Clinical Pharmacology Studies Summary

Summary of Biomarker Assays used in mRNA-1273 Program

Version 1.0

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LIST OF ABBREVIATIONS

Abbreviation	Definition
Ab	Antibody
ACE2	Angiotensin-converting enzyme 2
AU/mL	Absorbance units per milliliter
BAb	Binding antibody
BAL	Bronchoalveolar lavage
cDNA	Complementary DNA
CI	confidence interval
COI	Cutoff index
CoVs	Coronaviruses
COVID-19	coronavirus disease 2019
DMEM	Dulbecco's modified Eagle's medium
DSMO	Dimethyl sulfoxide
EUA	Emergency Use Authorization
ECLIA	Electrochemiluminescence immunoassay
ELISA	Enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
FRNT	focus reduction neutralization test
GCLP	Good Clinical Laboratory Practice
GMP	Good Manufacturing Practice
HRP	Horseradish peroxidase
ICS	Intracellular cytokine stimulation
ID_{50}	50 % inhibitory dose
ID_{80}	80 % inhibitory dose
IgG	Immunoglobulin G
Live MN	Live virus microneutralization
LNP	lipid nanoparticle
MN	microneutralization
mRNA	messenger RNA
MSD	MesoScale Discovery
N	Nucleocapsid
nAb	neutralizing antibody(ies)
NGS	Nextgeneration sequencing
NIH	National Institutes of Health
OWS	Operation Warp Speed
PBMC	Peripheral blood mononuclear cell
PRNT	plaque reduction neutralization test
PsVNA	Pseudotyped virus neutralization assay
RBD	receptor-binding domain
RLU	relative luminescence units
rt	real-time
RLU	relative luminescence units
RT-qPCR	reverse transcriptase quantitative polymerase chain reaction
RT-PCR	reverse transcriptase polymerase chain reaction
S	Spike
S-2P	S-protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

Th1 (2)	T helper cell 1 (2)
TMB	3,3′,5,5′tetramethylbenzidine
VAERD	Vaccine-associated enhanced respiratory disease
VIP	Vaccine Immunology Program
VRC	Vaccine Research Center
WGS	Whole genome sequencing

1 IMMUNOGENICITY ASSESSMENT LIFECYCLE

This document describes the lifecycle roadmap of mRNA-1273 drug product staging the various steps involved in analytical method development while attempting to navigate the accelerated process for vaccine development due to the global coronavirus disease 2019 (COVID-19) public health crisis.

In early January 2020, a novel coronavirus (CoV), severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was identified as the cause of a respiratory virus outbreak occurring in Wuhan, Hubei Province, China, in Dec 2019, and the disease has since spread globally (WHO 2020). ModernaTX, Inc. (Sponsor) developed mRNA-1273, a novel lipid nanoparticle (LNP)encapsulated mRNA-based vaccine against SARS-CoV-2. mRNA-1273 contains a single messenger RNA (mRNA) that encodes the full-length SARS-CoV-2 Spike (S) protein modified with 2 proline substitutions within the heptad repeat 1 domain (S2P) to stabilize the S-protein into the prefusion conformation. The CoV S-protein mediates attachment and entry of the virus into host cells, making it a primary target for neutralizing antibodies that prevent infection (Corti et al 2015; Wang et al 2015; Yu et al 2015; Johnson et al 2016; Chen et al 2017; Wang et al 2018; Kim et al 2019; Widjaja et al 2019).

Good Manufacturing Practice (GMP) production of mRNA-1273 was initiated in parallel with preclinical evaluation. Prior to vaccination of the first human subject, expression and antigenicity of the S-2P antigen delivered by mRNA was confirmed in vitro, and immunogenicity of mRNA-1273 was documented in several animal models. Nonclinical immunogenicity assays for mRNA-1273 included assessment of sera for binding immunoglobulin G (IgG) antibody titers against SARS-CoV-2 S-protein and S-2P-protein as well as S-protein subunit (S1 and S2) and subdomains (receptor-binding domain [RBD] and N-terminal domain), as measured by enzyme-linked immunosorbent assay (ELISA). Vaccine-induced neutralizing activity was assessed by pseudotyped virus neutralization assay (PsVNA), plaque/focus reduction neutralization test (P/FRNT), and live virus microneutralization (Live MN). Additionally, evaluation of T-cell cytokine responses to pools of overlapping peptides from the S-protein was also assessed. These nonclinical immunogenicity assays were developed by Vaccine Research Center (VRC) at the National Institutes of Health (NIH) (Corbett et al 2020).

The immunogenicity assay development strategy for clinical samples was phase-dependent and flexible to accommodate for the evolving COVID-19 pandemic and the compliance towards regulatory requirements. Study 101 (Study 20-0003) was initiated on 16 March 2020 (NCT04283461), followed by Study 201 (mRNA-1273-P201), which began 29 March 2020 (NCT04405076) and Study 301 (mRNA-1273-P301), which began 27 Jul 2020 (NCT04470427).

For Study 101, the following fit-for-purpose immunoassays were used to assess sera for binding IgG antibody titers, neutralizing activity and T-cell cytokine response (performed at the National Institute of Allergy and Infectious Diseases VRC). These bioassays were not formally qualified

or validated at the time; however, they were demonstrated to be scientifically sound, suitable, and reliable for their intended purposes.

Quantitative ELISAs were used to detect IgG antibody to the SARS-CoV-2 virus S-2P protein as well as S-protein RBD-subdomain in human serum (Section 2.1.1.1.1). Vaccine-induced neutralizing activity was assessed by pseudotyped lentivirus reporter single-round-of-infection neutralization assay (PsVNA [Section 2.1.2.2]) and by live wild-type SARS-CoV-2 virus focus/plaque reduction neutralization test (F/PRNT [Section 2.1.2.4 and Section 2.1.2.3]) assay. These experimental assays were developed using a fit-for-purpose approach due to the early phase of study. In addition, in Study 101, convalescent sera obtained from 41 patients who recovered from SARS-CoV-2 infection were used during assay development to generate a relative benchmark (based on levels elicited by natural infection).

To address concerns about the theoretical risk of enhanced disease after injection with mRNA1273, a SARS-CoV-2 intracellular cytokine stimulation (ICS)- assay was developed using peripheral blood mononuclear cells (PBMCs) isolated from participants in Study 101. The intent of evaluating the ICS assay was to evaluate if the mRNA-1273 vaccine induced a Th-2 directed response (which has been linked with vaccine-associated enhanced respiratory disease [VAERD]) or a T helper cell 1 (Th1) directed response (which has been linked with lack of VAERD). This was done through the evaluation of CD4+/CD8+ T cells which can be segregated into Th1/Th2 directed responses. In the SARS-CoV-2 ICS assay, PBMCs were stimulated with custom ordered peptides peptide pools ranging from the N-terminus of SARS-CoV-2 S-protein up to the furin cleavage site (S1 pool) and the C-terminus of the SARS-CoV-2 S-protein up to the furin cleavage site (S2 pool) and antigen-specific cytokine frequencies are reported (Section 2.3).

Suitability of the immunogenicity analytical methods for clinical use were subsequently demonstrated in Study 201, the phase-specific immunogenicity assays were qualified for the primary analysis of immunogenicity data (05 Nov 2020 data cutoff), and thereafter validated for subsequent immunogenicity data analysis. All SARS-CoV-2 viral RNA assays used were validated with the exception of Live MN which was qualified for all data analyzed in Study 201. In Study 301, all analytical assays were validated, following regulatory expectations, except for BioFire® Respiratory Panel qualitative reverse transcriptase-polymerase chain reaction (RT-PCR) which was qualified at the time and used to assess other viral and bacterial respiratory organisms. The immunogenicity analytical assays used in Study 201 and Study 301 and their development status and supporting documentation per clinical phase are summarized in Table 1.

In Study P201 and Study 301, baseline SARS-CoV-2 status and asymptomatic/symptomatic SARS-CoV-2 infection were evaluated using a SARS-CoV-2 RT-PCR assay (validated by Eurofins-Viracor) using nasopharyngeal swabs from individuals suspected of COVID-19 (Section 2.2.1). To test for antibodies generated by an intercurrent SARS-CoV2 infection in Study 301 participants, an in-vitro diagnostic assay, Elecsys® anti-SARS-CoV-2 (developed by Roche Diagnostics) was used. This assay evaluated anti-SARS-CoV-2 IgG antibody presence in

study participants. The commercially available ELECSYS Anti-SARS-CoV-2 in vitro diagnostic electrochemiluminescence immunoassay (ECLIA) is a qualitative assay which evaluates the SARS-CoV-2 baseline infection status by detecting anti-IgG specific SARS-CoV-2 Nucleocapsid (N) protein antibodies present in human serum and plasma. This assay was validated by PPD Global Central Labs-US laboratory for its use in Study 301 (Section 2.1.1.3). Sera from study participants in Study 201 and Study 301 were additionally evaluated using a quantitative ELISA method for the detection of IgG specific to SARS-CoV-2 -N-protein. This assay, developed by PPD Laboratories, was qualified for primary analysis in Study 201 and validated for subsequent analysis of Study 201 data and Study 301 (Section 2.1.1.2).

A quantitative RT-PCR analysis of RNA from SARS-CoV-2 virus in swab and saliva was additionally carried out in Study 301. This quantitative RT-PCR from swab and saliva samples was carried out to measure viral load at SARS-CoV-2 infection diagnosis over time to evaluate the effect of mRNA on the viral infection kinetics. This assay was validated by Eurofins-Viracor (Section 2.2.2).

To evaluate vaccine immunogenicity, an ELISA method for the detection of IgG specific to S-2P-protein in human serum was developed, qualified, and validated by PPD® Laboratories. This assay was qualified for primary analysis in Study 201 (VSDVAC58) and thereafter validated for subsequent analysis of Study 201 and Study 301 data (VSDVAC65; Section 2.1.1.1). For Study 301, an additional assay detecting IgG against S-protein was developed with the aim of increasing automatization of immunogenicity evaluation. This multiplex assay using a MesoScale Discovery (MSD) platform was developed by the NIH VRC Vaccine Immunology Program (VIP) and measured IgG-specific S, N, and RBD proteins. The anti-S-protein bAb assay within the multiplex MSD was qualified and validated only for the detection of IgG S-protein binding antibody (bAb) and was used to evaluate vaccine immunogenicity along with the previously validated immunogenicity ELISA for detection of IgG specific to SARS-CoV-2 S-2P-protein (Section 2.1.1.4).

Neutralizing antibodies against SARS-CoV-2 S-protein were analyzed throughout the clinical development of mRNA-1273. A Live MN assay was developed to quantify SARS-CoV-2 neutralizing antibodies in serum or plasma samples from individuals who have received a SARS-CoV-2 vaccine or were exposed to SARS-CoV-2. This assay was qualified for primary analysis in Study 201 by Battelle Memorial Institute (Section 2.1.2.1). These Live MN assays are labor intensive and require high biosafety level containment (level 3) handling by trained personnel and pose challenges for high throughput. A pseudovirus neutralization assay in 293T/angiotensin-converting enzyme 2 (ACE2) cells had been developed by VRC at NIH and used in Study 101. Therefore, further development of this assay was performed for its use in clinical testing by the “Neutralizing Antibody Core” Laboratory, under the Good Clinical

Laboratory Practice (GCLP) oversight of the Quality Assurance for Duke Vaccine Immunogenicity Programs at Duke University Medical Center. This PsVNA assay was qualified and validated by the "Neutralizing Antibody Core" Laboratory, at Duke University Medical Center and used in Study 301. An additional validation of this assay was performed with mRNA-1273 Study 101 samples (Section 2.1.2.2). Concordance (b) (4) of the PsVNA assay with bAb assays (ELISA and MSD assays for SARS-CoV-2 IgG antibody quantification) were evaluated and the (b) (4) value for seroresponse was adjusted based on the concordance analysis (Section 2.1.3).

During mRNA-1273 clinical development, increasing concerns arose around emerging SARS-CoV-2 variants that may be associated with increased transmission rates and mortality and/or a decrease in the efficacy of the authorized COVID-19 vaccines (Haynes et al 2021). In order to determine the strain of SARS-CoV-2 which caused COVID-19 cases in 301 and to compare with the mRNA-1273 vaccine sequence, a whole SARS-CoV-2 genome analysis (whole genome sequencing [WGS] assay) and a whole SARS-CoV-2 S--protein analysis (next generation sequencing [NGS] assay) were performed. These assays were developed by Eurofins Genomics and validated by Eurofins-Viracor, and used in Study 301 (Section 2.2.3).

Additionally, the BioFire FilmArray® Respiratory Panel (RP) assay for the identification of a variety of respiratory targets, including SARS-CoV-2, was used as a qualitative assay in Study 301. This bioassay can simultaneously detect nucleic acids from 20 viral and bacterial organisms in nasopharyngeal swab samples. BioFire FilmArray® RP, qualified by Eurofins-Viracor was used in Study 301 for the detection of other viral and bacterial organisms associated with respiratory disease (Section 2.2.3).

All bioassays used for the evaluation of mRNA-1273 drug product throughout its clinical development process underwent an integrated approach of an analytical lifecycle (development, qualification, and validation) that was best suited for the intended use of each assay at various clinical stages (Table 1). The qualification and validation reports for each bioassay are located in Module 5, Section 5.3.1.4.

Table 1: Table of Pharmacology Bioassays per Clinical Phase Development

Context of Use (Endpoint)	Assay Name (Section)	Parameter	Methodology	Development Status (Vendor) And Supporting Documentation	SOP Used	Supporting Licensure
STUDY 101						
Immunogenicity						
Quantitative bAb (Secondary endpoint and Exploratory endpoint)	ELISA: S-2P-protein IgG (Section 2.1.1.1.1)	Anti S-2P IgG bAb	ELISA	Developed (VRC VIP at the NIH)	N/A[a]	NO
	ELISA: RBD IgG (Section 2.1.1.1.1)	Anti-RBD IgG bAb	ELISA	Developed (VRC VIP at the NIH)	N/A[a]	NO
Quantitative nAb (Exploratory endpoint)	PsVNA (Section 2.1.2.2)	Anti-S IgG nAb	PsVNA	Developed (VRC VIP at the NIH)	N/A[a]	NO
	PRNT (Section 2.1.2.3)		PRNT	Developed (VRC VIP at the NIH)	N/A[a]	NO
	FRNT (Section 2.1.2.4)		FRNT	Developed (VRC VIP at the NIH)	N/A[a]	NO
T-cell response						
(Exploratory endpoint)	ICS Assay (Section 2.3)	Intracellular cytokine stimulation	Intracellular cytokine staining	Developed (VRC VIP at the NIH)	N/A[a]	NO

Context of Use (Endpoint)	Assay Name (Section)	Parameter	Methodology	Development Status (Vendor) And Supporting Documentation	SOP Used	Supporting Licensure
STUDY 201						
SARS-CoV-2 infection status assessments						
Baseline status and asymptomatic / symptomatic SARS-CoV-2 infection (Exploratory endpoints)	RT-qPCR (Upper Respiratory Samples) (Section 2.2.1)	RNA	RT-PCR	Commercial (LDT), Validated at CLIA Lab (Eurofins Viracor) Upper Respiratory samples: 21120.8918 VSR v3.0	21120.9204	YES
	ELISA: N-protein IgG (Section 2.1.1.2)	Anti-N IgG bAb	ELISA	Qualified (PPD Laboratories) [a] VSDVAC 64 QSR VSDVAC 64 QSR Adden. 1 Validated (PPD Vaccine Laboratories) VSDVAC 66 VSR VSDVAC 66 VSR Adden. 1	VSDVAC 66[b]	YES
Immunogenicity						
Quantitative bAb (Primary immunogenicity and exploratory endpoints)	ELISA: S-2P protein IgG (Section 2.1.1.1)	Anti-S-2P IgG bAb	ELISA	Qualified (PPD Laboratories) [a] VSDVAC 58 QSR Validated (PPD Vaccine Laboratories) [a] VSDVAC 65 VSR VSDVAC 65 VSR Adden. 2 VSDVAC 65 VSR Adden. 3 VSDVAC 65 VSR Adden. 4	VSDVAC 65[b]	YES
Quantitative nAb (Secondary immunogenicity and exploratory endpoints)	Live Virus MN (Section 2.1.2.1)	Anti-S IgG nAb	Live virus MN	Qualified (Battelle)[a] QA-5858 QSR	SOP BBR.C.X-339-01	YES

Context of Use (Endpoint)	Assay Name (Section)	Parameter	Methodology	Development Status (Vendor) And Supporting Documentation	SOP Used	Supporting Licensure
STUDY 301						
SARS-CoV-2 infection status assessments						
Baseline status and asymptomatic / symptomatic SARS-CoV-2 infection (Support of Primary and Secondary efficacy endpoints)	RT-qPCR (Upper Respiratory Samples) (Section 2.2.1)	RNA	RT-PCR	Commercial (LDT), Validated at CLIA Lab (Eurofins Viracor) Upper Respiratory samples: 21120.8918 VSR v3.0	21120.9204	YES
	ELECSYS: N-protein IgG (Section 2.1.1.3)	Anti-N IgG bAb	ECLIA	Commercial (Roche), Validated at performing CLIA Lab (PPD GCL) VR-GCL-US-2020-06-551-2 VSR V01	SOP-18550	YES
	ELISA: N-protein IgG (Section 2.1.1.2)	Anti- N IgG bAb	ELISA	Validated (PPD Vaccine Laboratories) VSDVAC 66 VSR VSDVAC 66 VSR Adden. 1	VSDVAC 66	YES
Viral infection kinetic assessment						
Viral load analysis (Exploratory endpoints)	RT-qPCR (Saliva Samples) (Section 2.2.2)	RNA	RT-PCR	Commercial (LDT), Validated at CLIA Lab (Eurofins Viracor) Swab and saliva specimen: 21120.9249VSR v1.0	21120.9204	YES
Genotype variant analysis						
Genetic and/or phenotypic assessment (Exploratory endpoints)	RT-PCR NGS (Section 2.2.4.1.1)	RNA	RT-PCR	Commercial (LDT), Validated at CLIA Lab (Eurofins Viracor) 21120.10208 GNS VSR v1.0	21120.10120	YES
	RT-PCR WGS (Section 2.2.4.1.3)		RT-PCR	Commercial (LDT), Validated at CLIA Lab (Eurofins Viracor) 21120.10562 WGS VSR v1.0	21120.10517	YES

Context of Use (Endpoint)	Assay Name (Section)	Parameter	Methodology	Development Status (Vendor) And Supporting Documentation	SOP Used	Supporting Licensure
Detection of other viral and bacterial organisms						
Other viral and bacterial respiratory organisms (Exploratory Endpoint)	RT-PCR BioFire (Section 2.2.3)	RNA	RT-PCR	Validated (Eurofins-Viracor) 21120.8062 QSR	SOP 21120.2380	YES
Immunogenicity						
Quantitative bAb (Secondary immunogenicity)	ELISA: S-2P protein IgG (Section 2.1.1.1)	Anti-S-2P IgG bAb	ELISA	Validated (PPD Vaccine Laboratories) VSDVAC 65 VSR VSDVAC 65 VSR Adden. 2 VSDVAC 65 VSR Adden. 3 VSDVAC 65 VSR Adden. 4	VSDVAC 65	YES
	MSD Multiplex: S-protein IgG (Section 2.1.1.4)	Anti-S IgG bAb	MSD Multiplex	Commercial (MSD), anti-S validated (VRC VIP at the NIH) B1004 VSR R1022 VSR of incurred Samples for OWS R1023 VSR Adden. 1 R1024 VSR of incurred Samples for OWS Adden. 1	SOP5525	YES
Quantitative nAb (Secondary immunogenicity)	Live Virus MN (Section 2.1.2.1)	SARS-CoV-2 virus neutralization	Live virus MN	Validated (Battelle) VA-5933 VSR VA-6003 VSR Phase 1 mRNA-1273 samples	SOP BBRC.X-339-10	YES
	PsVNA (Section 2.1.2.2)	SARS-CoV-2 pseudotyped virus neutralization assay	PsVNA	Validated (Duke University Medical Center) COVID0001 Method VSR v1.0 COVID0001 Method VSR Adden. 1 v1.1 COVID0002 Method VSR	CFAR02-A0026	YES

Abbreviations: Adden. = addendum; bAb = binding antibodies; CLIA = Clinical Laboratory Improvement Amendments; ECLIA = electrochemiluminescence immunoassay; ELISA = enzyme-linked immunosorbent assay; FRNT = focus reduction neutralization test; IgG = immunoglobulin G; MSD = MesoScale Discovery; N = nucleocapsid; N/A = not applicable ; nAb = neutralizing antibodies; NIH = National Institutes of Health; OWS = Operation Warp Speed; PRNT = plaque reduction neutralization test; PsVNA = pseudotyped virus neutralization assay; QSR = quality statistical report; RBD = receptor-binding domain; RT-PCR = reverse transcriptase polymerase chain reaction; S = spike; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SOP = standard operating procedure; V = version; VIP = Vaccine Immunology Program; VRC = Vaccine Research Center; VSR = validation statistical report

[a] These experimental assays were developed by VRC, VIP at the NIH using a fit-for-purpose approach due to the early phase of study and are not supporting licensure.

[b] Note Study 201 immunogenicity assays were qualified for the primary analysis of immunogenicity data (05 Nov 2020 data cutoff). However, this study is ongoing and validated results have been used for subsequent analysis of immunogenicity data.

2 ASSAY METHODOLOGY AND VALIDATION RESULTS

2.1 Antibody Analysis

2.1.1 Binding Antibodies

2.1.1.1 ELISA: S-2P-protein IgG

This quantitative ELISA was designed to detect the IgG antibody to the SARS-CoV-2 virus S-2P-protein in human serum. Microtiter plates were coated with commercially available SARS-CoV-2 full-length S-2P glycoprotein and serum containing the SARS-CoV-2 IgG antibody was added. Bound antigen-antibody complex was detected using purified goat anti-human IgG horseradish peroxidase (HRP) conjugate. Color development occurred during the addition of 3,3',5,5'tetramethylbenzidine (TMB) substrate and color intensity was measured spectrophotometrically (b) (4) The intensity of the color was directly proportional to the IgG antibody concentration. Quantitation of the human IgG antibody to SARS-CoV-2, or antibody concentration (AU/mL), was determined by interpolation from a standard curve analyzed on each assay plate.

This proprietary serological method, "*An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*" was developed, qualified (VSDVAC58 ELISA IgG S-protein Qualification Statistical Report Version 1.00), and validated (VSDVAC65 ELISA IgG S-protein Method Validation Report, Addendum 2, Addendum 3 and Addendum 4) by PPD® Laboratories.

Validation parameter results are presented in Table 2.

Table 2: ELISA: S-2P-protein IgG Analysis: Summary of Method Validation Parameters

Assay Characteristic	SARS-CoV-2 Spike IgG ELISA	Acceptance Criteria
(b) (4)	(b) (4)	
LLOQ (AU/mL; (b) (4))		
ULOQ (AU/mL; (b) (4))		
Precision ((b) (4) for samples with GMC within the LOQs)	(b) (4)	
Precision (% samples within LOQs with (b) (4))	(b) (4)	
Relative accuracy (overall % accuracy across samples with expected concentration within the LOQs)	(b) (4)	
Analyst ruggedness (maximum overall fold difference)		
Dilutional linearity (dilution bias per (b) (4) -fold)		
Selectivity (% recovery across samples with expected concentration within the LOQs)		
(b) (4) specificity (% results with (b) (4))		
(b) (4) specificity (% results with (b) (4))		

Abbreviations: AU = arbitrary unit; ELISA = enzyme-linked immunosorbent assay; (b) (4) ; (b) (4) (b) (4) GMC = geometric mean antibody concentration; IgG = immunoglobulin G; LLOQ = lower limit of quantitation; (b) (4) LOQ = limit of quantitation; OD = Optical Density; N/A = not applicable; (b) (4) (b) (4) (b) (4) ; ULOQ = upper limit of quantitation.
Source = VSDVAC 65 ELISA IgG Method Validation Report, Addendum 2 and Addendum 4.

[a] (b) (4)

[b] (b) (4) Quality control limits are (b) (4) limits.

2.1.1.1.1 Study 101: ELISA S-2P-protein IgG and RBD IgG

The IgG ELISA testing of clinical samples in Study 101 evaluated the presence of binding IgG antibodies to either of 2 SARS-CoV-2 antigens, S-2P and RBD, each in singleplex format.

In singleplex format either SARS-CoV-2 S-2P at a working concentration of (b) (4) , or SARS-CoV-2 RBD antigen at a working concentration of (b) (4) were coated onto (b) (4) (b) (4) plates overnight for (b) (4) hours at (b) (4) . Antigen concentrations were defined during assay development and antigen lot titration. Plates were washed and blocked (b) (4) (b) (4) for (b) (4) . (b) (4) serial (b) (4) fold dilutions covering the range of (b) (4) of the test sample (diluted in (b) (4)) were incubated at (b) (4) for (b) (4) followed by (b) (4) anti-human antibody detection (b) (4) and (b) (4) addition ((b) (4) (b) (4)). Color development was stopped by addition of (b) (4) and plates were read

within (b) (4) at (b) (4) via the (b) (4) plate reader. Endpoint Titer dilution from raw OD data was interpolated using the plate background OD (b) (4)

(b) (4)

2.1.1.2 ELISA: N-protein IgG

This quantitative ELISA was designed to detect IgG antibody to the SARS-CoV-2 virus N-protein in human serum. Microtiter plates are coated with commercially available SARS-CoV-2 N-glycoprotein and serum containing the SARS-CoV-2 IgG antibody is added. Bound antigen-antibody complex is detected using purified goat anti-human IgG HRP conjugate. Color development occurs during the addition of TMB substrate and color intensity is measured spectrophotometrically (b) (4) . The intensity of the color is directly proportional to the IgG antibody concentration. Quantitation of the human IgG antibody to SARS-CoV-2, or antibody concentration (AU/mL), is determined by interpolation from a standard curve analyzed on each assay plate.

This proprietary serological method, "*An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum*" was developed, qualified (VSDVAC 64 ELISA IgG N-protein Qualification Statistical Report Version 1.00), Addendum 1 and validated (VSDVAC 66 ELISA IgG N-protein Method Validation Report) and Addendum 1, by PPD® Laboratories.

Validation parameter results are presented in Table 3.

Table 3: ELISA: N-protein IgG Analysis: Summary of Method Validation Parameters

Assay Characteristic	Study 301 (Validated)	Acceptance Criteria
(b) (4)	(b) (4)	
LLOQ (AU/mL; (b) (4))		
ULOQ (AU/mL; (b) (4))		
Precision ((b) (4) for samples with GMC within the LOQs)		
Precision (% samples within LOQs with (b) (4)		
Relative accuracy (overall % accuracy across samples with expected concentration within the LOQs)		
Analyst ruggedness (maximum overall fold difference)		
Dilutional linearity (dilution bias per (b) (4) fold)		
Selectivity (% recovery across samples with expected concentration within the LOQs)		
(b) (4) specificity (% results with (b) (4))		
(b) (4) specificity (% results with (b) (4))		

Abbreviations: AU = arbitrary unit; ELISA = enzyme-linked immunosorbent assay; (b) (4)
GMC = geometric mean antibody concentration; IgG = immunoglobulin G; LLOQ = lower limit of quantitation; (b) (4) (b) (4) ; LOQ = limit of quantitation; N/A = not applicable; NE = not evaluable; ULOQ = upper limit of quantitation.
Source = VSDVAC 66 ELISA IgG N-protein Method Validation Report Addendum 1

2.1.1.3 ELECSYS: N-protein IgG

The Elecsys® Anti-SARS-CoV-2 is a commercially available in vitro diagnostic ECLIA developed by Roche Diagnostics and intended for the qualitative detection of antibodies to the SARS-CoV-2 N-protein antigen in human serum and plasma. The ECLIA is intended for use on COBAS E® analyzers.

The assay is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, which indicates recent or prior infection, and should not be used to diagnose or exclude acute SARS-CoV-2 infection. The qualitative results are reported as reactive (ie, positive for anti-SARS-CoV-2 N antibodies; cutoff index [COI] ≥ 1.0) or nonreactive (ie, negative for anti-SARS-CoV-2 N antibodies; COI < 1.0).

On 02MAY2020, the US Food and Drug Administration (FDA) issued an Emergency Use Authorization (EUA) for use of the ELECSYS Anti-SARS-CoV-2 for the qualitative detection of antibodies to SARS-CoV-2 in human serum or plasma.

The performance characteristics of this *Roche ELC kit for the measurement of anti-SARS-CoV-2 in serum and plasma* (ELECSYS Anti-SARS-CoV-2 Package Insert) was validated (VR-GCL-US-2020-06-551-2 Elecsys IgG N-protein Method Validation Report Version 01) by PPD Global Central Labs.

Validation parameter results are presented in Table 4.

Table 4: ELECSYS: N-protein IgG Analysis: Summary of Method Validation Parameters

Assay Characteristic	Study 301 (Validated)	Acceptance Criteria
Sample Stability	Up to 28 days frozen (-20°C ± 5°C) and up to 7 days at ambient (15°C to 25°C) and refrigerated (2°C to 8°C) temperature	N/A
Limitation/interference	No interference observed	N/A
Analytical specificity (95 % CI)	99.5 % (98.63-99.85)	N/A
Specificity (95 % CI)	99.80 % (99.69, 99.88)	N/A
Sensitivity (≥14 days post PCR) (95 % CI)	99.5 % (97.0, 100 %)	N/A
(b) (4)		

Abbreviations: CI = confidence interval; N/A = not applicable; PCR = polymerase chain reaction.
Note: Highlighted in grey are all parameters obtained from the manufacturer's performance evaluation
Source = ELECSYS Anti-SARS-CoV-2 Package Insert and VR-GCL-US-2020-06-551-2 Elecsys IgG N-protein Method Validation Report Version 01

2.1.1.4 MSD Multiplex: S-protein IgG

The multiplex assay was used in the detection of IgG against SARS-CoV-2 S-protein. The MSD® 384-well custom serology assay (4-plex) SARS-CoV-2 assay is an ECLIA intended for the multiplex simultaneous quantitative detection of IgG antibodies to SAR-CoV-2 distinct antigens in human serum.

The MSD platform utilizes MULTI-SPOT® microtiter plates that are fitted with a series of electrodes on the bottom of each well. Using an MSD (b) (4) detection system, an electrical current is applied to the plates and areas of well surface which form antigen anti-human IgG antibody SULFO-TAG™ complex and emit light in the presence of the Electrochemical luminescence substrate. The MSD 4-plex SARS-CoV-2 assay detects SARS-CoV-2 antigen S-protein, RBD, and N-protein with an additional BSA control. Plates are provided with antigens on spots in the wells of a 384-well plate. Antibodies in the serum samples bind to the antigen(s) on the spots and anti-human antibody (IgG) conjugated with MSD SULFO-TAG™ are used for detection. The plate is read on an MSD instrument, which measures the light emitted from the MSD SULFO-TAG.

This proprietary serological method, "*Multiplex (4-plex) Assay for the detection of IgG antibodies against SARS-CoV-2 proteins in human sera*" was developed and validated for human sera samples (MSD IgG S-protein Validation Report and Addendum 1) and for samples for Operation Warp Speed (OWS) (R1022 MSD IgG S-protein of Incurred Samples for OWS Validation Report and Addendum 1) by the NIH VRC VIP.

Validation parameter results are presented in Table 5.

Table 5: MSD Multiplex S-protein IgG Analysis: Summary of Method Validation Parameters

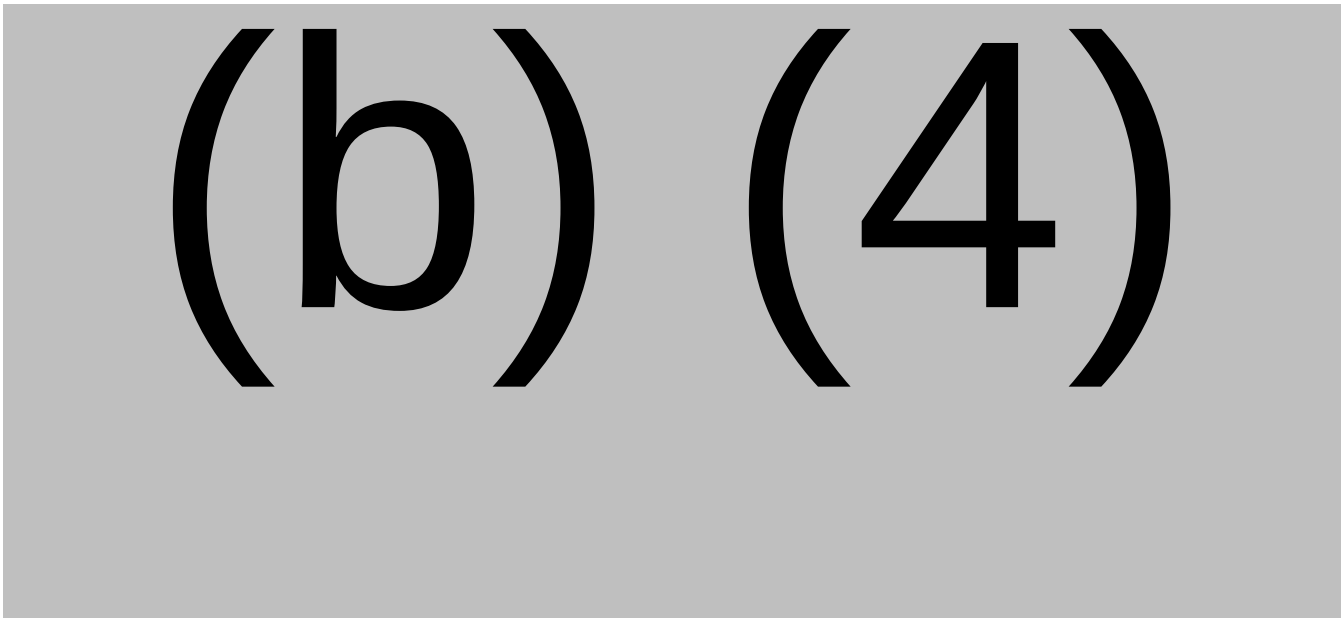
Assay Characteristic	Study 301 (Validated)	Acceptance Criteria
Human Sera Samples		
LLOQ (AU/mL)	(b) (4)	
ULOQ (AU/mL)		
Intra-assay precision (%CV of AU/mL)		
Inter-assay precision (%CV of AU/mL)		
Linearity (b) (4) for linear)		
%recovery (% CVs)		
Total assay variability		
Selectivity (% samples >lower linearity range[(b) (4)		
Sample stability		
(b) (4)		
Incurred Samples for OWS		
LLOQ (AU/mL)	(b) (4)	
ULOQ (AU/mL)		
Intra-assay precision (%CV of AU/mL)		
Inter-assay precision (%CV of AU/mL)		
Linearity (b) (4) for linear)		
%recovery (% CVs)		
Total assay variability		

Abbreviations: AU = absorbance units; CV = coefficient of variation; F/T = freeze/thaw; IgG = ; LLOQ = Lower limit of quantitation; MSD = MesoScale Discovery; N/A = not applicable; OWS = Operation Warp Speed; RT = room temperature; ULOQ = upper limit of quantitation; vs = versus.
Source = B1004 V MSD IgG S-protein Validation Report and Addendum 1 and R1022 MSD IgG S-protein of Incurred Samples for OWS Validation Report and Addendum 1

2.1.2 Neutralizing Antibodies

2.1.2.1 Live Virus MN

The MN assay was developed to quantify SARS-CoV-2 neutralizing antibodies in serum or plasma samples from individuals who have received a SARS-CoV-2 vaccine or were exposed to SARS-CoV-2.



This proprietary serological method, "*Microneutralization Assay for SARS-CoV-2 antibodies in human serum obtained from individuals vaccinated with mRNA-1273*" was developed by ModernaTX, Inc. A subsequent development plan was established and the assay was qualified (QA-5858 Qualification of the SARS-CoV-2 Microneutralization Assay) and validated (VA5933STATSCSR Validation Report) by Battelle Memorial Institute. A partial validation with serum from individuals vaccinated with mRNA-1273 (VA-6003 Partial Validation Phase 1 mRNA-1273 Samples) was also performed by Battelle Memorial Institute.

Validation parameter results are presented in Table 6.

Table 6: Live Virus MN: Summary of Method Validation Parameters

Assay Characteristic	Study 201 (Validated)	Acceptance Criteria
(b) (4)		
Endpoint titer		
LOD (95 % CI)	(b) (4)	
LLOQ		
ULOQ		
Dilution linearity (90 %CI) (90 %CI for slope)		
Precision (% endpoints withing 2-fold of median)		
Specificity (95 % lower CI)		
Validation of method with serum from individuals vaccinated with mRNA-1273		
LOD (b) (4) (b) (4) [(b) (4)	(b) (4)	
LLOQ (GM MN_{50})		
ULOQ (GM MN_{50})		
Dilution linearity (90 %CI for slope)		
Precision (total assay variability: operator and residual precision) (%CV samples combined into a single model)		
Precision (incurred sample testing) (% CV samples)		

Abbreviations: CI = confidence interval; CV = coefficient of variation; (b) (4) (b) (4) ; GM = geometric mean; LOD = limit of detection; LLOQ = lower limit of quantification; MN = microneutralization; ULOQ = upper limit of quantification; N/A = not applicable.
Source = VA5933STATSCSR Validation Report and VA-6003 Partial Validation Phase 1 mRNA-1273 Samples
[a] In Study 201 immunogenicity data in Part B was analyzed using the validated assay (VA-6003).

(b) (4)

2.1.2.2 PsVNA

The SARS-CoV-2 Spike-PsVNA in 293/ACE2 cells was used to evaluate neutralizing antibody activity against SARS-CoV-2 in serum samples from clinical study participants after vaccination or natural infection.

The quantification of SARS-CoV-2 neutralizing antibodies utilizes lentivirus particles that express SARS-CoV-2 spike protein on their surface and contain a firefly luciferase reporter gene for quantitative measurements of infection by relative luminescence units (RLU). The virus is applied to transduced 293T cells expressing high levels of ACE2 (293T/ACE2 cells), with or without pre-incubation with antibodies (control antibodies or serum samples); the presence of neutralizing antibodies reduces infection and results in lower RLUs. Serial dilution of antibodies or serum samples can be used to produce a dose-response curve. Neutralization is measured as the serum dilution at which the RLU is reduced by 50 % (ID_{50}) or 80 % (ID_{80}) relative to mean RLU in virus control wells (cells + virus but no control antibody or sample) after subtraction of the mean RLU in cell control wells (cells only).

This proprietary serological method "*SARS-CoV-2 Spike-PsVNA in 293T/ACE2 Cells*" was developed, qualified and validated (COVID0001 Method Validation Statistical Report v1.0 and Addendum 1 v1.1), validated with control samples and mRNA-1273 Phase 1 samples (COVID0002 Method Validation Report) by the "Neutralizing Antibody Core" Laboratory, under the GCLP oversight of the Quality Assurance for Duke Vaccine Immunogenicity Programs at Duke University Medical Center.

Validation parameter results are presented in Table 7.

Table 7: PsVNA: Summary of Method Validation Parameters

Assay Characteristic [a]	Study 301 (Validated)	Acceptance Criteria
ID_{50}		
LLOQ	(b) (4)	
ULOQ		
ID_{80}		
LLOQ	(b) (4)	
ULOQ		

Assay Characteristic [a]	Study 301 (Validated)	Acceptance Criteria
	(b) (4)	
Validation of method with serum from individuals vaccinated with mRNA-1273		
ID_{50}		
LLOQ	72	N/A
ULOQ	2189	N/A
	(b) (4)	
ID_{80}		
LLOQ	26	N/A
ULOQ	673	N/A
	(b) (4)	

Abbreviations: ACE2 = angiotensin-converting enzyme 2; CV = coefficient of variation; conc. = concentration; (b) (4) (b) (4); ID = infective dose; LLOQ = lower limit of quantification; MN = ; ULOQ = upper limit of quantification; N/A = not applicable.

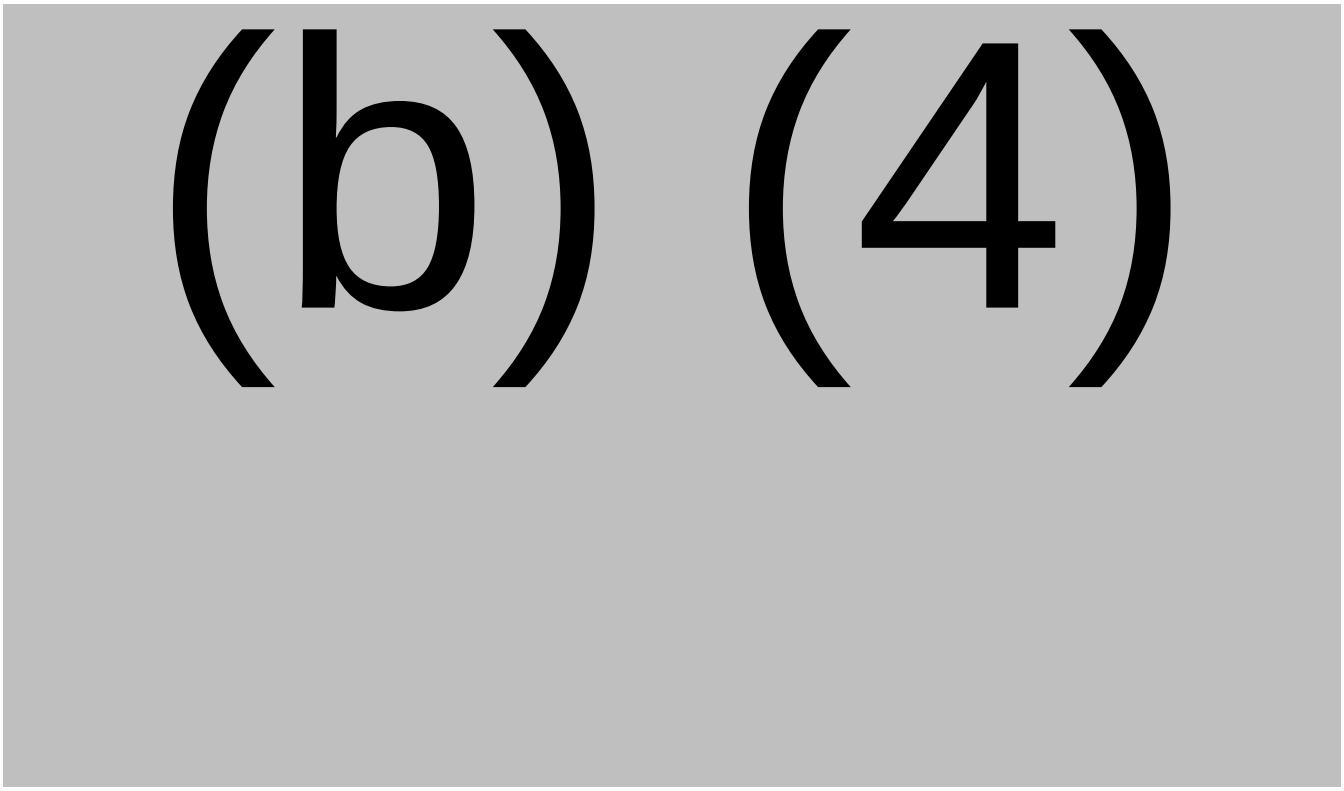
[a] validation experiments described in this Report excluded COVID-19 convalescent serum samples from HIV-1-infected individuals.

[b] Negative samples are samples that failed to achieve (b) (4) % neutralization at a (b) (4) dilution.

Source = COVID0001 Method Validation Statistical Report Adden. 1 v1.1 and COVID0002 Method Validation Report

2.1.2.3 PRNT

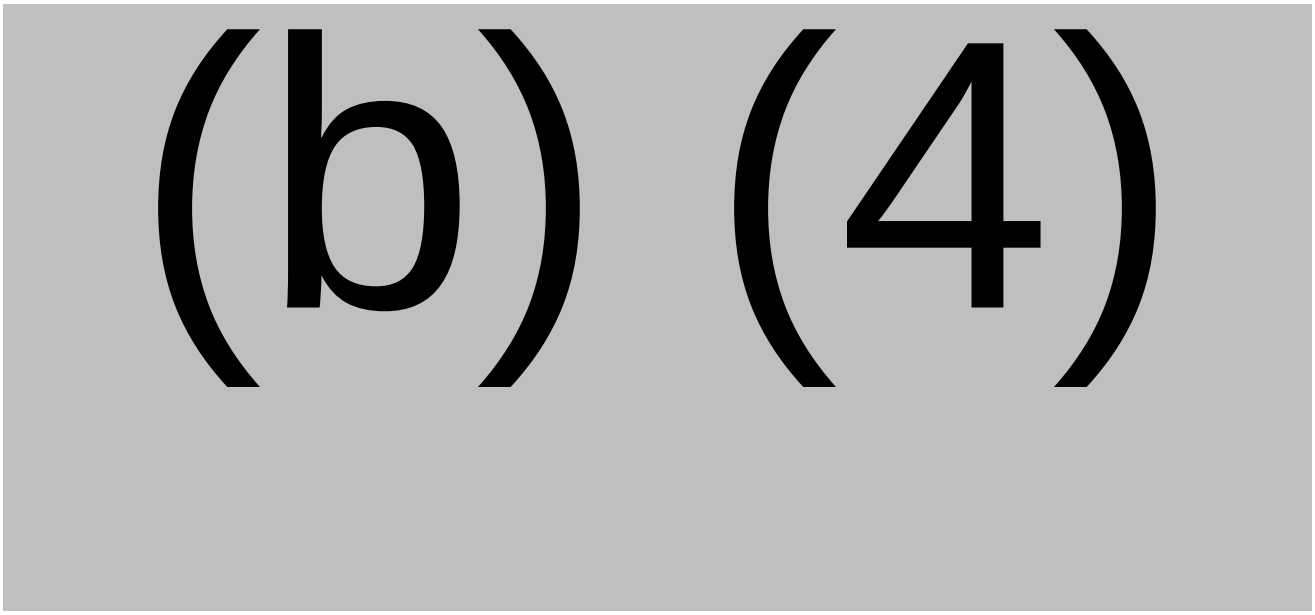
The PRNT assay was developed to quantify SARS-CoV-2 neutralizing antibodies in serum or plasma samples from individuals who have received a SARS-CoV-2 vaccine or were exposed to SARS-CoV-2 in Study 101.



The SARS-CoV-2 PRNT assay is a fit-for-purpose research assay that has not been qualified or validated.

2.1.2.4 FRNT

The FRNT assay was developed to quantify SARS-CoV-2 neutralizing antibodies in serum or plasma samples from individuals who have received a SARS-CoV-2 vaccine or were exposed to SARS-CoV-2 in Study 101.



The SARS-CoV-2 FRNT assay is a fit-for-purpose research assay that has not been qualified or validated. 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.

2.1.3 Concordance Between Binding and Neutralizing Antibody Analysis

An analysis was performed to determine the concordance of binding assays for the detection of IgG specific to SARS-CoV-2 spike protein (including ELISA: S-2P-protein IgG assay [Section 2.1.1.1] and MSD Multiplex: S-protein IgG assay [Section 2.1.1.1]) with the PsVNA assay (Section 2.1.2.2) by Moderna TX (Moderna 1273 Biomarker Concordance Analysis Report). Models using different study time points, age and baseline status of SARS-CoV-2 status were analyzed. The concordance (b) (4) Thus, the concordance (b) (4) could be used to adjust fold difference criteria (e.g., (b) (4)) for purposes of clinical sample evaluations. Overall (all time points) (b) (4) are presented in Table 8.

Table 8: Concordance Analysis: (b) (4)

	MSD Multiplex: S-protein IgG assay[a] vs		ELISA: S-2P-protein IgG assay[b] vs	
	PsVNA50	PsVNA80	PsVNA50	PsVNA80
Overall (b) (4) (b) (4)	(b) (4)			

Abbreviations: CI = confidence interval; ELISA = enzyme-linked immunosorbent assay; IgG =immunoglobulin G; MSD = MesoScale Discovery; (b) (4) ; PsVNA = pseudovirus neutralization assay; S = spike; S-2P = S-protein modified with 2 proline substitutions within the heptad repeat 1 domain; vs = versus.
Source = Moderna 1273 Biomarker Concordance Analysis Report for Study 301
Note: (b) (4)

[a] Determined by using Total %CV (R1024 MSD IgG S-protein of Incurred Samples for OWS Validation Report Adden 1.)
[b] Determined by using the dilution specific "Overall Precision," (b) (4) (VSDVAC 65 ELISA IgG S-protein Method Validation Report Adden. 2).

The overall critical fold rises were determined and are presented in Table 9.

Table 9: Critical Fold Rise

	Critical Fold Rise	Definition Significance
ELISA: S-2P-protein IgG assay[a]		
(b) (4)		
MSD[b]		
(b) (4)		
PsNVA[c]		
ID_{50}	(b) (4)	
ID_{80}		

Abbreviations: CI = confidence interval; ELISA = enzyme-linked immunosorbent assay; ID = infectious dose; IgG =immunoglobulin G; MSD = MesoScale Discovery; (b) (4) ; PsVNA = pseudovirus neutralization assay; S = spike; S-2P = S-protein modified with 2 proline substitutions within the heptad repeat 1 domain; vs = versus.
Source = Moderna 1273 Biomarker Concordance Analysis Report for Study 301
Note: PsVNA ID_{50} obtained from the (b) (4) PsVNA ID_{80} is obtained (b) (4)

[a] Determined by using the dilution specific "Overall Precision," % GCV (VSDVAC 65 ELISA IgG S-protein Method Validation Report Adden. 2)
[b] Determined by using Total %CV (R1024 MSD IgG S-protein of Incurred Samples for OWS Adden 1)
[c] Determined by using intermediate precision of the assay (COVID002 Method Validation Statistical Report)

2.2 SARS-CoV-2 VIRAL RNA Assessment

2.2.1 Baseline SARS-CoV-2 Status (RT-qPCR [Upper Respiratory Samples])

The SARS-CoV-2 specific reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) detect SARS-CoV-2 RNA in nasopharyngeal swab from individuals suspected of COVID-19 and is intended for the qualitative detection of SARS-CoV-2 status of a patient through the analysis of viral RNA.

The SARS-CoV-2 specific RT-qPCR assay was developed by Eurofins Genomics and validated by Eurofins-Viracor to detect SARS-CoV-2 RNA in upper respiratory (nasal/nasopharyngeal wash and swab) and bronchoalveolar lavage samples and in nasal swab. This assay is intended for qualitative detection of RNA from SARS-CoV-2 virus from specimens collected from individuals with symptoms of COVID-19. The SARS-CoV-2 RT-qPCR assay is performed as a multiplex reaction with the MS2 internal control assay. Oligonucleotide primers and TaqMan probes developed by Eurofins-Viracor are used for the detection of 2 regions of the viral N-protein gene region of SARS-CoV-2 and an internal extraction and amplification control target (the RNA bacteriophage MS2) are used.

On 08 April 2020, the FDA issued an EUA for emergency use of the SARS-CoV-2 RT-PCR test will be performed at Eurofins-Viracor Clinical Diagnostics. The performance characteristics of this *SARS-CoV-2 specific RT-qPCR assay* was validated for upper respiratory wash/swab (21120.8918 SARS-CoV-2 [COVID-19] RT-qPCR Validation Report Version 3.0) by Eurofins-Viracor.

Validation parameter results are presented in Table 10.

Table 10: RT-qPCR Assay (upper respiratory samples): Summary of Method Validation Parameters

Assay Characteristic	Study 301 (Validated)	Acceptance Criteria
[redacted]	[redacted]	[redacted]

Source = 21120.8918 SARS-CoV-2 (COVID-19) RT-qPCR Validation Report.

2.2.2 Viral Load (RT-qPCR [Saliva Samples])

The SARS-CoV-2 specific RT-qPCR detect SARS-CoV-2 RNA in swab and saliva specimens and is intended for the quantitative detection of RNA from SARS-CoV-2 virus to analyze viral load of SARS-CoV-2 infection diagnosis over time.

Nucleic acid extraction for swab and saliva specimens using saliva collection kits made by Isohelix were performed following instructions in Eurofins-Viracor-developed SOPs for RT-qPCR. The SARS-CoV-2 RT-qPCR assay is performed as a multiplex reaction with the MS2 internal control assay. Oligonucleotide primers and TaqMan probes developed by Eurofins-Viracor are used for the detection of the viral N-protein gene region of SARS-CoV-2 and an internal extraction and amplification control target (the RNA bacteriophage MS2) are used.

The performance characteristics of this *SARS-CoV-2 specific RT-qPCR assay* was validated for swab and saliva specimen (21120.9249 SARS-CoV-2 RT-qPCR Swab and Isohelix-Saliva Validation Report Version 1.0) by Eurofins-Viracor.

Validation parameter results are presented in Table 11.

Table 11: RT-qPCR (saliva sample) Assay: Summary of Method Validation Parameters

Assay Characteristic	Study 301 (Validated)	Acceptance Criteria
(b) (4)		

Source = Eurofins-Viracor report 21120.9249 SARS-CoV-2 RT-qPCR Swab and Isohelix-Saliva Validation Report Version 1.0.

2.2.3 Evaluation of Viral and Bacterial Infection Assessment (RT-PCR BioFire)

The BioFire FilmArray® RP is a diagnostic multiplexed nucleic acid test intended for the simultaneous qualitative detection and differentiation of nucleic acids from 20 viral and bacterial respiratory organisms.

The disposable closed system pouch is run on the FilmArray® 2.0 system which lyses samples, extracts, and purifies all nucleic acids, and performs nested multiplex PCR. Endpoint melting curve data is used to detect target-specific amplicons and analyzes data to generate a result for each analyte. Nasopharyngeal swabs were used as qualitative diagnostic assays for the detection of 20 different viruses and bacteria associated with respiratory tract infection (Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Human Rhinovirus, Enterovirus, Influenza A, Influenza A/H1, Influenza A/H1-2009, Influenza A/H3, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*). Assay result readouts were negative or positive for each pathogen in the BioFire FilmArray® RP. Samples were collected according to the Schedule of Events listed in the protocol.

The performance characteristics of this BioFire FilmArray® RP assay in nasopharyngeal swabs and oropharyngeal swabs was qualified (Qualification Report of the BioFire FilmArray® Respiratory Panel Version 1.0) by Eurofins-Viracor.

Validation parameter results are presented in Table 12.

Table 12: RT-PCR BioFire FilmArray® Assay: Summary of Method Validation Parameters

Assay Characteristic	Study 301 (Qualified)	Acceptance Criteria
(b) (4)		

Source = Qualification Report of the BioFire FilmArray® RP.

2.2.4 Genotypic and Phenotypic Assessment

2.2.4.1.1 Next-Generation Sequencing (RT-PCR NGS)

The NGS assay was performed to evaluate the genetic/phenotypic relationship of isolated SARS-CoV-2 strains to the mRNA-1273 vaccine sequence by sequencing the whole SARS-CoV-2 S-protein genome.

The NGS assay performed in Study 301 was done using residue samples initially collected for the qualitative detection of RNA from SARS-CoV-2 from nasal swabs (Section 2.2.1). No additional samples were collected for the NGS assay.

Viral RNA from nasal swabs was extracted using the NucliSENS® easyMag® extraction kit. Extracted RNA was used as template in a Qiagen QIAquick One-Step RT-PCR reaction for complementary DNA (cDNA) synthesis using SARS-CoV-2 S gene Conventional RT-PCR primer mixes. The Agilent 2200 or 4200 TapeStation in conjunction with D5000 ScreenTapes, D5000 reagents and the TapeStation Analysis software was used to assess postamplified and purified PCR reactions for the presence, size, and concentration of any products generated. Library preparation was performed using Illumina Nextera XT Library Prep Kit. The Agilent 2200 or 4200 TapeStation in conjunction with D5000 ScreenTapes, D5000 reagents, and the TapeStation Analysis software was used to assess purified libraries for presence, average fragment size, and concentration of the fragment distributions generated. Analysis of NGS data for the SARS-CoV-2 S gene NGS assay was done using Qiagen CLC Genomics Workbench Version 20.0.1 using NC_045512.2 as the reference strain. The custom workflow in CLC Genomics Workbench processed the sequencing data as follows: paired fastq files were imported, primer sequences were trimmed from 5'-ends of reads, reads were mapped to the full SARS-CoV-2 reference genome (NC_045512.2), single nucleotide and insertion/deletion variants relative to reference were called and annotated, and a consensus sequence of the spike gene (bases 21615 to 25436) was generated. The analysis workflow reported annotated variant tables, spike-gene coverage tables, and S-gene consensus sequences. Upon TapeStation D5000 assessment and subsequent analysis of data using the TapeStation Analysis software, if the viral load was insufficient to obtain a correct band for the SARS-CoV-2 S gene targets (S1 [1026bp], S2 [893bp], S3 [1178 bp], and S4 [1264bp]), these results were considered negative. Positive results for the RT-PCR reactions were identified by 1) the presence of a band at the appropriate size for the SARS-CoV-2 S gene PCR products (S1 [1026bp], S2 [893bp], S3 [1178], and S4 [1264bp] relative to the D5000 ladder and 2) a peak table reporting a concentration for the specific bands for the sample.

The performance characteristics of this SARS-CoV-2 specific RT-qPCR assay were validated (Validation Report for Establishing the Performance Characteristics of the SARS- CoV-2 S Gene NGS) by Eurofins-Viracor.

Validation parameter results are presented in Table 13.

Table 13: RT-qPCR SARS-CoV-2 S-Gene NGS Assay (Upper Respiratory Samples): Summary of Method Validation Parameters

Assay Characteristic	Study 301 (Validated)	Acceptance Criteria
(b) (4)		

Source = 21120.10208 Validation Report for Establishing the Performance Characteristics of the SARS-CoV-2 S Gene Next-Generation Sequencing Version 1.0.

2.2.4.1.2 Next Generation Sequencing LabCorp

An additional S-protein gene sequencing assay was developed for further throughput optimization. Samples were shown to be extracted in up (b) (4) and sequenced in (b) (4). As such, throughput can (b) (4) (b) (4) depending on the needs of the project. This assay was used for exploratory purposes.

Viral RNA from nasal swabs stored in UTM was extracted using the Kingfisher Flex platform. Following extraction, nested RT-PCR reactions were performed to amplify the entire SARS-CoV-2 S-coding region. The resulting amplicon was purified and normalized before undergoing NGS library preparation (Kapa). Resulting barcoded libraries were normalized, pooled, and underwent 2x150 bp paired end sequencing on the Illumina MiSeq platform. FASTQ files were analyzed using a semiautomated bioinformatics pipeline. Briefly, reads were quality trimmed and overlapping paired reads were joined. Reads were aligned in a codon aware manner that maintained reading frame through the S gene. Codon and amino acid variants were determined and reported along with consensus sequences. Results were reviewed to ensure appropriate coverage levels and quality metrics were obtained for each run and each individual sample.

2.2.4.1.3 Whole Genome Sequencing Assay (RT-PCR WGS)

Whole genome sequencing (WGS) assay was performed to evaluate the genetic/phenotypic relationship of isolated SARS-CoV-2 strains to the mRNA-1273 vaccine sequence by sequencing the whole SARS-CoV-2 genome.

The WGS assay was performed using residue samples initially collected for the qualitative detection of RNA from SARS-CoV-2 from nasal swabs (Section 2.2.1). No additional samples were collected for the WGS sequencing assay.

Viral RNA from a nasal swab was extracted using the Kingfisher Flex platform and Gold Standard Diagnostics NovaPrime® RNA extraction kit. Extracted RNA was used as a template in a 1-step RT-PCR reaction for cDNA synthesis. Each cDNA was subjected to amplification using ARTIC SARSCoV2 primer pools. These primer pools were designed to amplify approximately 90 amplicons each, with each amplicon averaging approximately 400 bp. Mapping these amplicons to a reference sequence illustrated the 'tiled' approach used for primer design resulting in coverage of the entire SARS-CoV-2 genome. Purification of the ARTIC PCR reaction was performed manually with Beckman-Coulter SPRIselect magnetic beads. The concentration of amplified amplicons in each sample was quantified using the Qubit Flex fluorometer. Preparation of libraries was performed using the NEBNext Ultra II FS library prep kit in conjunction with the BRAVO liquid handling platform. Automated purification of the library reactions was performed using the Agilent BRAVO liquid handler and Beckman-Coulter SPRIselect magnetic beads. The fragment size distribution of the final pooled library was confirmed using the Agilent TapeStation 4200 or ThermoFisher BioAnalyzer 2100 DNA fragment analyzer prior to preparation for sequencing. Pooled libraries were denatured and sequenced on the NextSEQ 500 or 550 instrument using a NextSEQ Mid Output 500/550 flow cell and reagents running a 2x 150 cycle paired-end sequencing protocol.

A Twist SARS-CoV-2 RNA positive control was processed in parallel with each verification run for positive control of the reverse transcription, ARTIC PCR amplification, and library preparation.

This proprietary serological method, "*SARS-CoV-2 WGS assay*" was developed, qualified, and validated by Eurofins-Viracor (SARS-CoV-2 WGS Verification Report Version 1.0) and used in Study 301.

Validation parameter results are presented in Table 14.

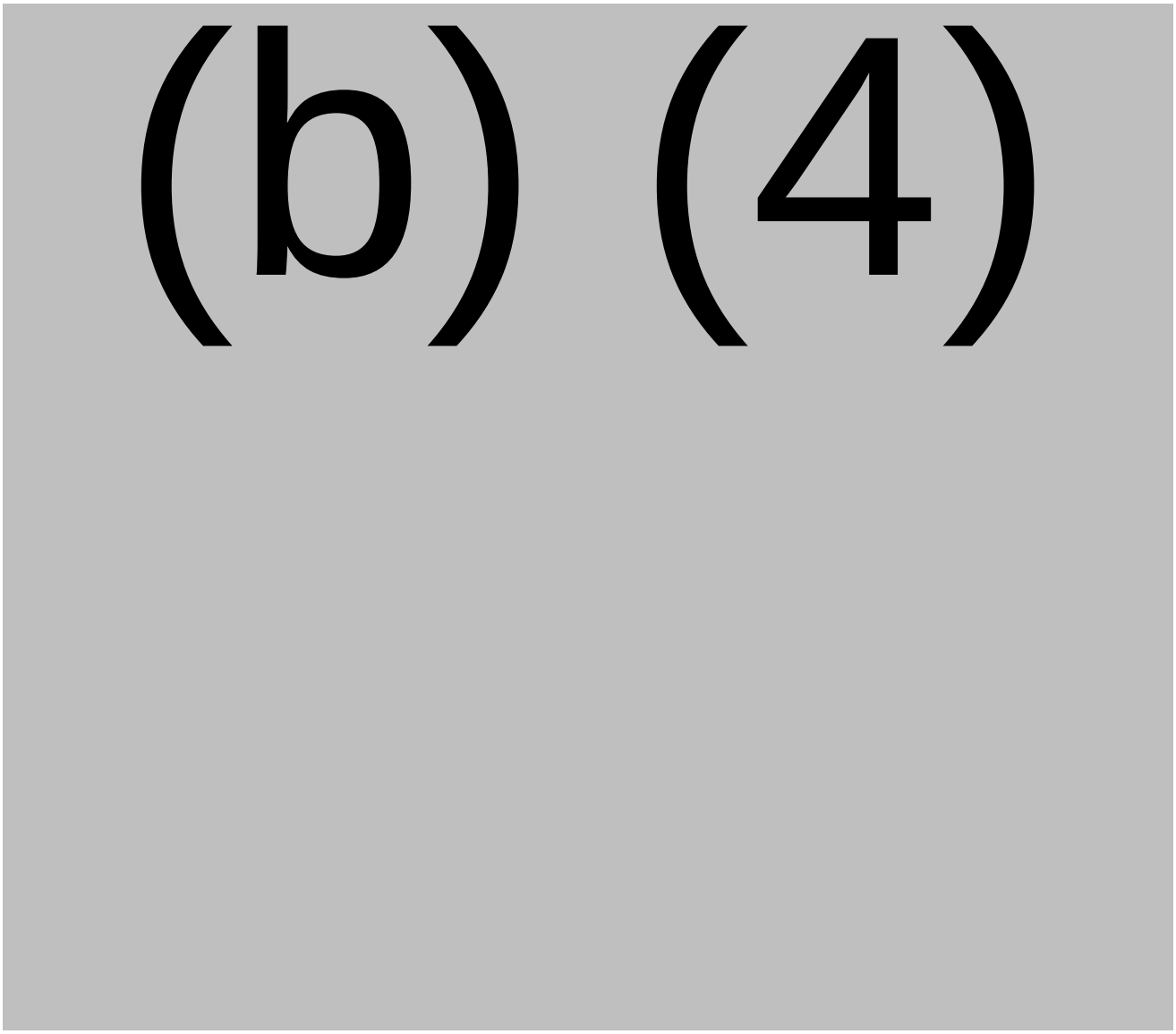
Table 14: RT-qPCR WGS Assay (Upper Respiratory Samples): Summary of Method Validation Parameters

Assay Characteristic	Study 301 (Validated)	Acceptance Criteria
LOD	100 copies/ARTIC PCR assay	N/A
(b) (4)		

Abbreviations: LOD = limit of detection; N/A = not applicable; PCR = polymerase chain reaction; RT-qPCR = reverse transcriptase quantitative polymerase chain reaction
Note: For all acceptance criteria, samples were required to be successfully sequenced, that is have (b) (4) sequencing genome coverage with (b) (4)
Source = SARS-CoV-2 WGS Verification Report Version 1.0.

2.3 Intracellular Cytokine Stimulation Assessment

An ICS assay was used to evaluate T-cell responses elicited by the mRNA-1273 vaccine in clinical samples collected in Study 101.



The SARS-CoV-2 ICS assay is a fit-for-purpose research assay that has not been qualified or validated. The intent of evaluating the ICS assay was to evaluate if the mRNA-1273 vaccine induced a Th-2 directed response (which has been linked with VAERD) or a Th1 directed response (which has been linked with lack of VAERD). This was done through the evaluation of CD4+/CD8+ T cells which can be segregated into Th1/Th2 directed responses.

3 STANDARD OPERATING PROCEDURES

All performance evaluations of bioassays supporting licensure were done using the following SOPs:

BINDING ANTIBODIES	
ELISA: S-2P-protein IgG	VSDVAC 65 Method Validation Plan Addendum 4
ELISA: N-protein IgG	VSDVAC 66 Method Plan Addendum 3
ELECSYS: N-protein IgG	SOP-18550 Anti-SARS-CoV-2 in Serum and Plasma by Electrochemiluminescence on Roche Cobas 8000 (e801)
MSD Singleplex: S-protein IgG	SOP5525 Multiplex (4-Plex) Assay for the Detection of IgG Antibodies Against SARS-CoV-2 Proteins in Human Sera
NEUTRALIZING ANTIBODIES	
PsVNA	CFAR02-A0026 Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped Virus and 293T/ACE2 Cells
Live Virus MN	SOP BBR.C.X-339-10 Microneutralization Assay for SARS-CoV-2
SARS-CoV-2 VIRAL RNA ASSESSMENT	
RT-qPCR (Upper Respiratory Samples)	21120.9204 Client Specific SARS-CoV-2 RT-PCR Performance
RT-qPCR Multiplex (Saliva Samples)	21120.9204 Client Specific SARS-CoV-2 RT-PCR Performance
RT-PCR BioFire	SOP 21120.2380 Procedure for BioFire FilmArray Respiratory Panel and Pneumonia Panel
RT-PCR NGS	21120.10120 SARS-CoV-2 Next Generation Sequencing Procedure
RT-PCR WGS	21120.10517 SARS-CoV-2 NGS Whole Genome Sequencing Procedure

Abbreviations: ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; MN = microneutralization; MSD = MesoScale Discovery; N = nucleocapsid; NGS = next generation sequencing; PsVNA = pseudovirus neutralization assay; RT-PCR = reverse transcriptase-polymerase chain reaction; S = spike; S-2P = S-protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2; SOP = standard operating procedure; WGS = whole genome sequencing.

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