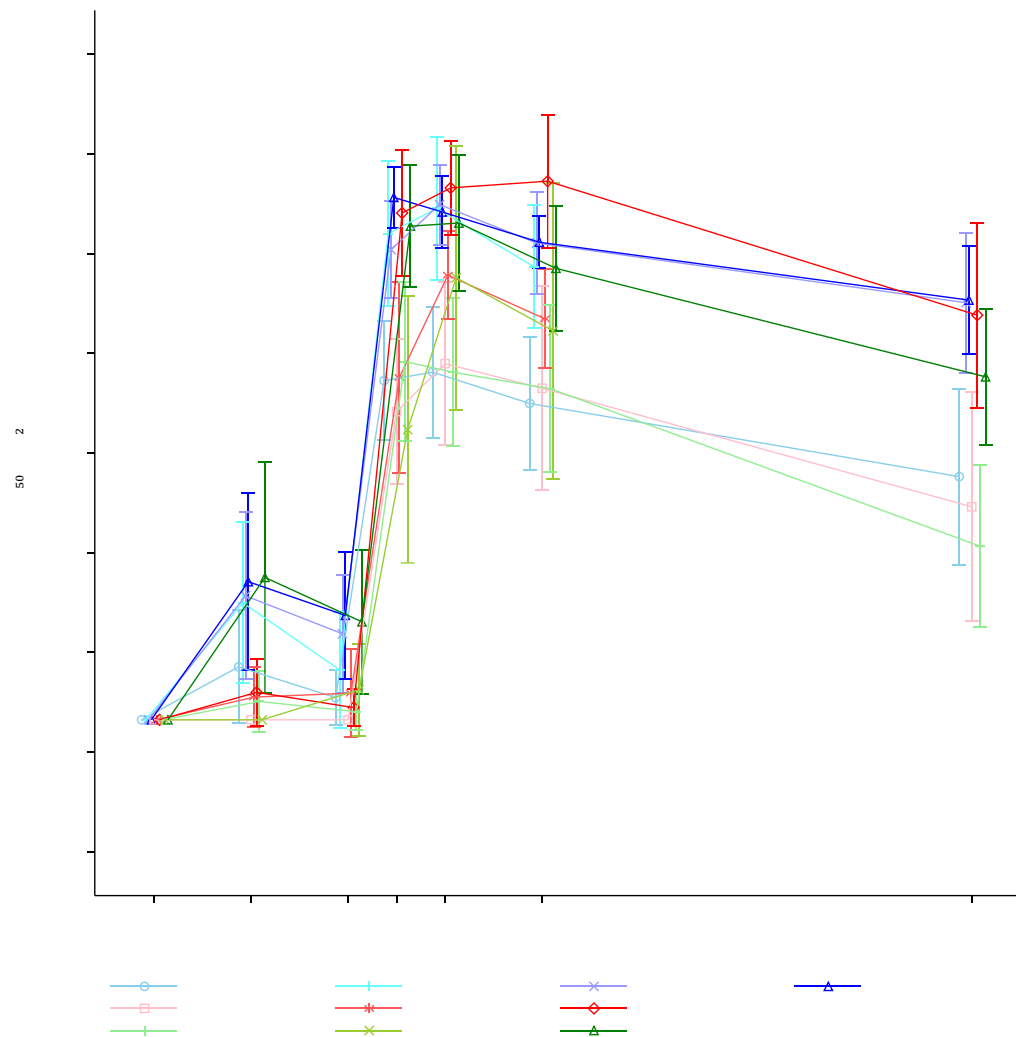
Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Figure 34. Pseudovirus Neutralization Assay Titers by Time Point and Vaccination Group - ID₅₀



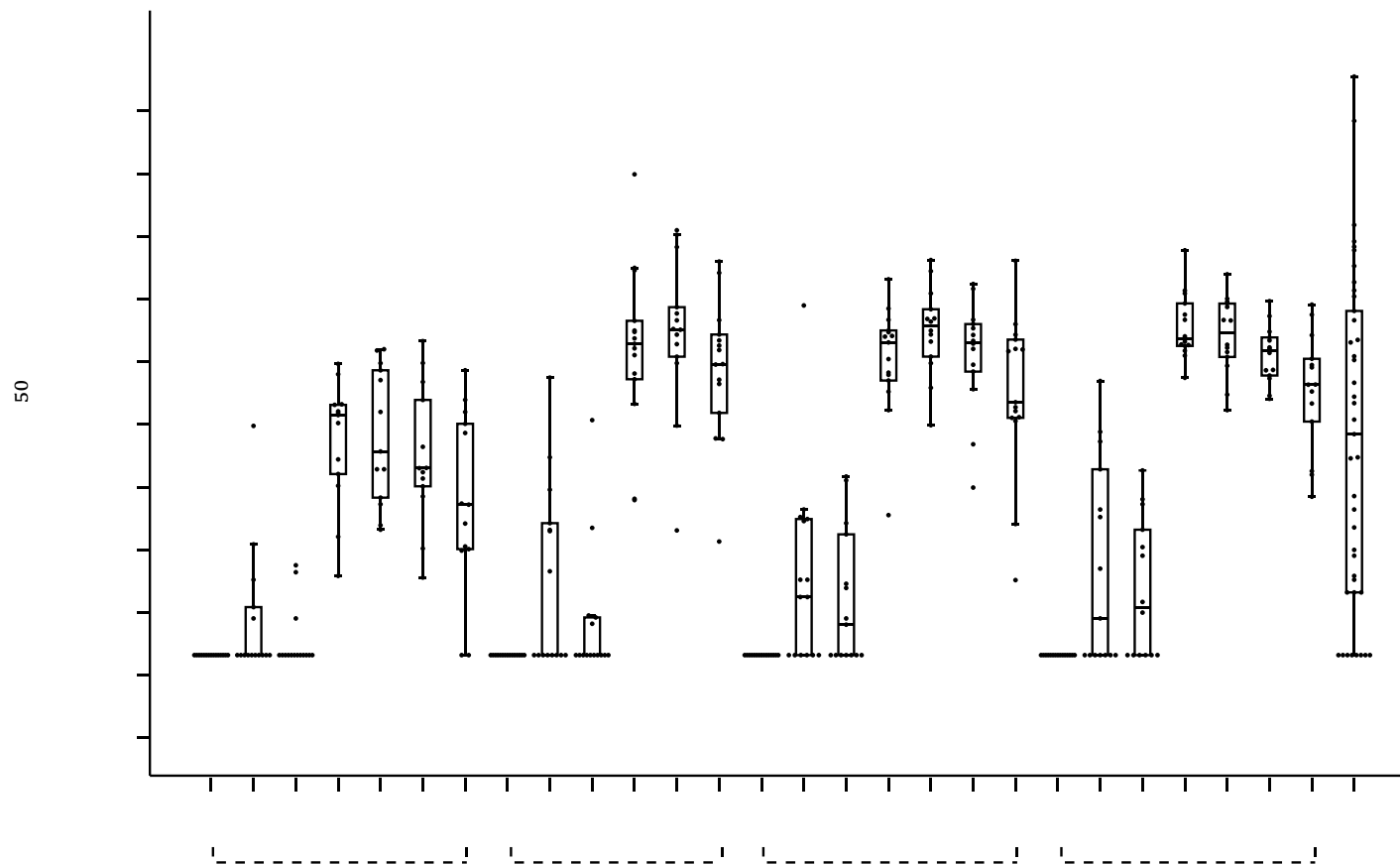
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 35. Pseudovirus Neutralization Assay GM by Time Point and Treatment Group - ID₅₀



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

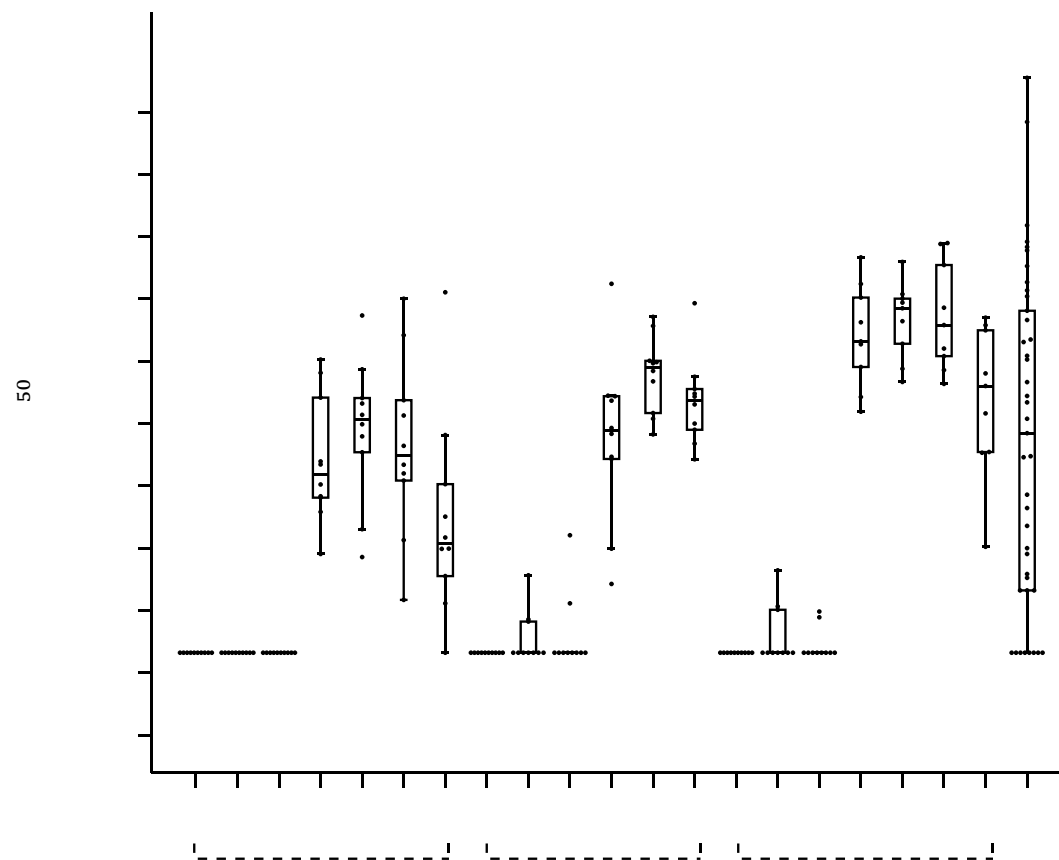
Figure 36. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID₅₀ – Age 18-55



Note: Boxes and horizontal bars denote interquartile range (IQR) and median ID₅₀, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median +/- 1.5 x IQR. The convalescent sera panel includes specimens from 41 individuals.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

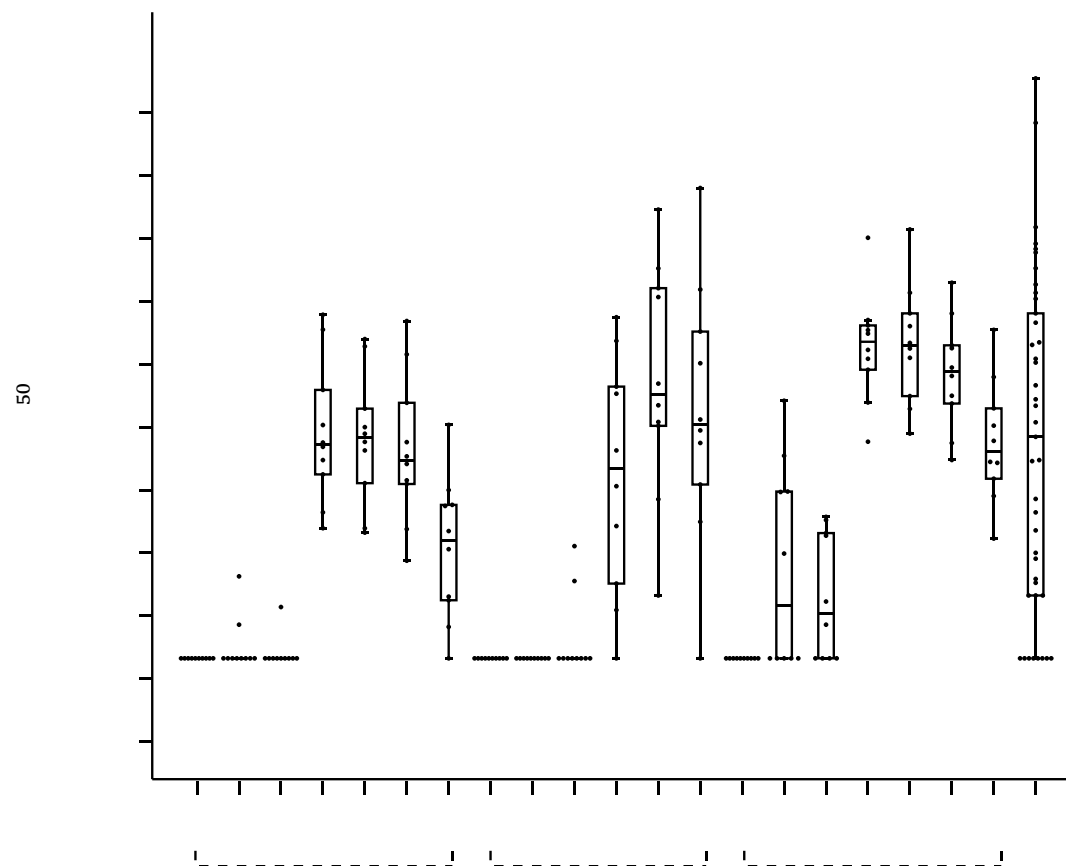
Figure 37. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID₅₀ – Age 56-70



Note: Boxes and horizontal bars denote interquartile range (IQR) and median ID₅₀, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 38. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID₅₀ - Age ≥ 71



Note: Boxes and horizontal bars denote interquartile range (IQR) and median ID₅₀, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Table 35. Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₈₀ – Age 18-55

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	15	15	15	15	60	41
	GM	10	10	10	10	10	41
	95% CI	NE	NE	NE	NE	NE	26, 66
Day 15 (14 Days Post Vaccination 1)	n	15	15	15	15	60	
	GM	11	11	13	13	12	
	95% CI	9, 13	9, 13	8, 19	10, 18	11, 14	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	15	14	59	
	GM	10	12	10	12	11	
	95% CI	NE	9, 14	10, 11	10, 16	10, 12	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	13	15	15	14	57	
	GM	53	130	134	158	112	
	95% CI	34, 83	88, 192	96, 188	131, 190	93, 136	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	14	55	
	GM	60	160	164	153	126	
	95% CI	37, 96	98, 262	122, 219	122, 193	103, 155	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	13	15	14	14	56	
	GM	52	109	145	123	102	
	95% CI	31, 87	70, 168	102, 205	94, 160	83, 125	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13		15	14	42	
	GM	29		80	83	59	

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	50 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=60)	Convalescent Sera
	95% CI	17, 50		52, 122	58, 119	45, 78	
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable							

Table 36. Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₈₀ – Age 56-70

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GM	10	10	10	10	41
	95% CI	NE	NE	NE	NE	26, 66
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GM	10	10	10	10	
	95% CI	NE	NE	NE	NE	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GM	10	10	10	10	
	95% CI	NE	NE	NE	NE	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	9	29	
	GM	41	50	166	68	
	95% CI	21, 79	25, 99	99, 279	46, 101	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	9	29	
	GM	53	102	204	101	
	95% CI	28, 98	77, 133	146, 284	74, 137	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	9	29	
	GM	42	74	140	74	
	95% CI	21, 85	52, 106	89, 221	54, 103	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		9	19	
	GM	24		70	40	
	95% CI	12, 46		45, 109	25, 62	

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	50 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 37. Pseudovirus Neutralization Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₈₀ – Age ≥ 71

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	10	30	41
	GM	10	10	10	10	41
	95% CI	NE	NE	NE	NE	26, 66
Day 15 (14 Days Post Vaccination 1)	n	10	10	10	30	
	GM	10	10	12	11	
	95% CI	NE	NE	10, 15	10, 11	
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	10	30	
	GM	10	10	11	10	
	95% CI	NE	NE	9, 14	10, 11	
Day 36 Post Vaccination 1 (7 Days Post Vaccination 2)	n	10	10	10	30	
	GM	58	28	140	61	
	95% CI	34, 99	11, 69	82, 239	40, 93	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	10	30	
	GM	61	74	194	96	
	95% CI	34, 110	26, 210	121, 311	63, 146	
Day 57 Post Vaccination 1 (28 Days Post Vaccination 2)	n	10	10	10	30	
	GM	47	55	117	67	
	95% CI	25, 87	19, 158	74, 184	45, 101	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10		10	20	
	GM	15		52	28	
	95% CI	10, 23		32, 87	19, 43	

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	50 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=30)	Convalescent Sera
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Figure 39. Pseudovirus Neutralization Assay Titers by Time Point and Vaccination Group - ID₈₀

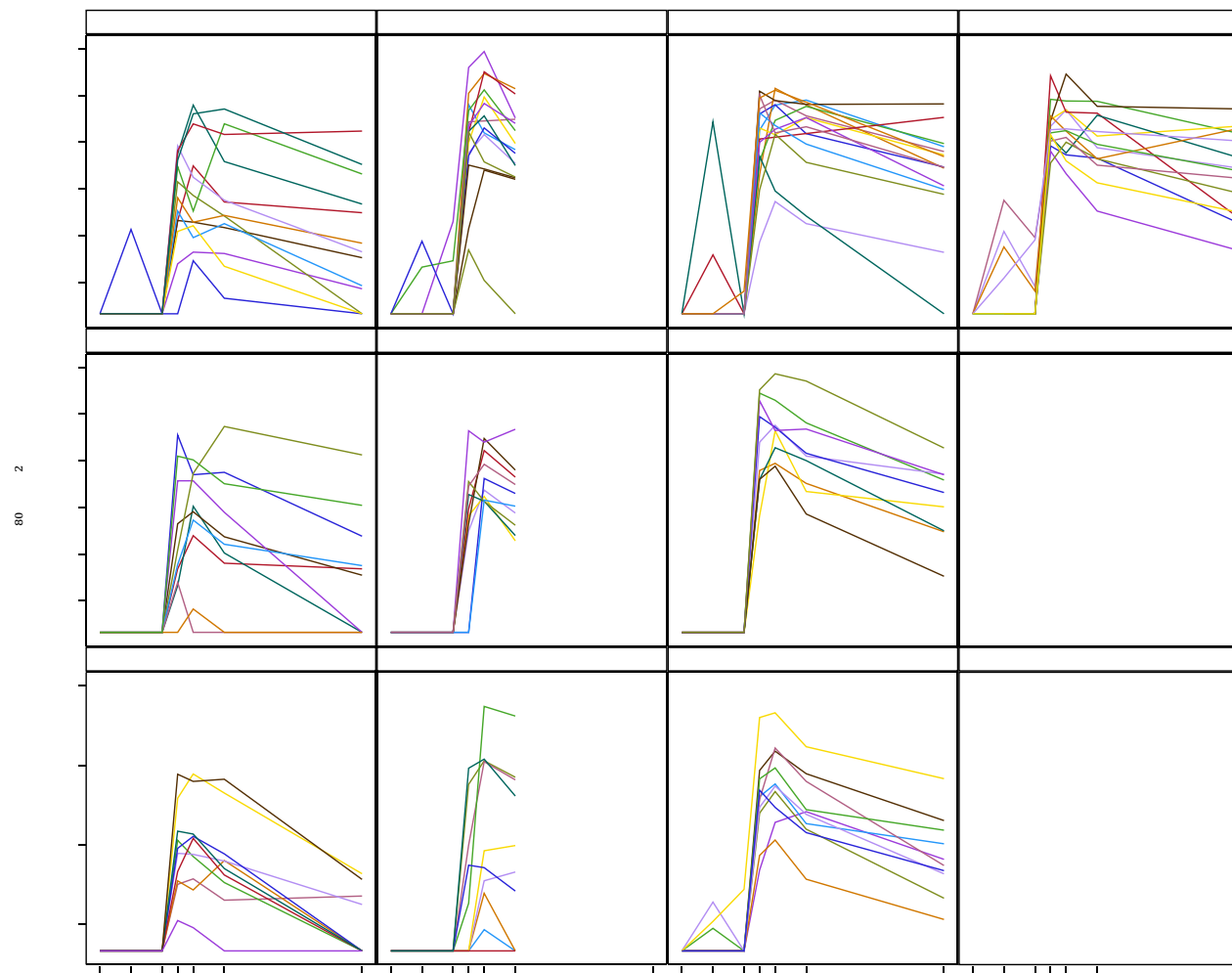
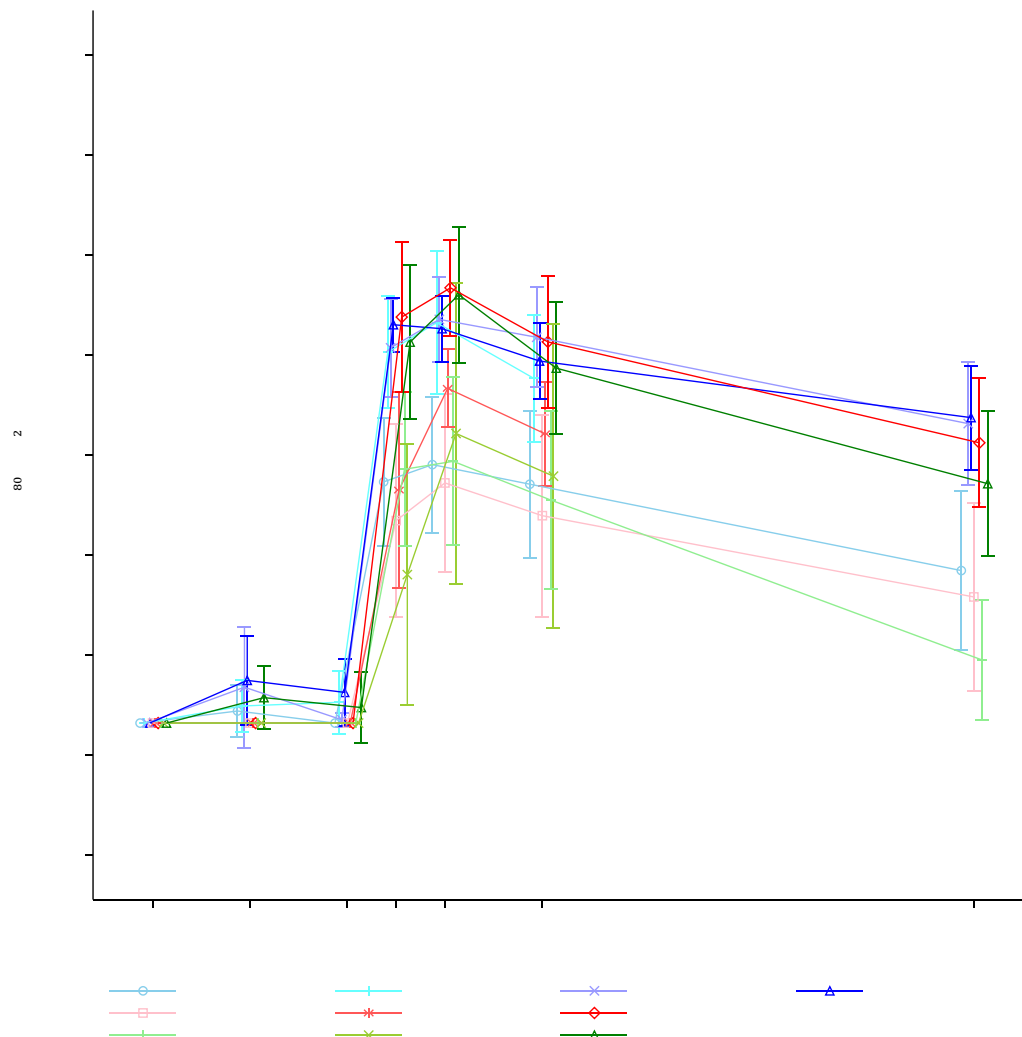
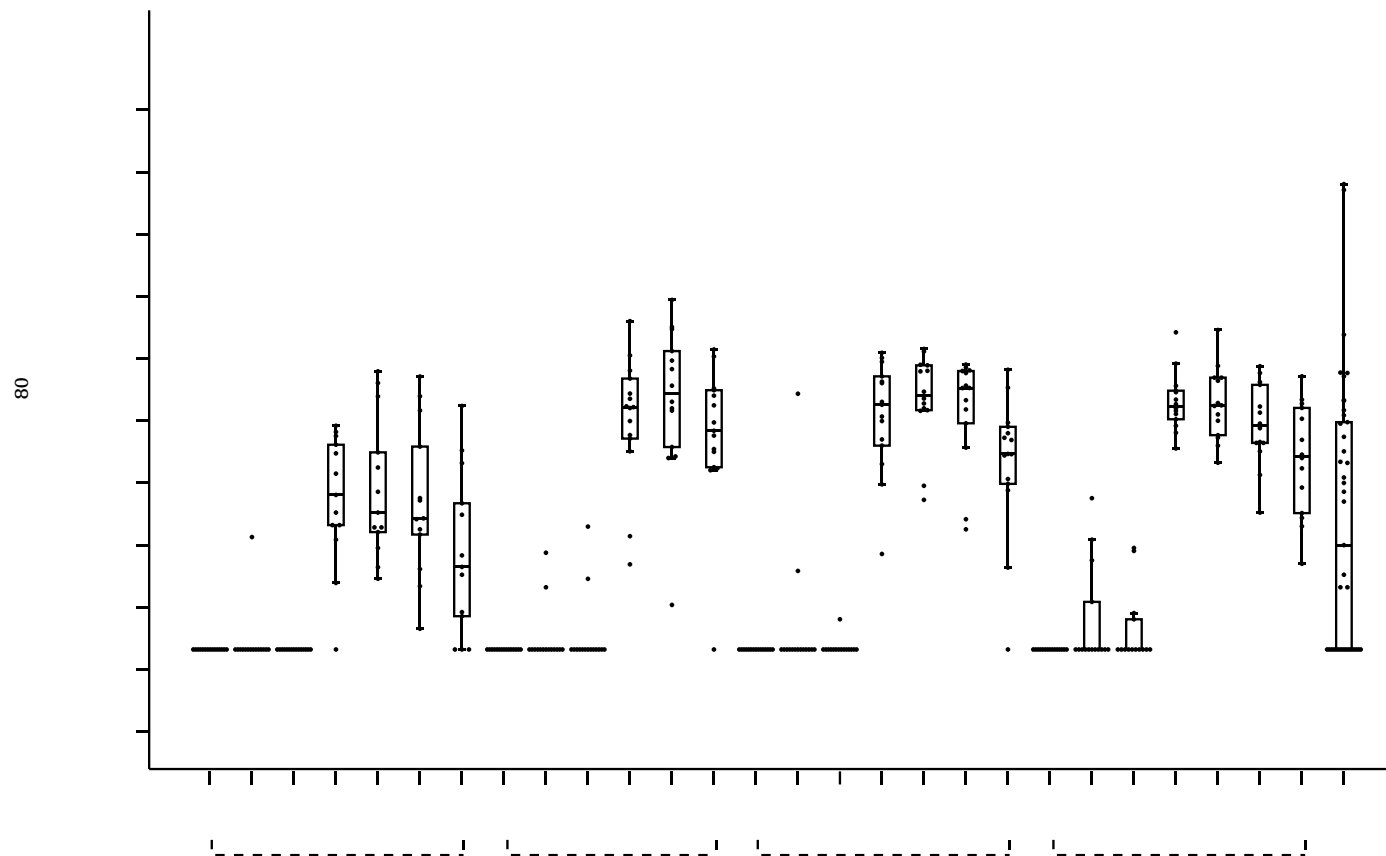


Figure 40. Pseudovirus Neutralization Assay GM by Time Point and Treatment Group - ID₈₀



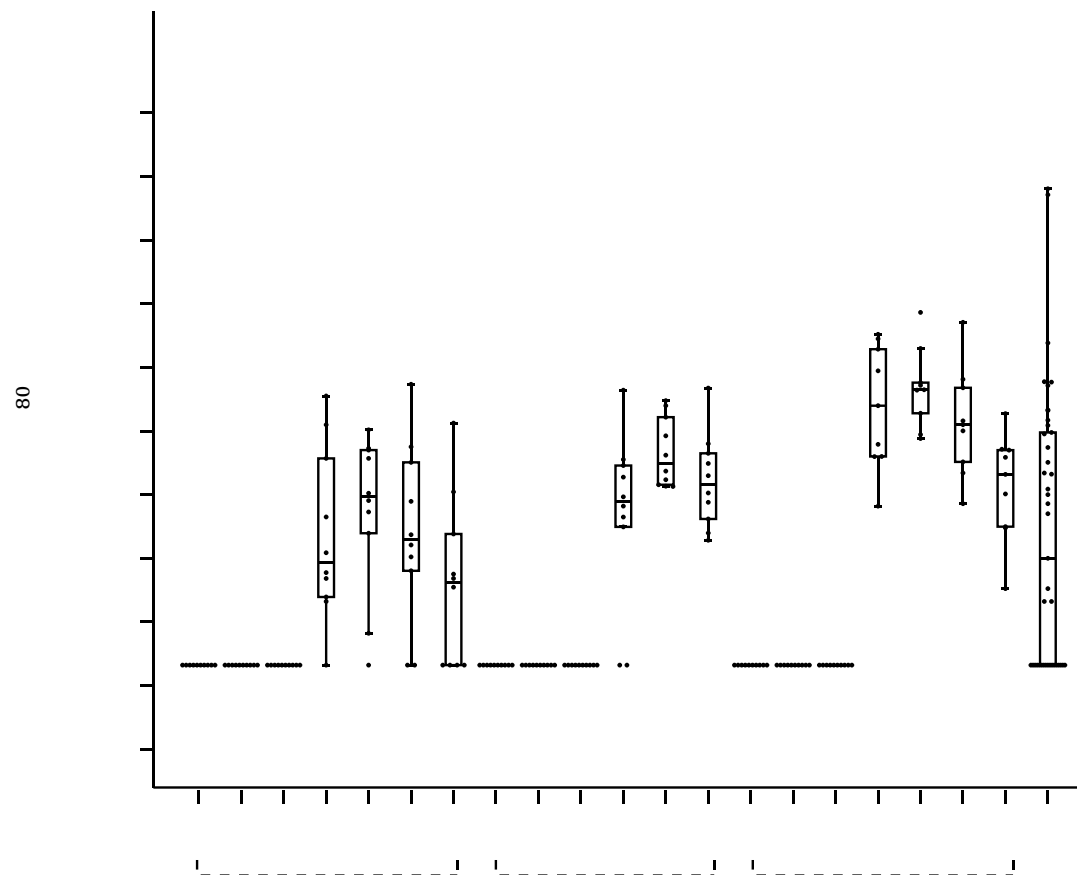
Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 41. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID₈₀ – Age 18-55



Note: Boxes and horizontal bars denote interquartile range (IQR) and median ID₈₀, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

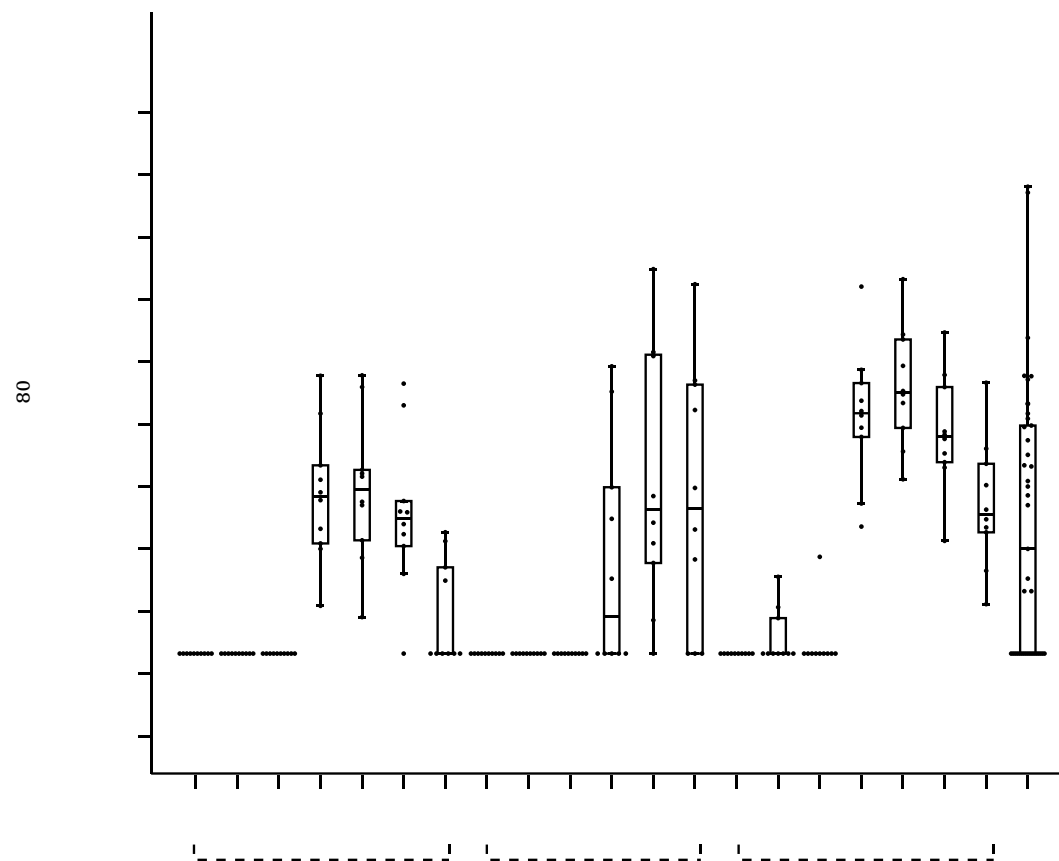
Figure 42. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID₈₀ – Age 56-70



Note: Boxes and horizontal bars denote interquartile range (IQR) and median ID₈₀, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 43. Pseudovirus Neutralization Assay Titers Distribution by Time Point and Treatment Group - ID₈₀ – Age ≥ 71



Note: Boxes and horizontal bars denote interquartile range (IQR) and median ID₈₀, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median $\pm 1.5 \times$ IQR. The convalescent sera panel includes specimens from 41 individuals.

Table 38. Plaque Reduction Neutralization Test Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group – PRNT₈₀ - 18-55 Years

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=30)
Day 1 (Pre-Vaccination 1)	n	15	15	30
	GM	4	4	4
	95% CI	NE	NE	NE
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	27
	GM	340	654	477
	95% CI	184, 627	460, 930	337, 676
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n		15	15
	GM		430	430
	95% CI		277, 667	277, 667
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable				

**Table 39: Plaque Reduction Neutralization Test Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point
and Vaccination Group - PRNT₈₀ – 56-70 Years**

Time Point	Statistic	100 µg mRNA-1273 56-70 years (N=10)
Day 1 (Pre-Vaccination 1)	n	10
	GM	4
	95% CI	NE
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	9
	GM	878
	95% CI	516, 1494
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	9
	GM	269
	95% CI	134, 542
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable		

**Table 40: Plaque Reduction Neutralization Test Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point
and Vaccination Group - PRNT₈₀ – ≥71 Years**

Time Point	Statistic	100 µg mRNA-1273 ≥71 years (N=10)
Day 1 (Pre-Vaccination 1)	n	10
	GM	4
	95% CI	NE
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10
	GM	317
	95% CI	181, 557
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10
	GM	165
	95% CI	82, 332
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable		

Figure 44. Plaque Reduction Neutralization Test Geometric Mean by Time Point and Vaccination Group - PRNT₈₀

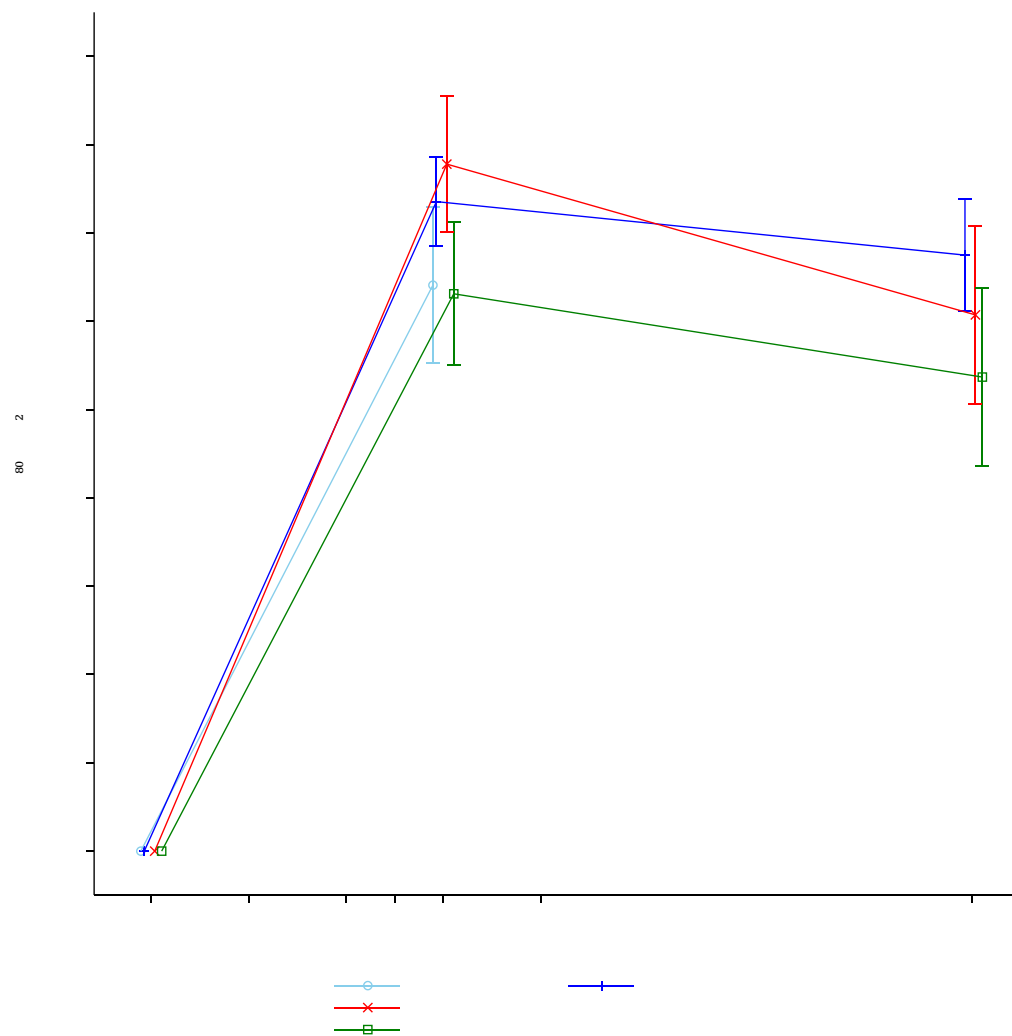


Figure 45: Plaque Reduction Neutralization Test Titers Distribution by Time Point and Treatment Group - PRNT₈₀

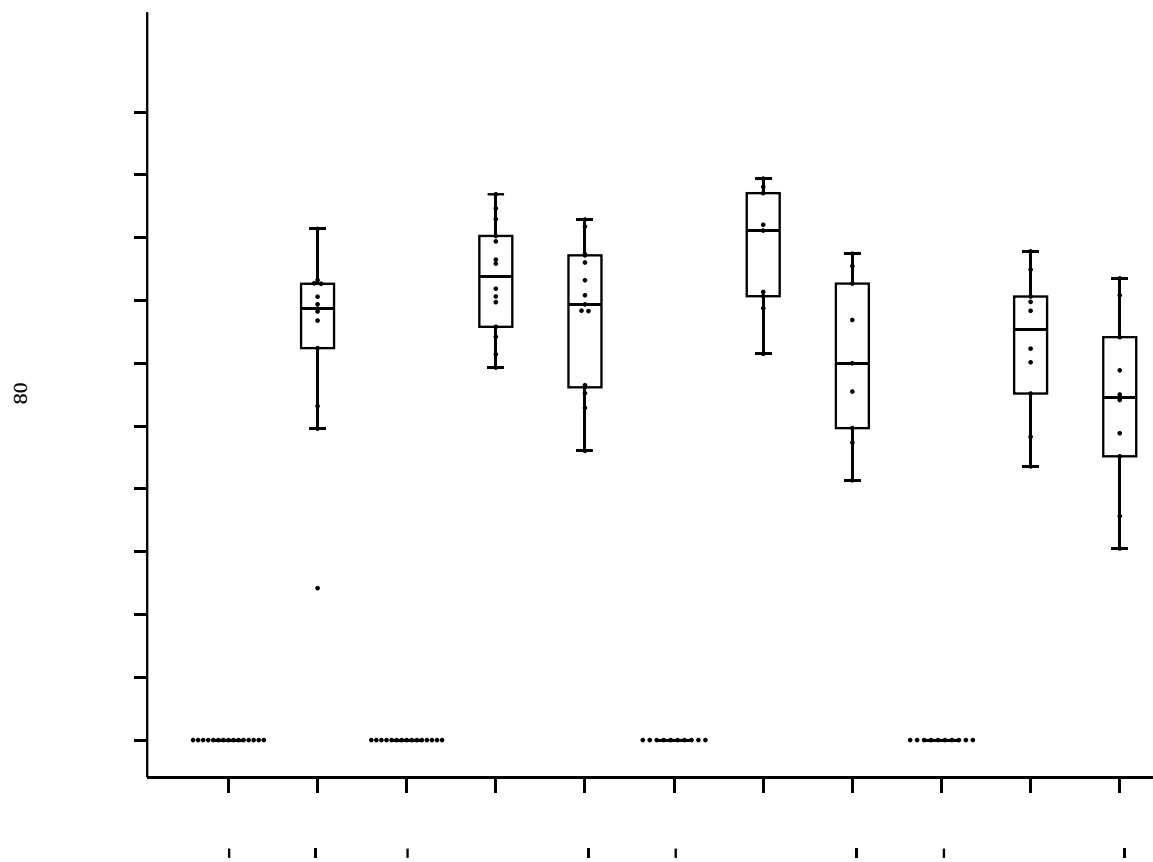


Table 41. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ - 18-55 Years

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=45)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	15	15	15	45	41
	GM	10	10	10	10	129
	95% CI	NE	NE	NE	NE	78, 214
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	14	44	
	GM	23	69	77	49	
	95% CI	13, 39	46, 102	43, 136	36, 67	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	41	
	GM	622	1388	1575	1123	
	95% CI	374, 1034	1056, 1825	1206, 2058	894, 1412	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13	15	14	42	
	GM	309	775	599	535	
	95% CI	184, 517	560, 1071	437, 821	422, 677	
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

Table 42. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ – 56-70 Years

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=20)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	20	41
	GM	10	10	10	129
	95% CI	NE	NE	NE	78, 214
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	20	
	GM	19	80	39	
	95% CI	11, 32	52, 123	24, 62	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	9	19	
	GM	550	1425	863	
	95% CI	302, 1001	980, 2072	578, 1290	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10	9	19	
	GM	227	685	383	
	95% CI	131, 392	436, 1077	251, 584	
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable					

Table 43. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ ≥ 71 Years

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=20)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	20	41
	GM	10	10	10	129
	95% CI	NE	NE	NE	78, 214
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	20	
	GM	18	39	27	
	95% CI	12, 29	18, 86	17, 42	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	20	
	GM	448	900	635	
	95% CI	299, 672	575, 1409	461, 874	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10	10	20	
	GM	183	552	318	
	95% CI	123, 272	321, 947	213, 475	
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable					

Figure 46: FRNT-mNG Geometric Mean by Time Point and Vaccination Group - ID₅₀

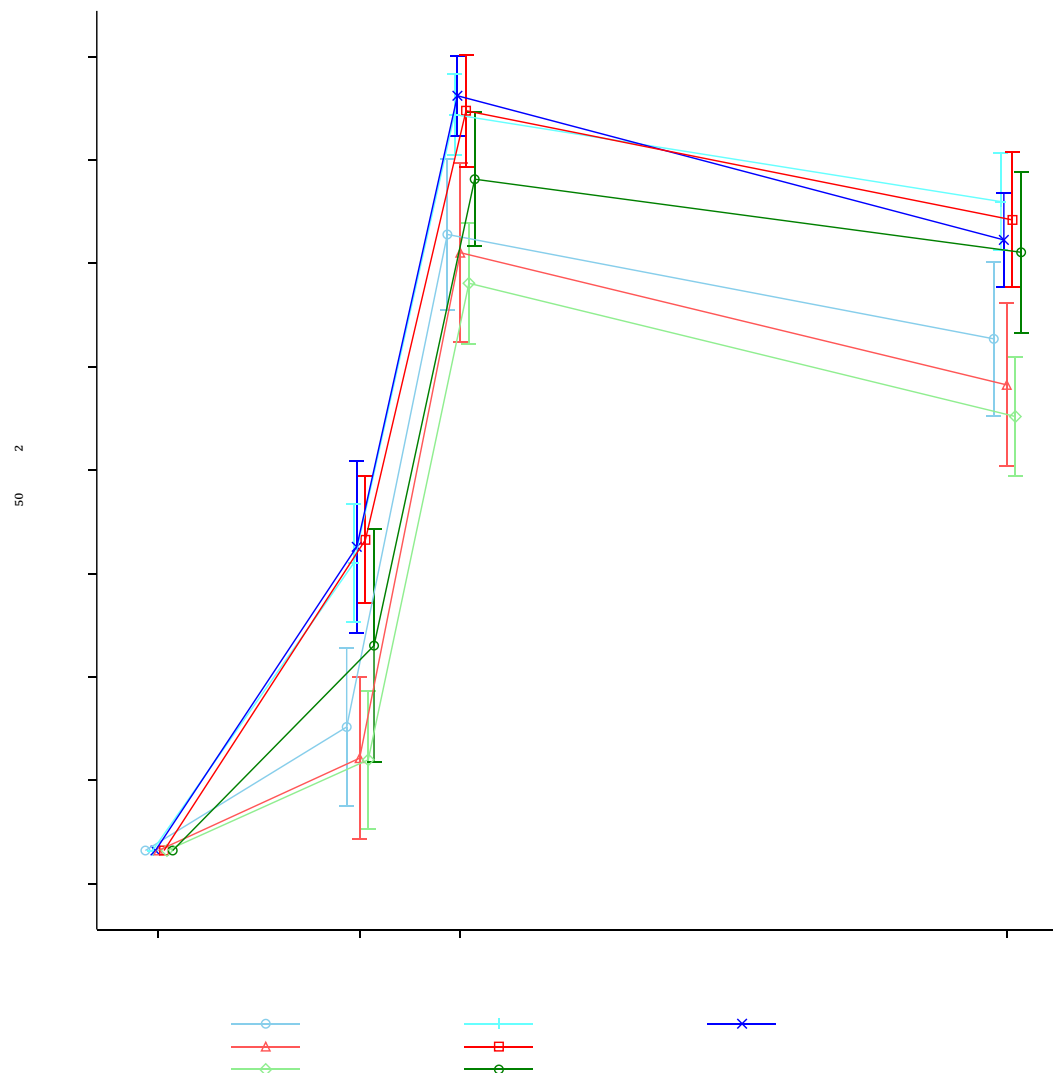


Figure 47: FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID₅₀ - 18-55 Years

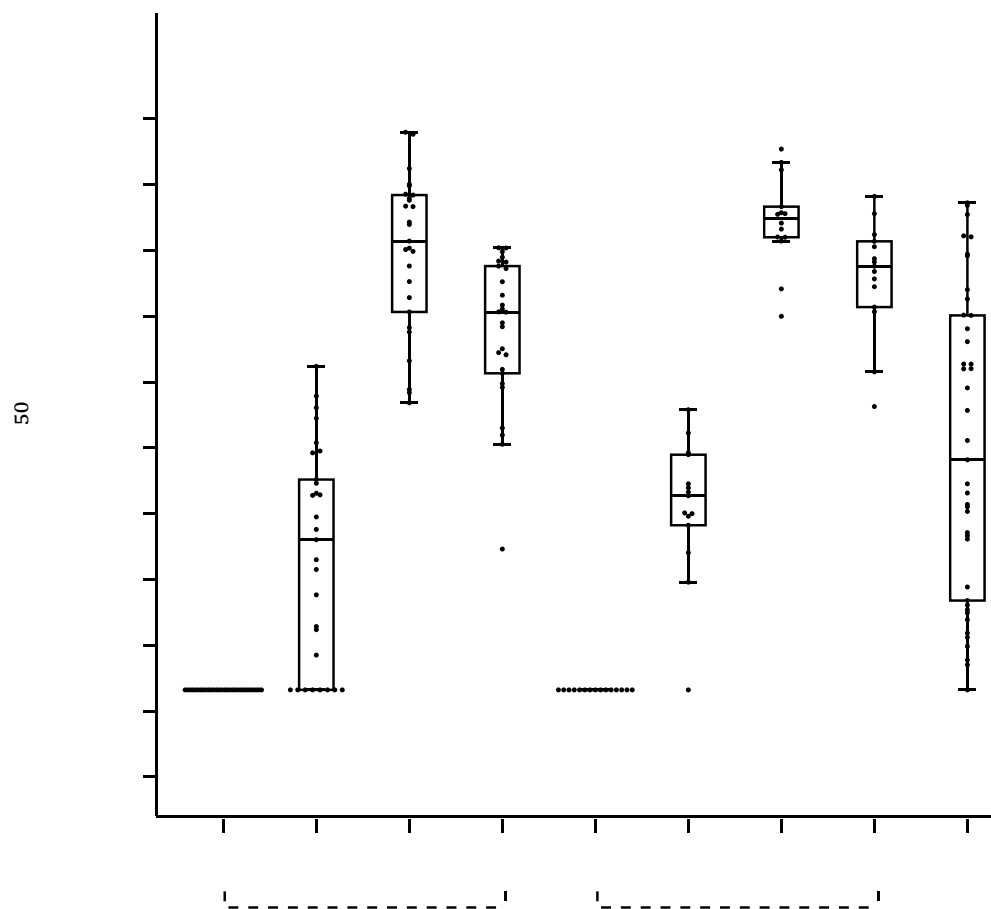


Figure 48. FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID₅₀ – 56-70 Years

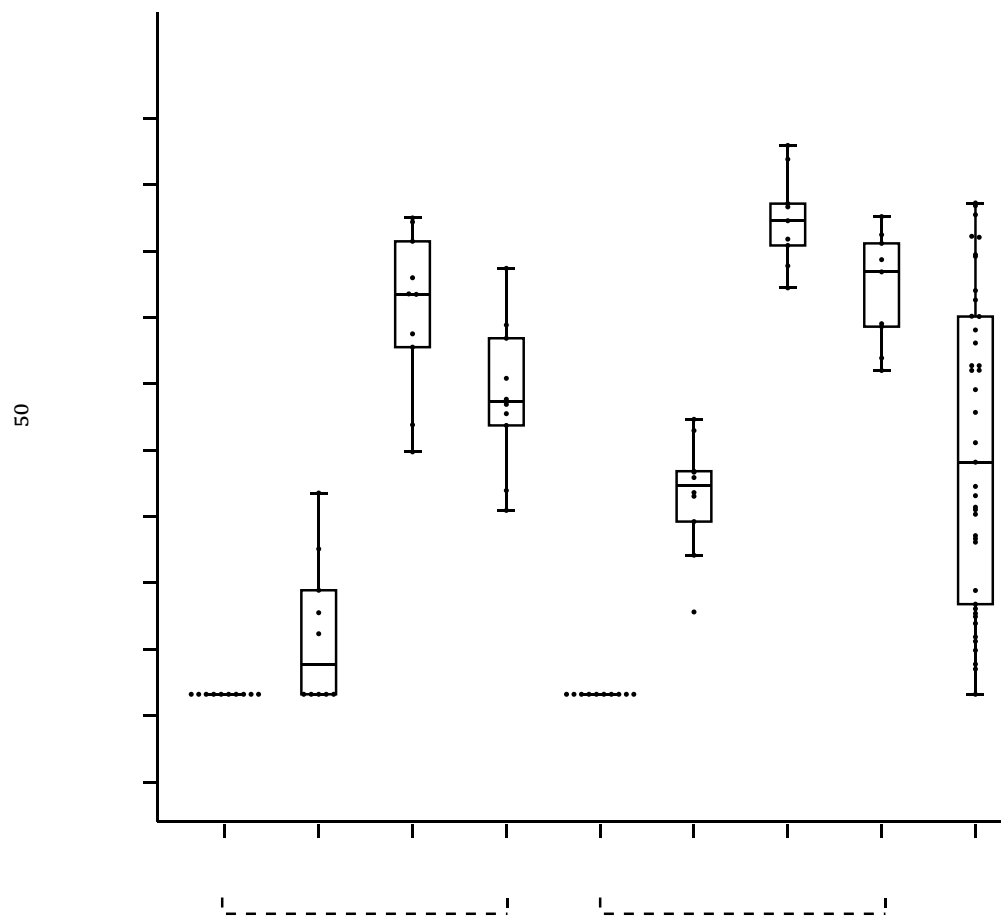
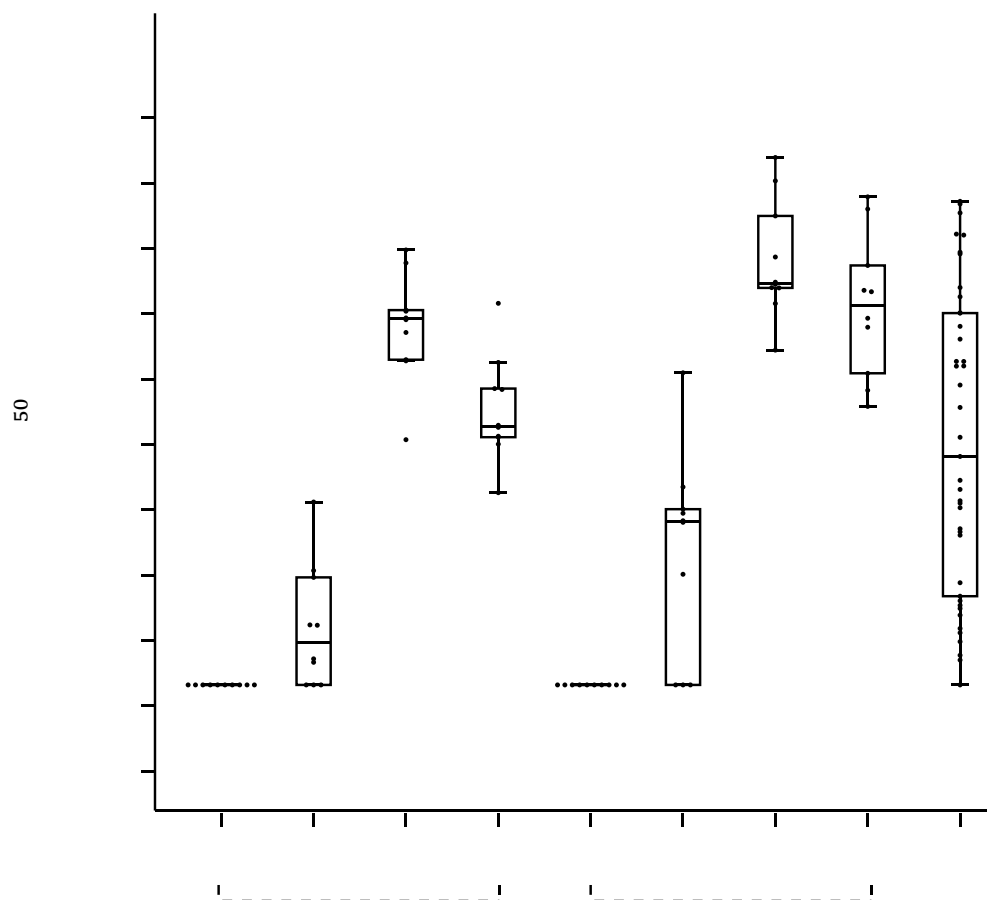


Figure 49. FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID₅₀ – ≥ 71 Years



**Table 44. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals
by Time Point and Vaccination Group – ID₈₀ – Age 18-55**

Time Point	Statistic	25 µg mRNA-1273 18-55 years (N=15)	100 µg mRNA-1273 18-55 years (N=15)	250 µg mRNA-1273 18-55 years (N=15)	18-55 years (N=45)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	15	15	15	45	41
	GM	10	10	10	10	37
	95% CI	NE	NE	NE	NE	24, 57
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	15	15	14	44	
	GM	12	25	20	18	
	95% CI	10, 15	18, 33	13, 32	15, 22	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	13	14	14	41	
	GM	234	525	487	396	
	95% CI	144, 381	416, 663	382, 619	322, 488	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	13	15	14	42	
	GM	89	237	196	164	
	95% CI	49, 160	170, 332	143, 270	127, 212	
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable						

**Table 45. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals
by Time Point and Vaccination Group – ID₈₀ — Age 56-70**

Time Point	Statistic	25 µg mRNA-1273 56-70 years (N=10)	100 µg mRNA-1273 56-70 years (N=10)	56-70 years (N=20)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	20	41
	GM	10	10	10	37
	95% CI	NE	NE	NE	24, 57
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	20	
	GM	10	17	13	
	95% CI	NE	11, 25	10, 16	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	9	19	
	GM	167	583	302	
	95% CI	101, 277	400, 851	198, 461	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10	9	19	
	GM	71	233	124	
	95% CI	34, 148	145, 375	75, 205	
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable					

**Table 46. FRNT-mNG Geometric Mean (GM) Results with 95% Confidence Intervals
by Time Point and Vaccination Group – ID₈₀ — Age ≥71**

Time Point	Statistic	25 µg mRNA-1273 ≥71 years (N=10)	100 µg mRNA-1273 ≥71 years (N=10)	≥71 years (N=20)	Convalescent Sera
Day 1 (Pre-Vaccination 1)	n	10	10	20	41
	GM	10	10	10	37
	95% CI	NE	NE	NE	24, 57
Day 29 Post Vaccination 1 (Pre-Vaccination 2)	n	10	10	20	
	GM	11	15	13	
	95% CI	9, 14	9, 25	10, 17	
Day 43 Post Vaccination 1 (14 Days Post Vaccination 2)	n	10	10	20	
	GM	169	392	258	
	95% CI	107, 268	252, 609	182, 365	
Day 119 Post Vaccination 1 (90 Days Post Vaccination 2)	n	10	10	20	
	GM	52	190	99	
	95% CI	30, 90	115, 317	63, 158	
Note: N=Number of Subjects. n=Number of subjects with results available at time point. NE=Not Estimable					

Figure 50. FRNT-mNG Geometric Mean by Time Point and Vaccination Group - ID₈₀

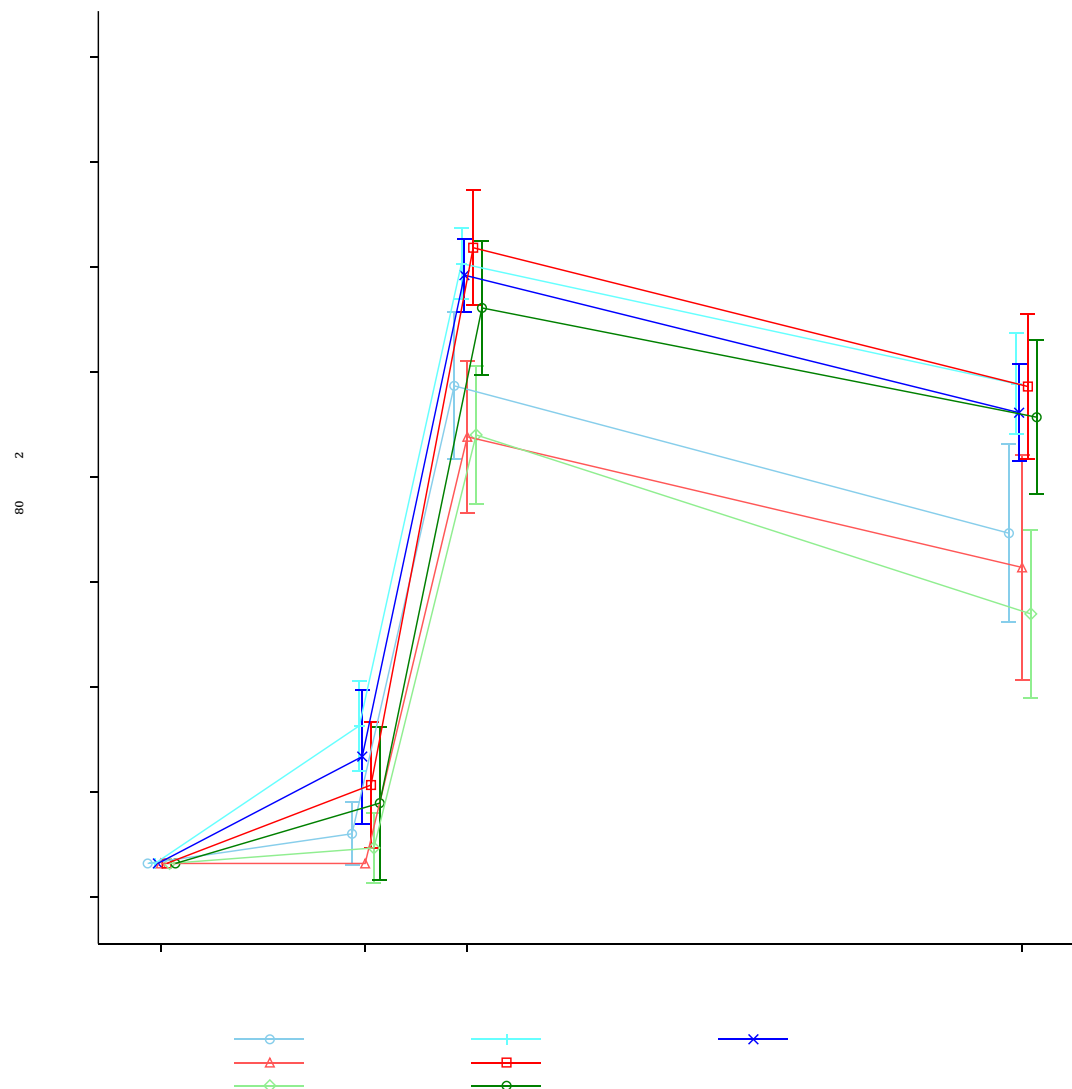


Figure 51. FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID₈₀ – Age 18-55

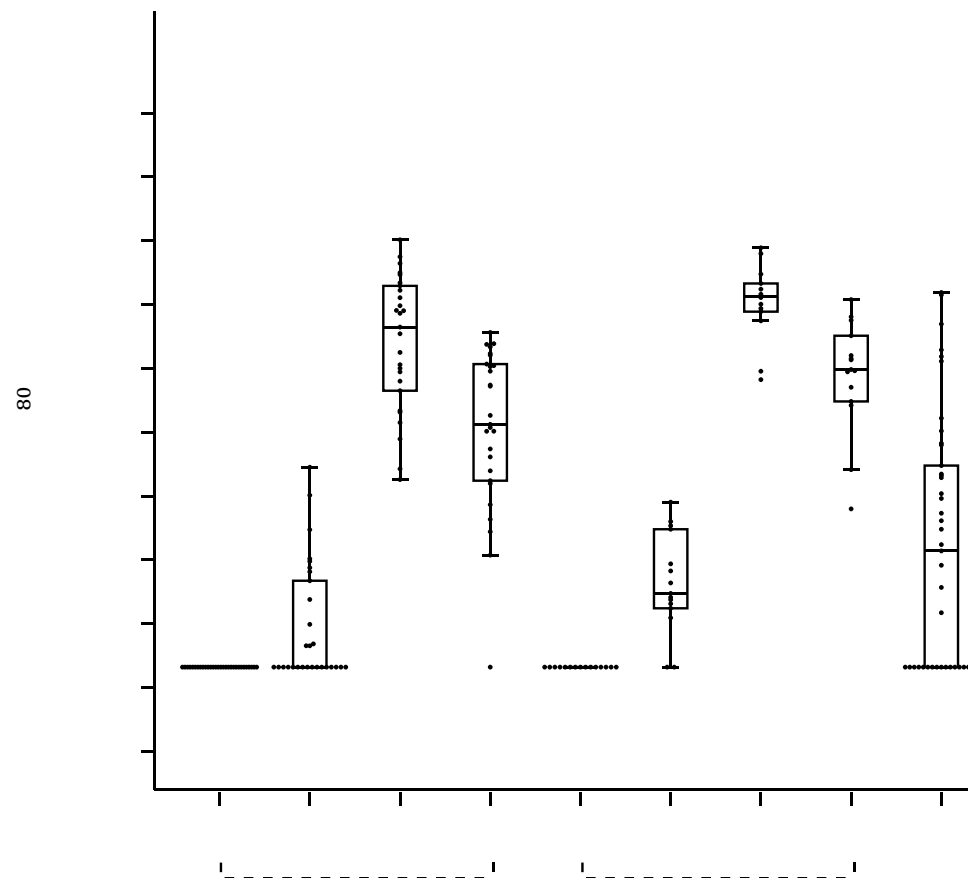


Figure 52: FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID₈₀ – Age 56-70

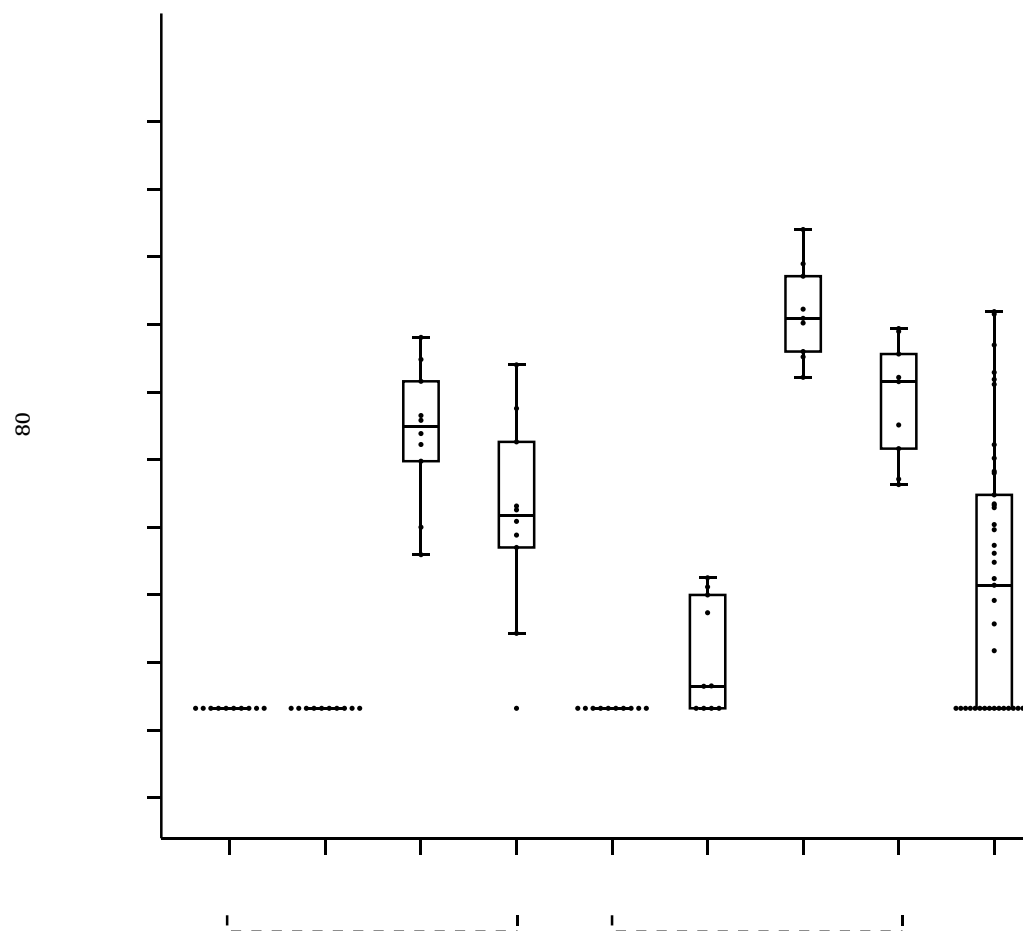


Figure 53: FRNT-mNG Titers Distribution by Time Point and Treatment Group - ID₈₀ – Age ≥ 71

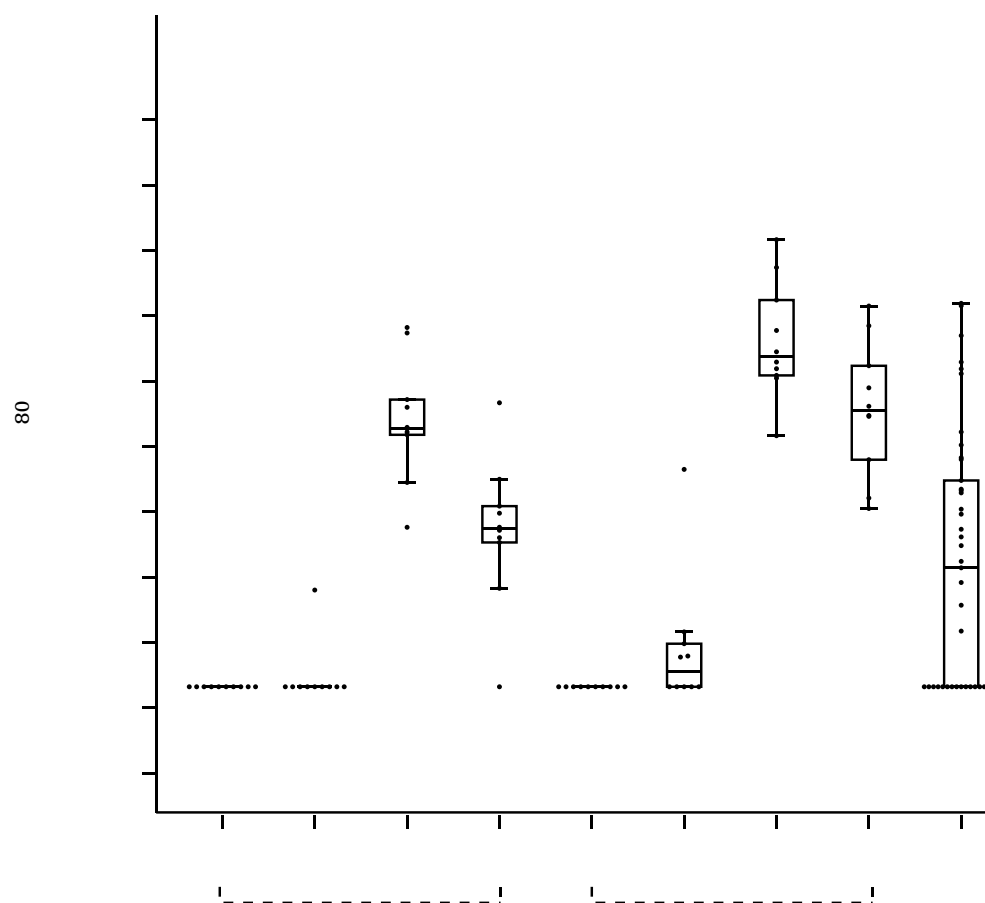
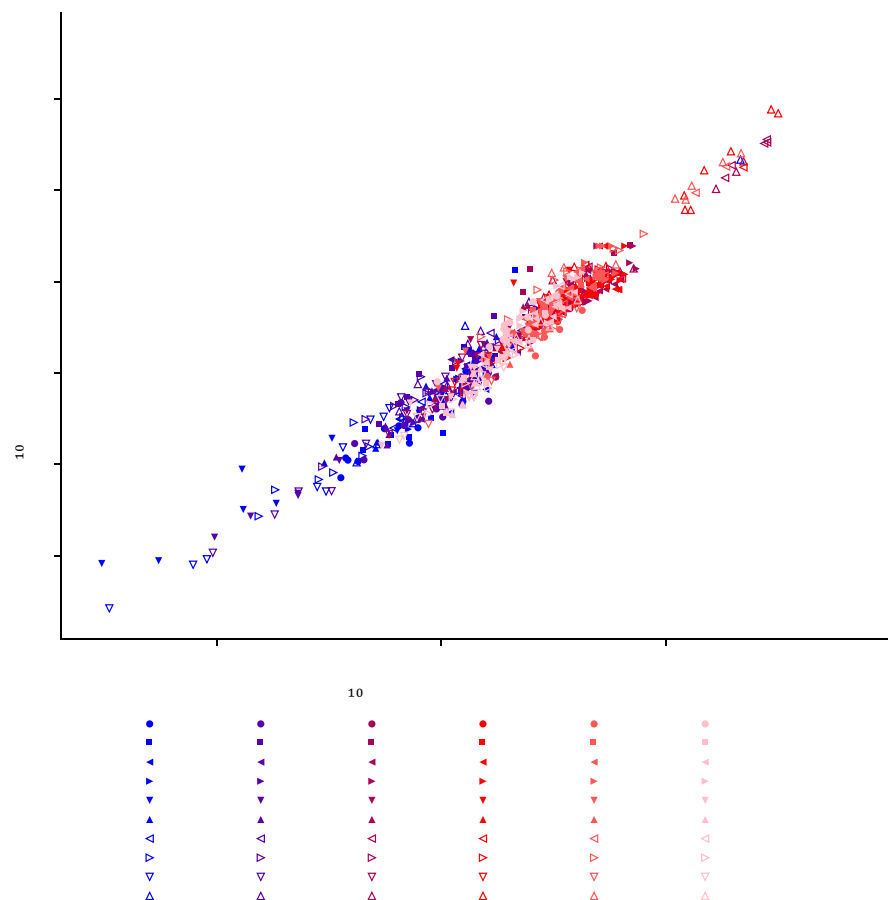


Figure 54. Binding to SARS-CoV-2 Spike Proteins in ELISA Expressed as Area-Under-the-Curve (AUC) is Highly Correlated with Binding Expressed as Endpoint Dilution Titer

A, vaccinee sera binding to S-2P expressed as endpoint vs AUC. **B**, vaccinee sera binding to RBD expressed as endpoint vs AUC.
C, convalescent sera binding to S-2P expressed as endpoint vs AUC. **D**, convalescent sera binding to RBD expressed as endpoint vs AUC.

A



B

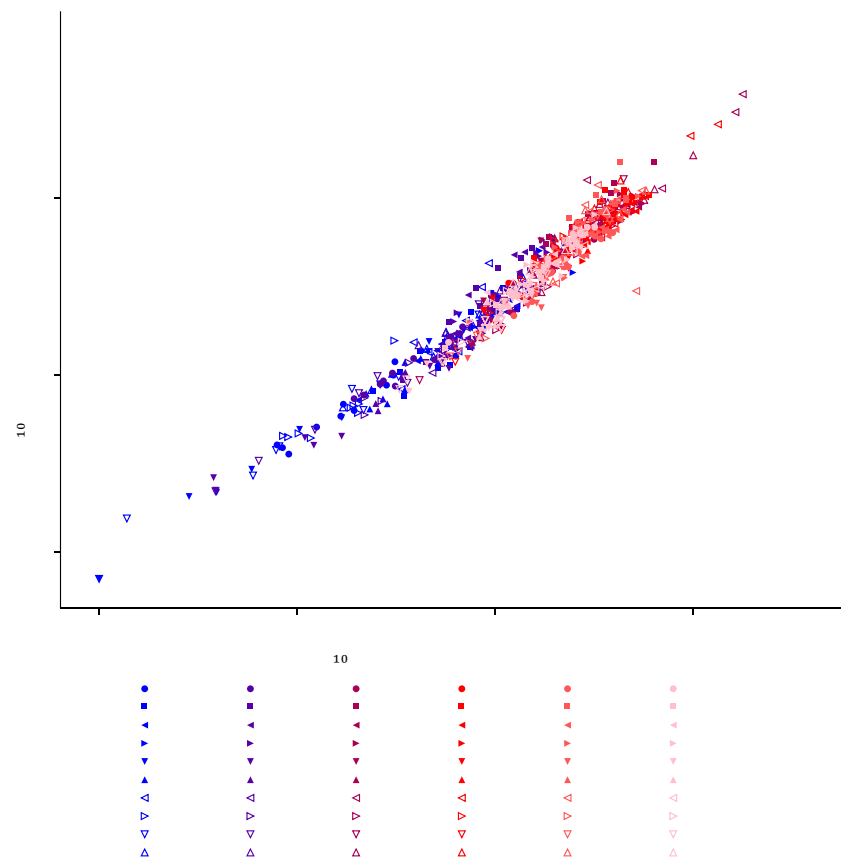
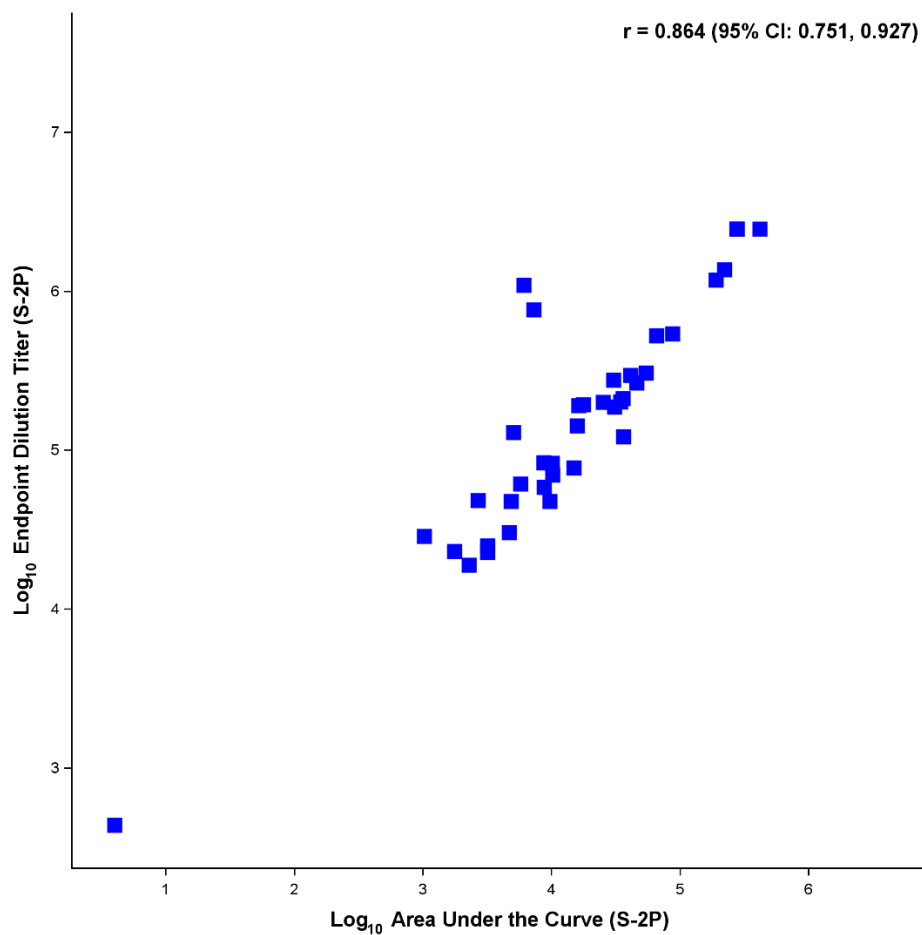


Figure 54 (continued)

C



D

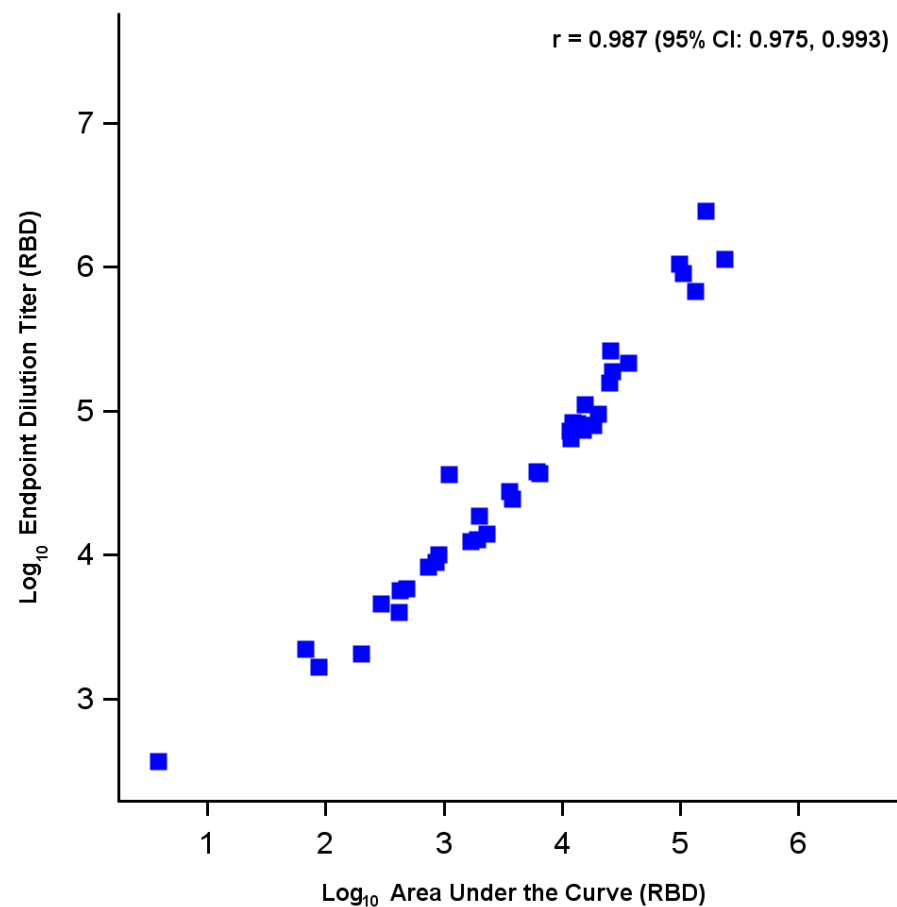
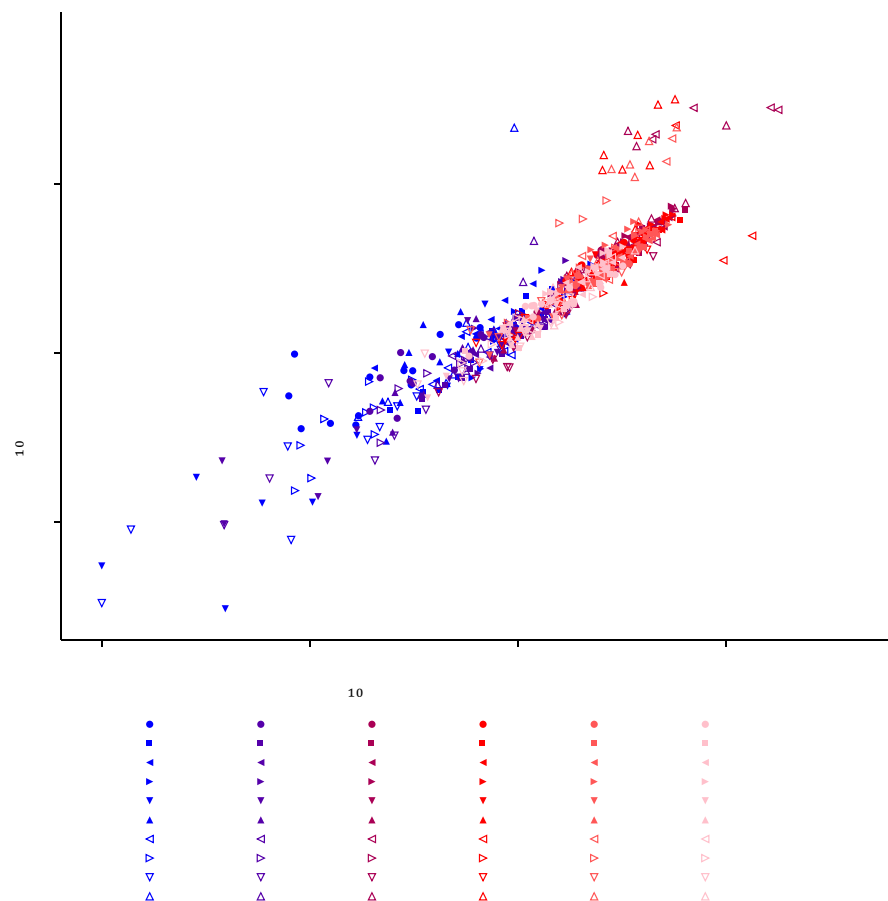


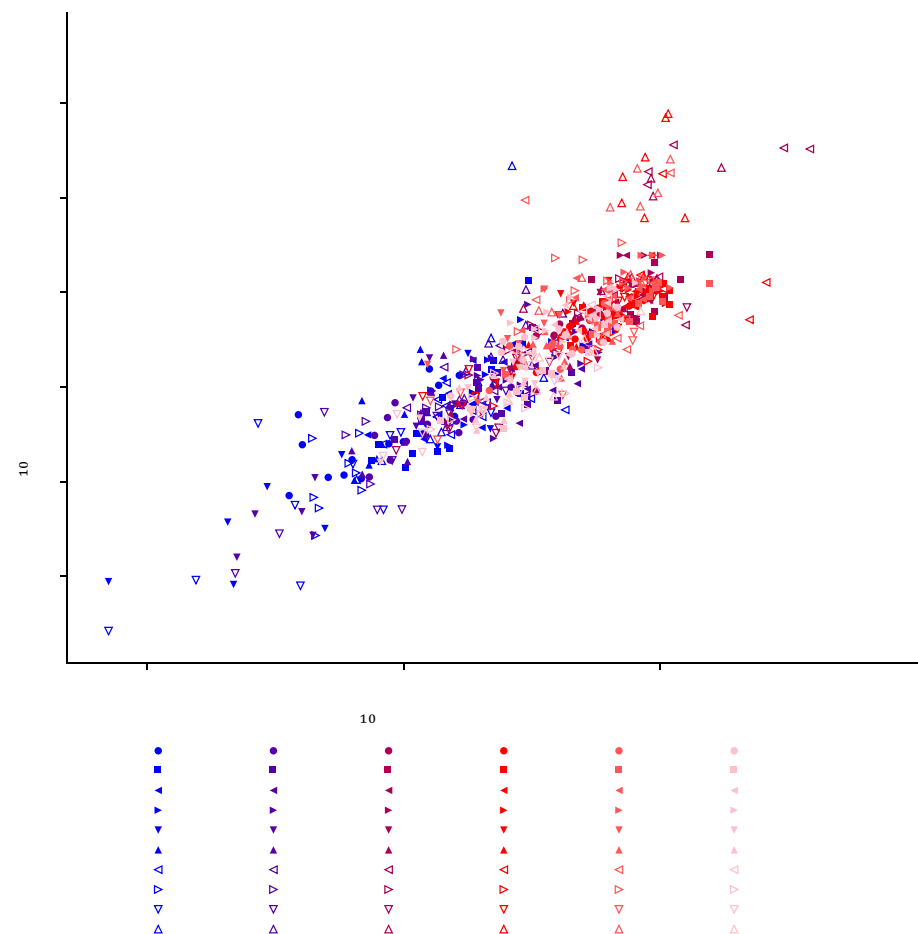
Figure 55. Binding to S-2P or RBD Proteins are Highly Correlated

A, vaccinee sera binding to S-2P vs. RBD, expressed as area under the curve (AUC). **B**, vaccinee sera binding to S-2P vs. RBD, expressed as endpoint dilution titer. **C**, convalescent sera binding to S-2P vs. RBD, expressed as AUC. **D**, convalescent sera binding to S-2P vs. RBD, expressed as endpoint dilution titer.

A



B



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 55 (continued)

C

D

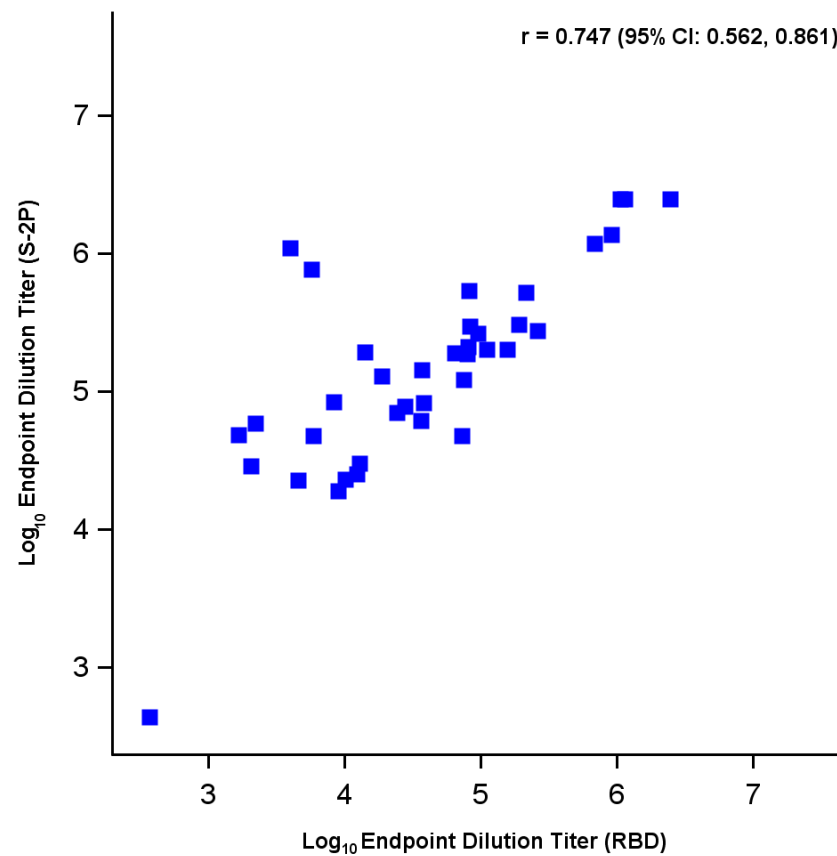
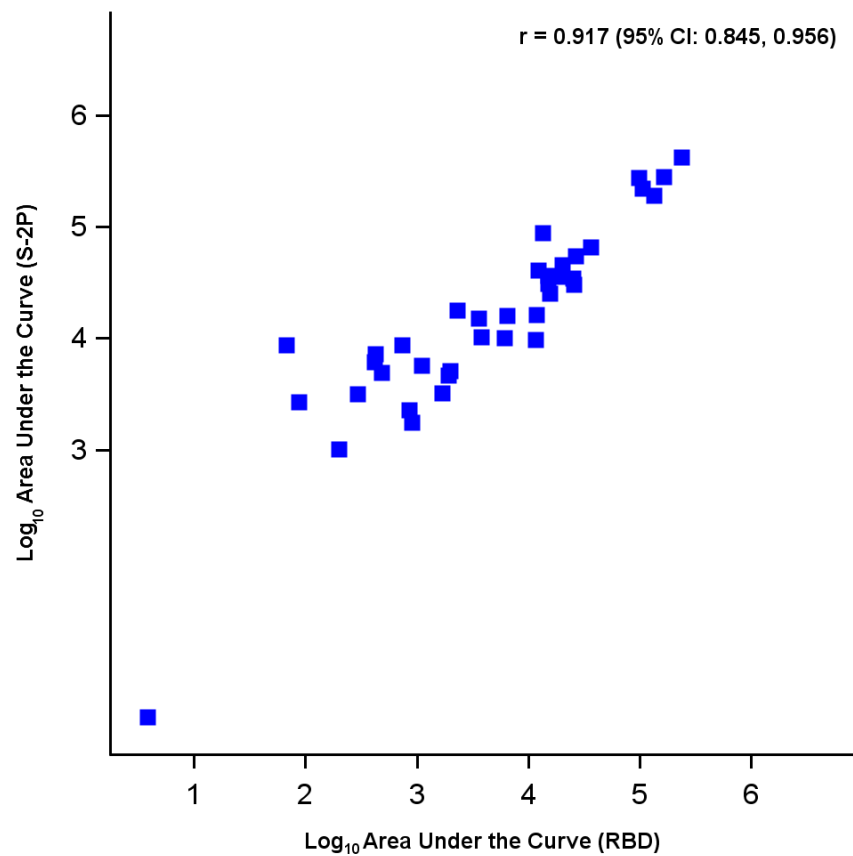
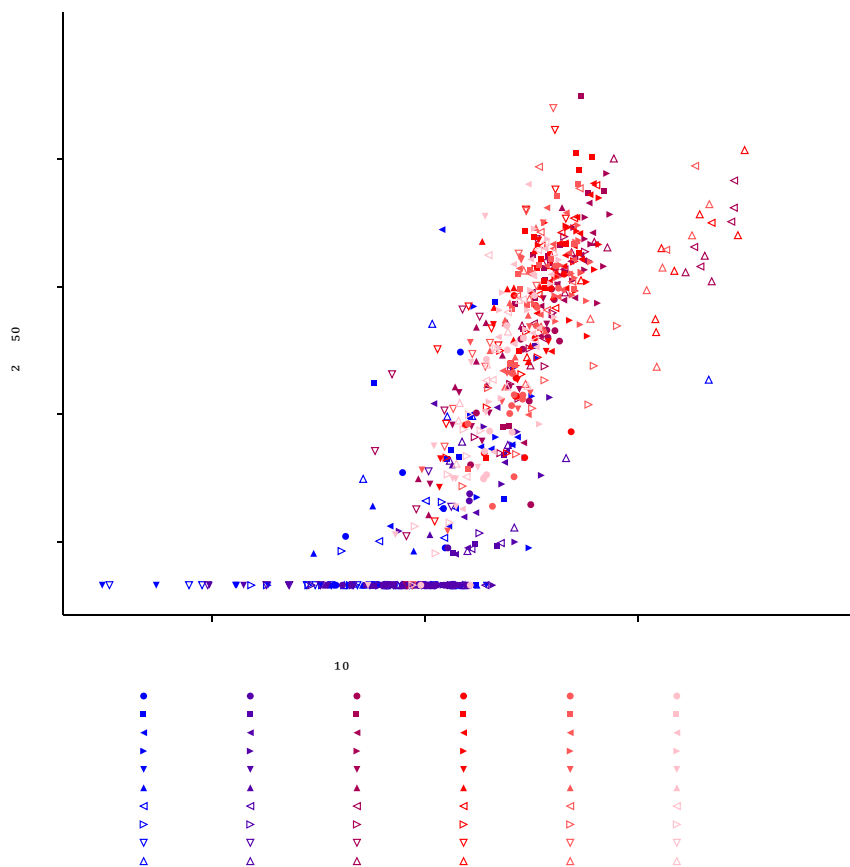


Figure 56. Pseudovirus Neutralization Correlates with Binding in ELISA

A, PsVNA vaccinee sera ID₅₀ vs. AUC S2P. **B**, PsVNA vaccinee sera ID₈₀ vs. AUC S2P. **C**, PsVNA convalescent sera ID₅₀ vs. AUC S2P. **D**, PsVNA convalescent sera ID₈₀ vs. AUC S2P

A



B

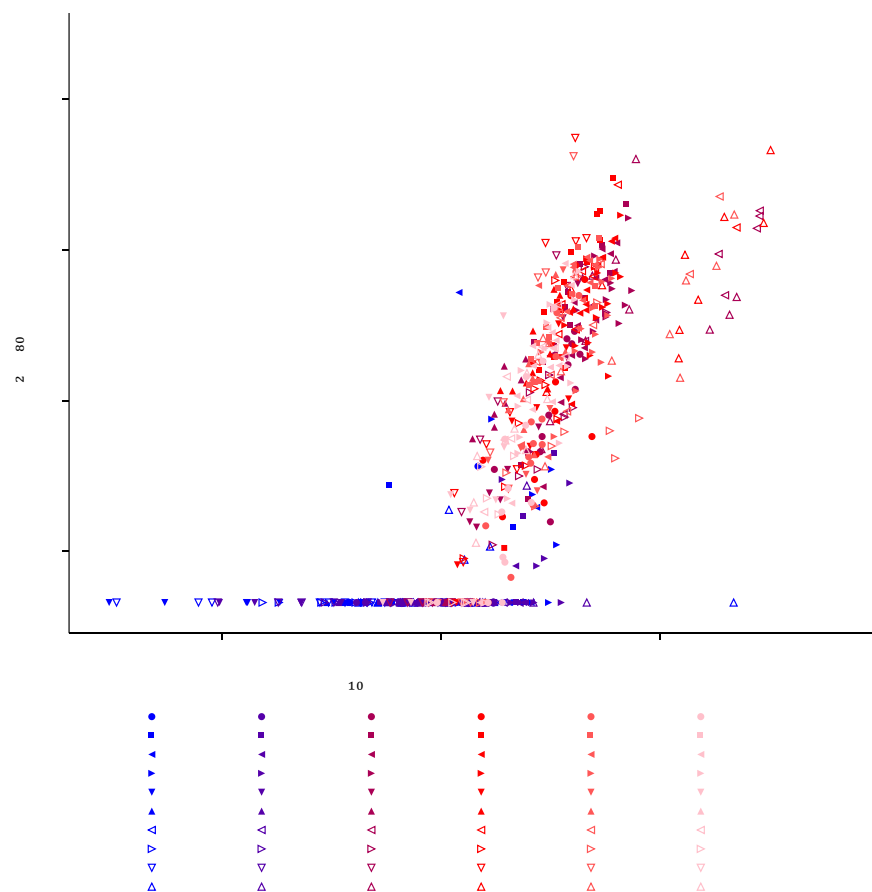
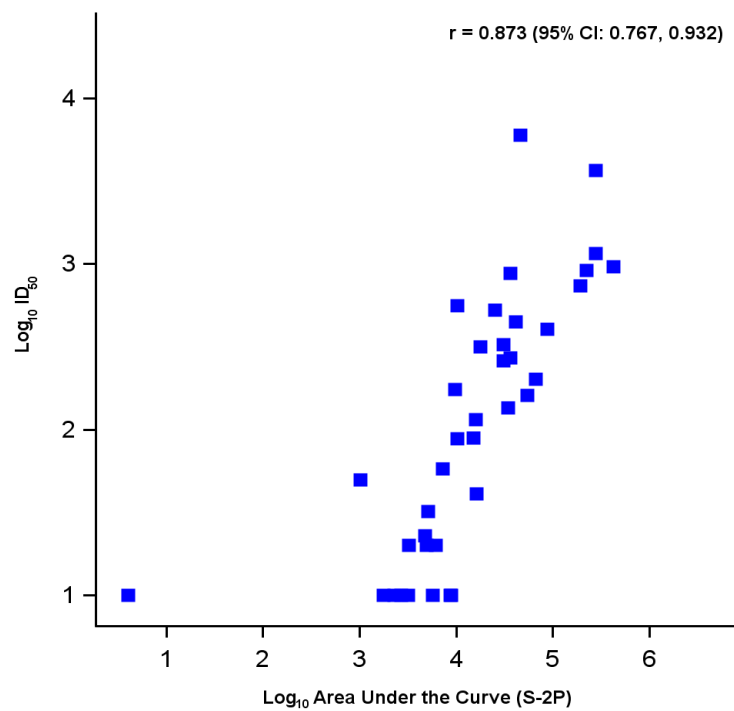


Figure 56 (continued)
D

C



D

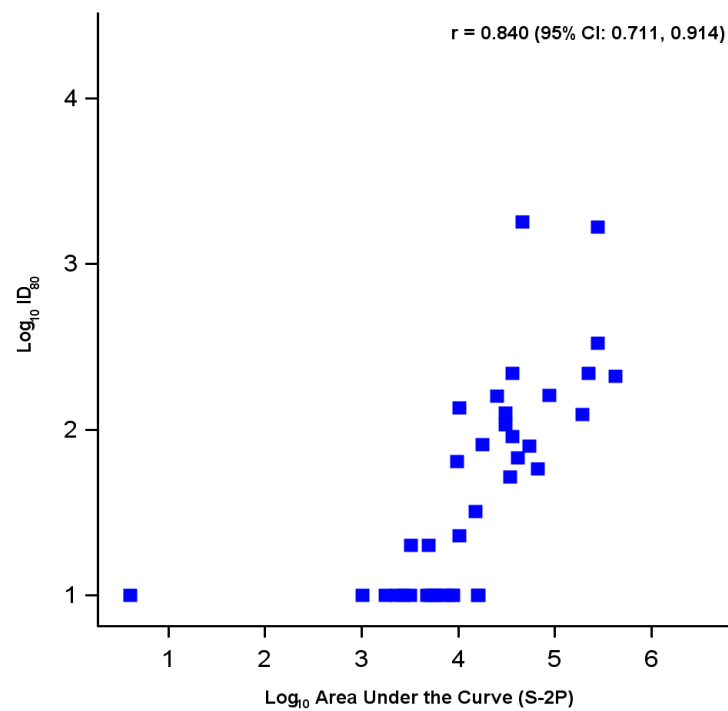
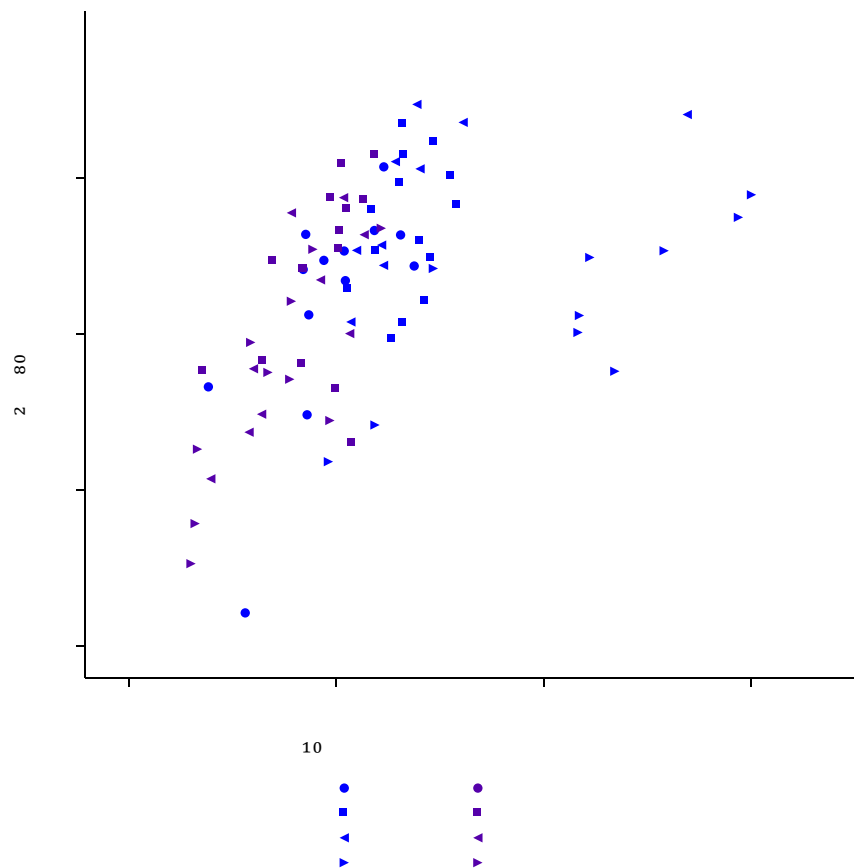


Figure 57. Live-Virus Neutralization (PRNT₈₀) Correlates with Binding in ELISA

A, PRNT₈₀ vs. S-2P binding (AUC) . **B**, PRNT₈₀ vs. RBD binding (AUC).

A



B

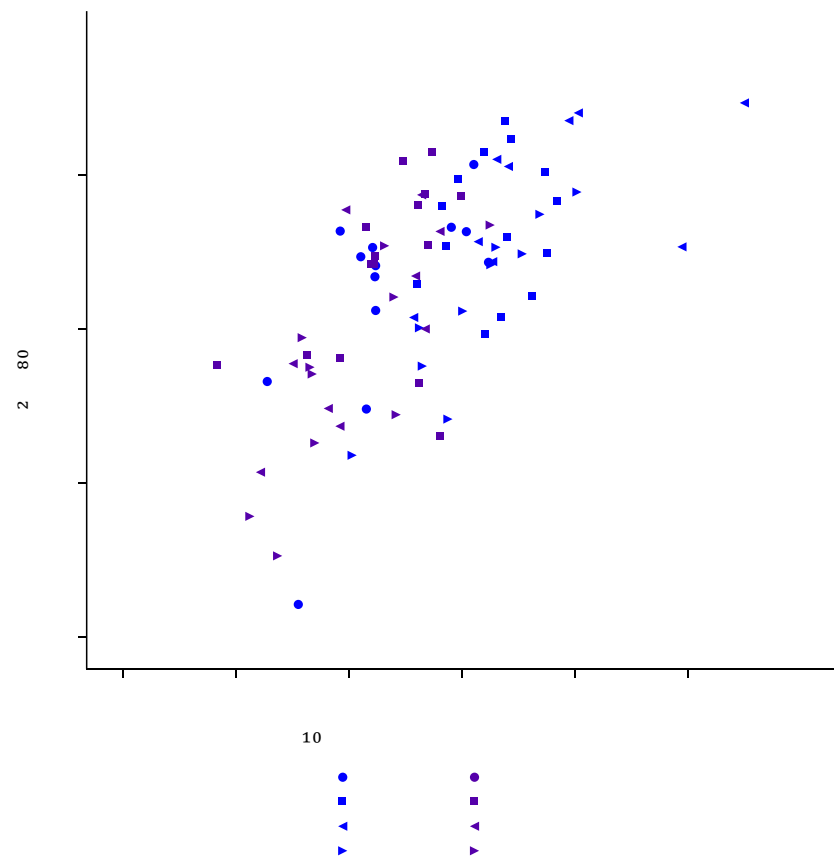
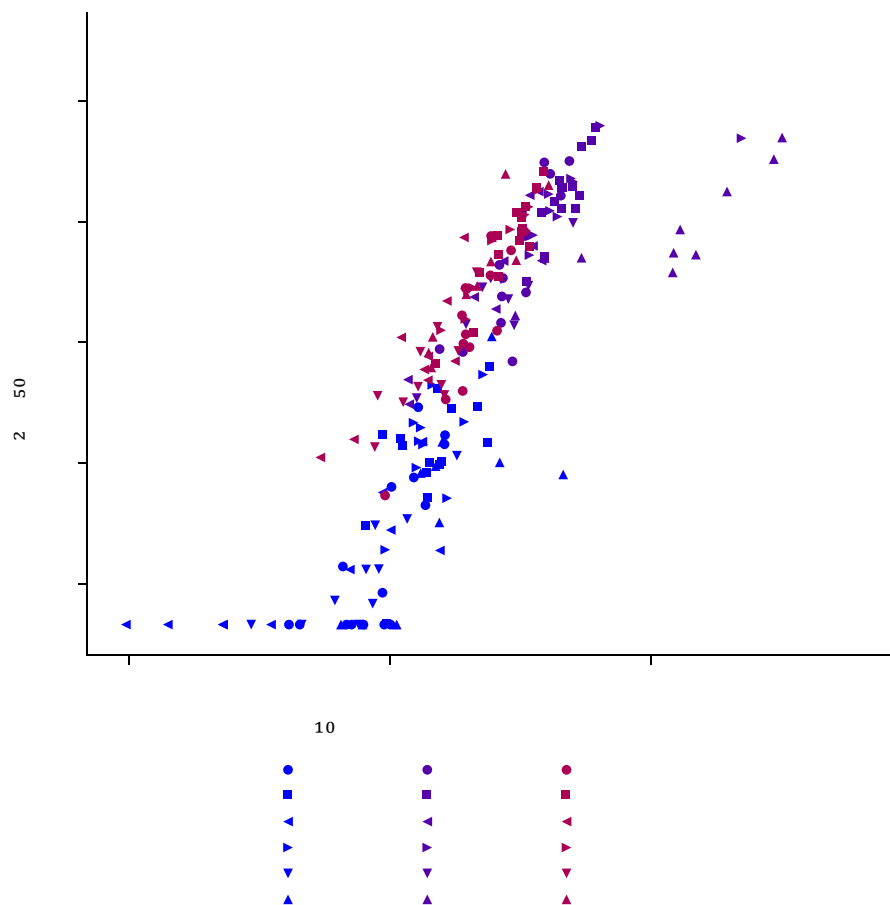


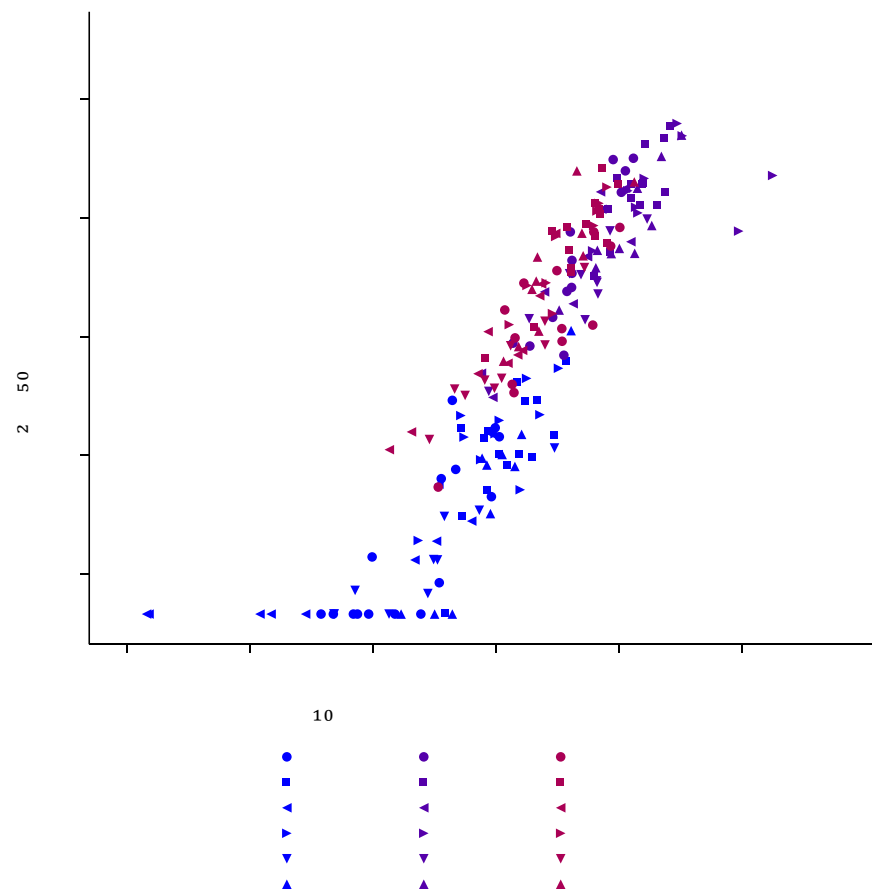
Figure 58. FRNT-mNG Correlates with Binding in ELISA

A, FRNT50 vs. S-2P binding (AUC) ; **B**, FRNT50 vs. RBD binding (AUC).

A



B



Report Date: 29 October 2020
Data Cutoff Date: 15 October 2020

Figure 59. Correlation Heatmap

